Early Intervention with High-Dose Acyclovir Treatment during Primary Herpes Simplex Virus Infection Reduces Latency and Subsequent Reactivation in the Nervous System In Vivo

N. M. Sawtell,1 R. L. Thompson,2 L. R. Stanberry,1,a and D. I. Bernstein1

There remains a lack of agreement on the effect of antiviral therapy on herpes simplex virus (HSV) latency and subsequent reactivation. To gain insight into this important issue, a single-cell polymerase chain reaction assay was used to quantify the effects of high-dose acyclovir on latency in a mouse model. Treatment with 50 mg/kg of acyclovir every 8 h reduced the number of latently infected neurons by >90% when treatment was begun before 24 h after infection and by 80% and 70% when begun at 48 or 72 h after infection, respectively. The biologic significance of these reductions was evaluated by using a well-established in vivo reactivation model. The number of animals in which virus reactivated was reduced significantly, even when acyclovir therapy was delayed until 72 h after infection, a time when animals had developed lesions. These findings indicate that potent antiviral therapy during early primary HSV infection can reduce the magnitude of the latent infection, such that a significant decrease in reactivation is observed.

During primary infection, herpes simplex virus (HSV) enters the nervous system where the viral genome can be maintained in a state of transcriptional repression, or “latency,” in neuronal nuclei (reviewed in [1, 2]). Reactivation from latency occurs in response to various types of physiologic stress and represents the initiation and progression of lytic gene expression to produce infectious progeny (reviewed in [1, 2]). Among the large number of people latently infected with HSV, only ~25% experience clinically manifested recurrences [2, 3]. Recent studies indicate that this figure underestimates the frequency of viral shedding from mucosal surfaces as a result of unrecognized symptomatic or apparent asymptomatic reactivation among seropositive individuals [4, 5]. However, there remains a wide range in the frequency of reactivation among seropositive individuals [4, 5]. Although immune competency is an important influencing factor [3], the pathobiologic basis for the differences in the frequency of reactivation among immunocompetent individuals has not been defined [3].

One hypothesis is that the magnitude of the latent pool (viral genome burden) is an important factor that influences the frequency of subsequent reactivation experienced by the infected host [2]. Two elements of the latent pool that determine its magnitude are the number of latently infected neurons and the number of viral genomes in each infected neuron. Of importance, viral replication at the body surface and within the ganglia appears to be a significant determinant of the magnitude of both of these elements [6]. Furthermore, there is evidence that the number of latently infected neurons [7] and the number of viral genome copies in individual latently infected neurons [8] influence in vivo reactivation competency. There are a number of animal model studies suggesting that factors altering the establishment of latency also alter the probability of recovering virus from ganglia cultivated in vitro [6–16] (reviewed in [17]). For example, because antiviral therapy administered during primary infection has the potential to decrease viral replication (reviewed in [9]), it is possible that, by influencing the size of the latent pool, therapy during primary infection could effectively decrease the number of subsequent recurrences for the patient.

Thackray and Field [10, 11] recently compared the ability of valacyclovir and foscarnet to reduce the establishment of latency when administered after a delay of 1–5 days postinfection (pi). It was found that antiviral treatment administered within 48 h pi consistently reduced the frequency of reactivation from the trigeminal ganglia (TG), as determined by conventional explant culture. In that study, latent sites were estimated by in situ hybridization for the latency-associated transcripts (LATs), an assay that marks a subset of neurons containing the viral genome [12, 13]. However, there was no significant correlation between the differences in the number of LAT sites and explant reacti-
vation [11]. The similarities and differences between explant and in vivo reactivation have yet to be fully defined. Selected virus mutants have been shown to reactivate more slowly [14] or with reduced efficiency [15, 16] in explant. Nonetheless, explant is a more permissive environment for viral replication than is the ganglionic environment in vivo [17]. This raises the possibility that explant studies underestimate the effect of antiviral treatment on subsequent reactivation frequency. Although a few studies have suggested a correlation between the size of the latent pool and the frequency of in vivo reactivation [7, 8, 18–20], there has been no comprehensive analysis of the impact of antiviral treatment on this relationship.

Indeed, controversy remains as to whether antiviral therapy initiated after the appearance of lesions can alter the subsequent reactivation frequency of the infected individual. Clearly, this issue has important implications for goals established for anti-HSV therapies and treatment of patients with first-episode infections. We have examined this issue using a mouse model of HSV latency and in vivo reactivation [21], together with a single-neuron polymerase chain reaction (PCR) assay [22]. In this report, the influence of the interval between virus inoculation and initiation of antiviral therapy on the number of latently infected neurons in the ganglia, the viral copy number profile, and the frequency of in vivo reactivation is presented.

Materials and Methods

Mice and virus inoculations. Male Swiss Webster mice (18–20 g; Harlan) were housed in American Association for the Accreditation of Laboratory Animal Care–approved facilities and had continuous access to food and water. HSV-1 strain 17 syn+ (originally obtained from J. Subak-Sharpe, Medical Research Council Virology Unit, Glasgow, Scotland) was propagated on rabbit skin cell (RSC) monolayers, and titers of stocks were determined by routine plaque assay. Mice were anesthetized with sodium pentobarbital (50 mg/kg body weight) administered by intraperitoneal injection. Corneas were scarified, and 10⁵ pfu was placed on each scarified corneal surface. This dose is nonlethal in 80% of animals, 100% of whom become latently infected [7].

Acyclovir treatment (dosage and timing). Acyclovir (Glaxo-Wellcome) was dissolved in 0.15 M sterile saline at 5 mg/mL just before use. Doses of 50 mg/kg were administered by intraperitoneal injection (250 μL) to mice every 8 h [23, 24]. Sham-treated mice were given 250 μL of 0.15 M sterile saline. Acyclovir treatment was initiated in groups of mice at the time of inoculation (time 0) and at 24, 48, 72, 96, 120, and 144 h pi. Treatment was terminated at the end of pi day 7. By this time, most animals, regardless of treatment, had spontaneously cleared the virus.

Replication kinetics in vivo. A total of 160 mice (20 mice/group) were inoculated, as described above. At each of the indicated pi times, 3 mice from each group were euthanized, and the appropriate tissues were removed, snap-frozen, and stored at −80°C. Each group of 3 pairs of eyes or TG was homogenized in 1 mL of MEM supplemented with 5% newborn calf serum (Gibco-BRL) and was centrifuged for 5 min at 5000 g. Serial 10-fold dilutions of the homogenates were assayed in triplicate for infectious virus titer on RSC monolayers. The remaining mice were maintained for latency studies, as described below.

Quantification of latent infections, using single-neuron PCR. Mice infected and treated, as described above, were maintained for a minimum of 40 days pi. Two sets of 3 mice (6 TG) from each group were analyzed. Enriched neuron populations were obtained, as described elsewhere [7, 8, 22]. In brief, animals were anesthetized with sodium pentobarbital and were perfused with Streck's tissue fixative. Fixed TG were dissected out and were dissociated into single-cell suspensions; collagenase and neurons were purified on Percoll (Pharmacia) gradients. Neurons were stained with Ponceau-S and were aliquoted into 200-μL PCR tubes. The tube contents were visualized by use of a microscope, and the number of neurons in each tube was adjusted to the desired number. After treatment with immobilized DNase I and subsequent inactivation of this enzyme, intracellular DNA was liberated with proteinase-K and was analyzed for the presence of the HSV-1 genome, using the quantitative PCR (QPCR) assay developed by Katz et al. [25]. Complete details of the procedure, as applied in this study, have been reported elsewhere [22]. HSV standards containing known numbers of viral genome equivalents were treated identically to test samples and were amplified with each PCR run. Products were electrophoresed, blotted, probed with an internal 32P-labeled oligonucleotide, and quantified on a phosphorimager (Molecular Dynamics), using ImageQuant software, as described elsewhere [22, 25]. Viral genome copy number was calculated from the standard curve run with the samples being quantified.

The frequency of HSV-positive neurons in each group was estimated from an initial screening. On the basis of these results, most groups were analyzed by examination of single-neuron samples. However, groups with a very low frequency of HSV-positive neurons in the preliminary analysis were analyzed further by the examination of 10-neuron samples, as described elsewhere [22]. Because this initial analysis revealed no significant differences between the 2 sets of 6 TG harvested from each treatment group, they were pooled for further analysis.

Reactivation in vivo. The frequency of in vivo reactivation of virus in treated and untreated latently infected mice was evaluated, using the hyperthermic stress model [21]. In brief, >40 days after inoculation, latently infected mice were placed in a 43°C water bath for 10 min to induce hyperthermia. Twenty-two hours later, mice were euthanized, and the TG were removed. TG were homogenized individually, and the homogenates were centrifuged to remove cellular debris. The supernatants were plated onto RSC monolayers and were allowed to adsorb for 2 h. Plates then were rinsed with fresh medium and were overlaid with medium containing 1% carboxymethylcellulose. Three days after plating, medium was removed, and plates were rinsed with PBS and were stained with crystal violet. Plaques were counted under a dissecting microscope.

Statistics. Statistical significance of the differences between groups was determined as indicated within the text. All tests were 2-tailed. Statistical software (GraphPad Prism) was used to analyze the data.

Results

Effect of acyclovir on acute replication of HSV in the eyes and TG. Viral replication in the eyes and TG is summarized...
in table 1. The data are presented as the area under the curve (AUC). This value reflects the total replication and was calculated (Prism statistical software) from the replication kinetic curves constructed from the virus titers in tissues pooled from 3 mice from each group on pi days 2, 4, 6, and 8. The ratio of the AUC of acyclovir-treated to sham-treated groups reveals that a major impact on replication in the eyes was achieved only in mice treated at the time of inoculation. In these mice, total HSV replication in the eyes was only 3.5% of that in sham-treated control mice. A delay of as little as 24 h pi resulted in viral replication equivalent to slightly >70% of that in the sham-treated controls. Total replication in the eyes was 100% of that in the sham-treated controls when antiviral treatment was delayed for \( \geq 96 \) h pi (table 1).

In the TG, a somewhat different picture emerged. When acyclovir treatment was initiated within 48 h pi, the total replication in the TG was reduced to \( \leq 4% \) of that in the sham-treated group (table 1). Thus, repression of replication in the TG occurred with treatment delayed 2 days beyond the time required for the same level of repression of replication in the eyes. Treatment beginning 72 h pi reduced total TG replication to 39% of that of sham-treated controls. A delay of treatment for \( \geq 96 \) h pi resulted in replication in the TG equivalent to that observed in sham-treated controls (table 1).

**Effect of acyclovir on the number of latently infected neurons.** The number of latently infected neurons in TG from acyclovir- and sham-treated mice was determined by use of a single-cell PCR assay. Forty days pi, 2 sets of 3 mice from each group were perfusion fixed, neurons were harvested, and single neurons were analyzed by QPCR for the HSV genome, as described in Materials and Methods and elsewhere [22]. Results from this analysis are summarized in figure 1. As the initiation of acyclovir treatment was delayed, progressively more latent infections were established in TG neurons. Treatment begun within the first 24 h pi reduced by \( \geq 90\% \) the number of ganglionic neurons that were latently infected, compared with that in sham-treated controls (\( P < .0001 \), Fisher’s exact test). Delaying treatment after infection for 48 or 72 h still reduced the percentage of neurons latently infected by 80% (\( P < .0001 \), Fisher’s exact test) and 72% \( (P = .0001 \), Fisher’s exact test), respectively, compared with that in the sham-treated group. However, at \( \geq 96 \) h, a \( <22\% \) reduction was observed, which was not significantly different from that in the sham-treated control group. Employing the estimate of 20,000 neurons per TG [22] and the percentage of neurons found to be positive for HSV DNA, we calculated that, in the sham-treated control group, there were \( \sim 6120 \) latently infected neurons per ganglion. When acyclovir treatment was administered at the time of virus infection or at 24 h pi, this number was reduced to 196 or 300, respectively. When treatment was delayed until 48 or 72 h pi, the size of the latent pool more than quadrupled to reach 1200 or 1720 neurons/ganglion, respectively (figure 1). Delay of treatment for \( \geq 96 \) h resulted in latent pools containing \( \sim 5000 \) infected neurons.

The effect of acyclovir treatment on the number of latently infected neurons and on the total replication (AUC) occurring in the eyes and TG during the course of the acute infection is presented as the percentage of sham-treated controls in table 2. The results show a break point between 72 and 96 h pi, with respect to the number of latent infections established. This break point is paralleled by a similar break point for replication in the TG but not for replication at the surface (eye).

**Effect of acyclovir on the viral genome copy number within individual neurons.** The single-neuron PCR assay that we recently developed for the analysis of HSV latency allows for the number of HSV genomes present in a single latently infected neuron to be determined. The measurement of the number of latent genomes present in many individual neurons allows for the generation of a profile of the latent pool. A reduced copy number profile has been correlated with a reduced capacity to reactivate [8]. Therefore, we determined the effect of administration of antiviral therapy on this profile. Viral copy number profiles are presented in figure 2. The ability of acyclovir to reduce the number of viral genome copies within the individual latently infected neurons required early intervention; a statistically significant impact was observed only in mice treated at the time of infection \( (P = .001 \), Mann-Whitney \( U \) test) or within 24 h pi \( (P = .0027 \), Mann-Whitney \( U \) test; figure 2). Although the mean copy number in the acyclovir treatment group in which treatment was initiated at 48 h pi was lower than that in 72-h group, the difference was not significant.

**Effect of acyclovir on the reactivation of HSV in mouse TG in vivo.** The hyperthermic stress induction procedure was used to determine the effect of acyclovir treatment during primary infection on the subsequent frequency of virus reactivation in vivo [21]. These data are summarized in figure 3. Virus reactivation was detected in 0% (0/30 TG; 0/15 mice) of TG examined from mice treated with acyclovir beginning 24 h pi, compared with 68% (22/32) of TG from sham-treated mice \( (P < .0001 \), Fisher’s exact test). There also were statistically sig-
Figure 1. Impact of delayed acyclovir (ACV) treatment on the no. of neurons containing the latent viral genome. Height of bars represents the percentage of trigeminal ganglia (TG) neurons containing the herpes simplex virus (HSV) genome, as determined by single-cell polymerase chain reaction (PCR) assay. No. of positive neurons/no. tested is listed for each group, as is the percentage of total TG neurons latently infected. An example of the single-cell PCR data is shown above the bar graph. The viral genome standards amplified with each run are to the left of the panel. Each lane contains one-tenth of the product amplified from a single TG neuron and probed, as described in Materials and Methods. Most of the neurons contain no detectable HSV DNA. Those that are positive show a variable signal intensity that reflects the no. of viral genome copies within the neuron.

There were significant reductions in the reactivation frequency in the groups treated with acyclovir beginning either 48 h (1/32 TG) or 72 h (3/32 TG) pi, compared with that in sham-treated controls (22/32 TG; \( P < .001 \), Fisher’s exact test). A trend toward fewer reactivation events also was seen in the group in which treatment was begun 96 h pi (11/24 TG), but this difference was not significantly different, compared with that in the sham-treated controls (\( P = .1 \), Fisher’s exact test). A comparison of the amount of virus produced in reactivating TG (figure 3) demonstrated no significant difference between the groups (\( P = .4 \), unpaired Student’s \( t \) test).

**Correlation between reactivation frequency and latent pool.** Both the percentage of neurons latently infected and the mean viral genome copy number were skewed downward by antiviral treatment, although with different sensitivities to timing of treatment. The relationships among frequency of reactivation, the percentage of latently infected neurons, and the mean viral genome copy number were tested, using the method of Pearson. As an independent parameter, the mean copy number did not show a significant correlation with reactivation frequency when examined across the entire data set (\( r^2 = .6421; \ P = .0553 \)). However, there was a significant positive correlation between the reactivation frequency and the percentage of neurons latently infected (\( r^2 = .9771; \ P = .0002 \)). There was an even greater positive correlation between the frequency of reactivation when the percentage of neurons latently infected was multiplied by the mean genome copy number (\( r^2 = .9909; \ P < .0001 \)), which suggests that both characteristics of the latent pool influence the frequency of reactivation (figure 4). This relationship predicts that a 50% reduction in the frequency of reactivation could be achieved by an \(~50\%\) reduction in the magnitude of the latent pool, a level attainable by antiviral intervention initiated between pi days 3 and 4.

**Discussion**

This study is different from antiviral evaluations in that it is the first to link in vivo reactivation frequency in the nervous
Table 2. Comparison of the number of latently herpes simplex virus (HSV)-infected neurons and of acute HSV replication in acyclovir- versus sham-treated animals.

<table>
<thead>
<tr>
<th>Acyclovir group,</th>
<th>Percentage of sham-treated animals (test/sham × 100)</th>
<th>Replication</th>
</tr>
</thead>
<tbody>
<tr>
<td>time treatment initiated after infection, h</td>
<td>Latently infected neurons</td>
<td>Eyes</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>71</td>
</tr>
<tr>
<td>48</td>
<td>20</td>
<td>72</td>
</tr>
<tr>
<td>72</td>
<td>28</td>
<td>81</td>
</tr>
<tr>
<td>96</td>
<td>79</td>
<td>99</td>
</tr>
<tr>
<td>120</td>
<td>78</td>
<td>105</td>
</tr>
<tr>
<td>144</td>
<td>84</td>
<td>100</td>
</tr>
</tbody>
</table>

NOTE. Total no. of latently infected neurons was estimated, as described in Materials and Methods. For viral replication, the area under the curve on days 0–8 was compared.

system with a precise quantification of the latent pool by 2 fundamental criteria, the number of neurons containing the viral genome and the viral genome copy number profile. The initiation of high-dose acyclovir treatment after infection was delayed in 24-h increments, starting at the time of infection or at 24, 48, 72, 96, 120, or 144 h pi. Thus, not only was the direct effect of the antiviral treatment on latency in the nervous system precisely evaluated, but the relationship between this primary effect, the timing of treatment, and the long-range biological outcome in the nervous system was determined.

Early studies were restricted in that methods for quantifying latency based on viral DNA were not available; thus, recovery of virus from explanted latently infected ganglia was used as an indirect measure. In these studies, only the administration of acyclovir at or soon after HSV inoculation resulted in a reduced frequency of in vitro explant reactivation [26–30]. More recently, 4 assays have been used to directly quantify the effect of nucleoside analogues on establishment of latency. These include (1) in situ hybridization for the LATs [10, 11, 31, 32], (2) whole-ganglia PCR for the viral genome [33, 34], (3) promoter/reporter mutants enumerating reporter-expressing cells [31, 32, 35], and (4) contextual expression analysis, the single-cell PCR assay used here [7, 22]. Most of these studies examined the effect of the antiviral when administered very early pi, from 0 to 24 h, and consistently showed a reduction in the level of latency but not complete prevention.

Our approach revealed several points of interest. First, antiviral treatment administered during the course of primary infection can profoundly influence the size of the viral latent gene pool and the long-term pathologic outcome in the host nervous system. The number of latently infected neurons could be reduced by >95%, compared with that in the sham-treated animals. As anticipated, the impact of treatment lessened as the delay in treatment increased. However, even as late as pi day 3, acyclovir treatment reduced the number of latently infected neurons by 78%. Of importance, this reduction in the size of the latent pool translated into a significant reduction in the frequency of reactivation in the nervous system in vivo. Indeed, 69% of ganglia from sham-treated animals reactivated, compared with only 9% of those treated with acyclovir starting at pi day 3. Thus, the 78% reduction in latent infections resulted in 86% fewer reactivations. Even delay in treatment until pi day 4 resulted in a 21% reduction in the number of latent infections and a 32% reduction in reactivation frequency, compared with those for sham-treated controls.

A second important observation involves the relationship between the magnitude of the latent pool and the frequency of reactivation. As shown in figure 4, our results reveal a direct correlation ($r^2 = .9914; P < .0001$) between the size and copy number profile of the latent pool and the frequency of in vivo reactivation. Ninety-nine percent of the variance in reactivation frequency can be explained by the variance in the magnitude of the latent pool. This finding is fully consistent with and provides

Figure 2. Herpes simplex virus genome copy no. in individual latently infected trigeminal ganglia neurons. Each point represents the viral genome copy no. in a positive neuron, as determined from the single-cell polymerase chain reaction (PCR) assay. The no. of copies was calculated from standards amplified with each PCR run. All samples were analyzed by Southern blots exposed to a phosphoimager screen. The intensities of the signals were quantified using ImageQuant software (Molecular Dynamics). The bar in each column indicates the mean genome copy no. “ACV h pi,” no. of hours postinoculation that acyclovir was first administered.
Figure 3. In vivo reactivation frequency and quantification of virus output in trigeminal ganglia (TG). A. Bar graph showing the percentage of TG positive (TG+) in each treatment group at 22 h after hyperthermic stress. Beneath each bar is the time of initiation of acyclovir treatment and the no. of virus-positive TG/no. tested. Differences between the sham-treated group and the 24-, 48-, and 72-h postinoculation treatment groups were significant (, , and , respectively, \( P < 0.0001 \)). B. Column scattergram showing the no. of plaque-forming units recovered from each ganglion 22 h after hyperthermic stress. Beneath each column are the time of initiation of acyclovir treatment and the mean (range) plaque-forming units of positive TG. Although there was a trend toward more virus in positive TG from the sham-treated group, compared with that from treated groups, differences were not significant. “h pi,” Hours postinoculation.

Figure 4. Representation of the relationship between the frequency of in vivo reactivation and the percentage of neurons latently infected (% LN) \( \times \) mean genome copy no. (MGCN). The percentage of reactivation and magnitude of the latent pool established in the treated TG was observed. In contrast to the surface replication, ganglionic replication was very low at this time, reaching levels of only 4% of those observed in the sham-treated controls. Thus the 95% reduction in latent sites appears to be reflected in the 96% reduction in TG replication. As the timing of treatment was delayed, replication in the TG increased. The increase in TG replication was accompanied by a parallel increase in the number of latent sites in the TG (table 2). In contrast, surface replication was not a faithful indicator of the level of establishment of latency. However, this parameter did appear to correlate with the copy number profile.

support for the hypothesis that variation in the magnitude of HSV latent infections in human TG is an important factor contributing to the widely variable frequency of recurrences observed among infected individuals. These findings also are consistent with our previous study, which showed a direct relationship between virus input titer, number of latently infected neurons in the TG, and in vivo reactivation frequency [7]. The fact that this important biologic relationship was not revealed in studies comparing LAT sites and in vitro reactivation [10, 11] suggests that either one or both of these assays may not be appropriate measures of the relevant biologic correlate.

We found what appears to be a breakpoint in the impact of acyclovir treatment on latent infections between pi days 3 and 4. The delay in initiation of treatment from day 3 to day 4 resulted in an increase in viral replication in the TG from 39% to 97%, compared with that of sham-treated controls, and an increase in the size of the latent pool from \( \sim 1700 \) to \( \sim 4800 \) latent sites per ganglion. With respect to disease outcome, this increase in the latent pool resulted in an increase in the percentage of TG in which virus reactivated from 9% to 46%, compared with sham-treated controls (68%). A similar breakpoint was not evident in the TG in either of the studies reported by Thackray and Field [10, 11], as reflected in LAT-positive sites. However, there are several important differences in the design of the studies that could account for this, including route of inoculation, virus strains, and dosing regimens. A final critical difference between the studies resides in the methods used to quantify both latency and reactivation.

One unanticipated result was the disparity between the effect of acyclovir treatment on the level of surface replication, when compared with the effect on the number of latent sites. The initiation of acyclovir treatment, even within 24 h pi, resulted in replication in the eyes \( \sim 70\% \) of that in the sham-treated controls. Despite this robust surface replication, a 95% reduction in the number of latent sites established in the treated TG was observed. In contrast to the surface replication, ganglionic replication was very low at this time, reaching levels of only 4% of those observed in the sham-treated controls. Thus the 95% reduction in latent sites appears to be reflected in the 96% reduction in TG replication. As the timing of treatment was delayed, replication in the TG increased. The increase in TG replication was accompanied by a parallel increase in the number of latent sites in the TG (table 2). In contrast, surface replication was not a faithful indicator of the level of establishment of latency. However, this parameter did appear to correlate with the copy number profile.
A repressed copy number profile was observed only while surface replication was low. This is consistent with our recent finding that replication within the TG is required for the establishment of high numbers of latent sites, whereas surface replication more closely correlates with a large number of viral genome copies in individual neurons [6].

A more practical implication of these data relates to how antivirals are currently evaluated. If, as our data strongly indicate, surface replication does not reflect the impact of a treatment on the latent pool, then the efficacy of antivirals to effect the number of latent sites in the TG may not be reliably measured by preclinical evaluations that are restricted to disease and/or peripheral viral replication. Direct quantitative assessment of the level of latency and quantitative assessment of in vivo reactivation potential seem essential if these studies are to be useful in predicting the effect on subsequent reactivation in humans.

It is of value to comment on the reproducibility of the inoculation and treatment procedures, as well as the single-neuron assay used to quantify latency in this study. How consistent are the number of latently infected neurons from experiment to experiment, and is the effect of the drug treatment consistent or variable? In 2 previous studies [7, 22], acyclovir treatment was administered early pi, and the effect on the latent pool was determined. A comparison of these previous results with those in this study shows excellent reproducibility. In these experiments, acyclovir reduced the number of latently infected neurons from 12000–12500 to 220–280 per ganglion when administered at the time of inoculation [7, 22]. These results are in good agreement with the results in this report, in which the number of latently infected neurons was reduced from ~6000 per ganglion (12,000 per TG pair) in the sham-treated group to ~200 per ganglion when acyclovir was administered at the time of infection.

In this HSV mouse model study, antiviral treatment delayed for as long as 3 days pi resulted in a biologically favorable outcome (i.e., a level of reduction in establishment of latency that significantly affected in vivo reactivation frequency). Of interest, systemic treatment had only a minor impact on surface replication when administration was delayed even 24 h pi. It is possible that combined topical treatment and systemic antiviral treatment could result in even greater efficacy at reducing the latent pool, but this remains to be determined. Although it is difficult to extrapolate how the timing of administration of antiviral compounds in animal models might correlate to the clinical setting, by 72 h pi, severe corneal involvement was readily apparent in these mice. In fact, most animals displayed overt surface lesions by pi day 2. It should be noted that these mice received a high inoculation titer on the scarified cornea, and the time course of the disease process is likely to be more rapid than commonly observed in humans.

Data on the effect of antiviral treatment of primary herpes infection on subsequent recurrences are limited but suggest that there is no impact (reviewed in [9]). It is likely that by the time the patient presents to the physician, significant viral replication and seeding of the TG has occurred. Nevertheless, the finding of Thackray and Field [29] and the findings presented here suggest that it is possible to reduce subsequent recurrences by treating the primary infection early and aggressively. The observation that viral replication, latent pool size, and in vivo reactivation frequency are directly correlated has implications beyond antiviral treatment. Any strategy that reduces replication of the virus early during primary infection likely will influence the extent of involvement of the nervous system and long-term disease outcome. Therefore, it is possible that, with more potent antivirals and early intervention, treatment of primary infection may reduce recurrences.

Acknowledgment

We thank Cheryl S. Tansky for expert technical assistance.

References

14. Maggioncalda J, Mehta A, Su YH, Fraser NW, Block TM. Correlation between herpes simplex virus type 1 rate of reactivation from latent...
fection and the number of infected neurons in trigeminal ganglia. Virology 1996;225:72–81.