Characterization of an in vivo reactivation model of herpes simplex virus from mice trigeminal ganglia

Bettina Birmanns, Israel Reibstein and Israel Steiner*

Laboratory of Neurovirology, Department of Neurology, Hadassah University Hospital, Jerusalem, 91120, Israel

Herpes simplex virus type 1 (HSV-1) is transcriptionally active during latent infection in human peripheral sensory ganglia. Viral gene expression includes the latency-associated transcripts (LATs) which have been linked to the ability of the virus to resume replication and reactivate. However, the molecular basis of reactivation and the mechanisms of action of these transcripts are unknown. In order to study these parameters, an in vivo reactivation model is needed. We investigated use of the mouse as the experimental animal, modifying the route of infection, the viral strain and the reactivation protocol. Following administration of human immunoglobulin 1 day prior to corneal infection, no infectious virus was detected in trigeminal ganglia (TG). However, latency was established in all infected animals as indicated by explant reactivation of TG, and in vivo reactivation was achieved in 30 to 40% of them. DNA quantification revealed that TG of immunized mice contained more HSV-1 DNA than did those of non-immunized mice. By in situ hybridization twice as many neuronal cells in TG of immunized mice were positive for LATs, compared with infected but non-immunized, mice. These findings suggest that suppression of primary infection facilitates reactivation by increasing HSV-1 copy number in latently infected nervous tissue.

Herpesviruses are capable of establishing and maintaining latent infections in their hosts (Stevens, 1989; Steiner & Kennedy, 1993). This feature provides clear biological advantages for the virus and contributes to its successful survival in a wide spectrum of organisms. Herpes simplex virus (HSV) type 1 establishes latent infection in the human nervous system and induces tissue damage and disease through replication and reactivation from latency. Following primary infection, latent HSV-1 persists in neurons within peripheral sensory ganglia and is transcriptionally active (Spivack & Fraser, 1987; Stevens et al., 1987; Steiner et al., 1988). The restricted gene expression during latency includes the latency-associated transcripts (LATs) which play an important role during latency since reactivation at explant of LAT(−) viruses is delayed and asynchronous compared to parental viral strains (Javier et al., 1988; Lieb et al., 1989; Steiner et al., 1989b). Similarly, the in vivo reactivation of these mutants is severely affected when examined in the rabbit experimental model (Hill et al., 1990; Trousdale et al., 1991). However, the mechanism(s) of action of the latency-associated gene expression of HSV-1 are still unknown.

Elucidating the molecular phenomena associated with the transition from the latent state into reactivation is dependent upon the availability of a reproducible and efficient in vivo reactivation model. Several in vivo reactivation models have been developed and include mice infected via the ear or cornea (Hill et al., 1975; Willey et al., 1984) and rabbits infected via the cornea, (Shimomura et al., 1983). These models have several disadvantages, such as spontaneous reactivations in the rabbit (Nesburn et al., 1967) and a low in vivo reactivation yield in the mouse (Hill et al., 1978). We were able to establish an in vivo reactivation model in mice by administration of human immunoglobulin 1 day prior to corneal infection. With this model, 30 to 40% in vivo reactivation was achieved in the latently infected mice. This system was further characterized and our findings may shed some light upon the mechanisms favouring in vivo reactivation.

Control female BALB/c mice (Hsd Sprague-Dawley Inc. or Hebrew University colony, Jerusalem, Israel) were injected intraperitoneally (i.p.) with increasing amounts of human hyperimmune serum (human immune globulin, USP; Gamastan, Miles Inc., Cutter Biological), up to 200 μl of serum per animal. No adverse reactions were observed and animals tolerated the human hyperimmune serum well. One day prior to corneal inoculation, mice were injected i.p. with 25, 50 or 100 μl of hyperimmune serum. Infection consisted of inoculation of 10^4 p.f.u. of HSV-1 strain F or KOS grown to stocks and titrated on CV-1 cells as described previously (Steiner et al., 1989b). Control animals were infected with the same virus strains without prior immunization. Starting from 1 day post-infection (p.i.) animals were sacrificed.
Fig. 1. HSV-1 titres in mouse TG during acute infection. Each point represents the geometric mean titre from eight ganglia tested individually at the indicated time p.i., from two experiments. Squares, HSV-1 (strain F) from non-immunized mice. No infectious HSV-1 (F) was detected during acute infection in TG from mice preimmunized with 25 µl of hyperimmune serum (closed circles).

Table 1. Disease frequency and survival rates of mice following corneal infection with and without prior administration of hyperimmune sera*

<table>
<thead>
<tr>
<th>HSV-1 strain</th>
<th>Titre (p.f.u.)</th>
<th>Non-immunized (%)</th>
<th>Immunized (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survival</td>
<td>Disease</td>
<td>Survival</td>
</tr>
<tr>
<td>F</td>
<td>$10^6$</td>
<td>80-90</td>
<td>80-100</td>
</tr>
<tr>
<td>KOS</td>
<td>$10^6$</td>
<td>30-40</td>
<td>100</td>
</tr>
<tr>
<td>KOS</td>
<td>$10^5$</td>
<td>50-60</td>
<td>100</td>
</tr>
<tr>
<td>KOS</td>
<td>$10^4$</td>
<td>70-90</td>
<td>100</td>
</tr>
</tbody>
</table>


Table 2. Explant and in vivo reactivation of latent HSV-1 from TG of mice immunized prior to viral inoculation

<table>
<thead>
<tr>
<th>HSV-1 strain reactivation</th>
<th>Titre (p.f.u.)</th>
<th>Explant reactivated* TG†</th>
<th>In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>$10^6$</td>
<td>19/20 (95%)</td>
<td>7/19 (37%)</td>
</tr>
<tr>
<td>KOS</td>
<td>$10^6$</td>
<td>10/10 (100%)</td>
<td>4/10 (40%)</td>
</tr>
<tr>
<td>KOS</td>
<td>$10^5$</td>
<td>14/16 (87%)</td>
<td>6/16 (37.5%)</td>
</tr>
<tr>
<td>Control†</td>
<td>$10^6$</td>
<td>20/20 (100%)</td>
<td>0/30 (0%)</td>
</tr>
</tbody>
</table>

* Expressed in numbers of animals.
† Strain F, non-immunized mice.

and TG removed for viral titration (Steiner et al., 1989b). Whereas viral replication was detected in the trigeminal ganglia (TG) of non-immunized control mice, no replication was detected in the TG of the mice immunized even with the lowest (25 µl) amounts of hyperimmune serum within 10 days p.i. with HSV-1 strain F (Fig. 1). This amount was therefore used for further experiments.

Similar results were obtained with HSV-1 strain KOS (data not shown).

Clinical signs of disease and survival were recorded in mice infected via the cornea using HSV-1 strains F or KOS with and without prior immunization with hyperimmune serum. Whereas in non-immunized animals survival ranged from 30 to 90%, depending on the HSV-1 strain and the amount of virus, 100% of the immunized mice survived (Table 1). None of the immunized animals had any signs of disease, but 80 to 100% of non-immunized mice exhibited ruffled skin, loss of weight, paralysis and other clinical signs. Following passage through CV-1 cells, HSV-1 strain KOS was highly virulent, causing up to 70% mortality in non-immunized animals (Table 1). Therefore only strain F was used for further experiments in non-immunized control mice.

Latent infection was examined by explant reactivation of TG, at least 4 weeks after viral inoculation and incubation of tissues on monolayers of CV-1 cells (Steiner et al., 1989b). The wells were inspected daily for signs of cytopathic effect (c.p.e.). Ganglia were transferred every 5 to 7 days to new cells and observed until reactivation occurred, or for 35 days. TG of preimmunized infected mice yielded reactivated HSV-1 at explant at comparable rates to control non-immunized and infected animals (Table 2). Between 87 and 100% of the TG in both groups were latently infected since they reactivated HSV-1. HSV-1 strains F and KOS produced similar results.

In vivo reactivation was induced by corneal scarification and u.v.-irradiation. Eyes of animals were swabbed daily and the swabs incubated on monolayers of CV-1 cells. Swabs and media were transferred every 2 days to fresh wells and the CV-1 cells were watched daily until the appearance of c.p.e., or for 21 days. Results were scored per animal, since each swab was used to culture both eyes in an individual animal. Between 37 to 40% of animals reactivated virus, in three separate experiments (Table 2). Virus shedding started on days 3 to 5 post-scarification, and lasted between 2 and 4 days in most animals. Exceptions included viral shedding at 1 day (2 mice) and 5 days in a single animal.

Several other means of inducing in vivo reactivation of HSV-1 from corneas of non-immunized mice failed. These included scarification of the cornea, u.v.-irradiation, or a combination of both, administration of immunosuppressive agents (methylprednisolone or cyclophosphamide), or i.p. injection or corneal application of anti-nerve growth factor (NGF) antibodies. In each experiment at least 10 mice were used and most experiments were carried out at least twice.

DNA was extracted from latently infected TG of preimmunized mice (Rock & Fraser, 1983; Mitchell et al., 1990), dot-blotted and hybridized to whole HSV-1
HSV-1 inoculation in order to suppress viral replication

immunized mice. There was no difference in the intensity of the signal between immunized and non-immunized mice TG (Table 3). Both F- and KOS-latently infected TG produced similar results. The signal was observed on and around the nucleus of ganglion cells. There was no difference in the intensity of the signal between immunized and non-immunized mice.

Several groups have used anti-HSV-1 serum prior to HSV-1 inoculation in order to suppress viral replication during primary infection (Sekizawa et al., 1980; Shimeld et al., 1989; Cook et al., 1991). However, preparation of anti-HSV-1 serum is time-consuming and expensive. We therefore chose to use human hyperimmune serum, which is commercially available and is relatively inexpensive. This preparation is used for viral titration because it prevents viral propagation via media in cell cultures, and it has been proved to be innocuous when injected into mice i.p. By immunizing mice with hyperimmune serum at the relatively low quantity of 25 μl, prior to HSV-1 inoculation, we were able to suppress primary infection, prevent disease and avoid mortality. Nevertheless, latent infection was effectively established in these animals at a rate similar to that in mice infected with HSV-1 without prior immunization, as measured by explant reactivation.

We have tried various protocols aimed at inducing in vivo HSV-1 reactivation in mice infected via the cornea. All failed except the one employing immunization of mice with hyperimmune serum prior to viral inoculation through the cornea. Although difficulty reactivating HSV-1 from mice latently infected by corneal inoculation seems to be inherent using these animals, and has become a constant frustrating challenge in various laboratories, the ability to induce in vivo reactivation following suppression of primary infection may shed light upon the mechanisms favouring HSV-1 reactivation from the human peripheral nervous system.

Viral replication is not a prerequisite for establishment of latent HSV-1 infection in peripheral sensory ganglia (PSG). HSV-1 mutants with deletions eliminating their capacity for in vivo replication establish effective in vivo latency (Russell et al., 1987; Coen et al., 1989; Steiner et al., 1990). The absence of viral replication does not necessarily prevent viral explant reactivation (Efstathiou et al., 1989; Steiner et al., 1990) and may in fact carry a theoretical advantage favouring in vivo reactivation (Steiner & Kennedy, 1991): since HSV-1 is a lytic virus, a full viral replication cycle during primary infection will culminate in the death of the infected cell and will therefore reduce the number of cells in the infected tissue which are available for establishment of latency. Thus, prevention of replication may increase the number of latently infected cells. Indeed, more cells within TG of preimmunized mice, where no viral replication was detected during primary infection, were latently infected compared with non-immunized mice, as measured by expression of LATs (Table 3). Similarly, the amount of HSV-1 DNA present in latently infected TG of pre-immunized mice was considerably higher than that present in non-immunized infected TG (Fig. 2) at a magnitude suggesting that the number of viral copies per cell was also increased.

It has been suggested (Roizman & Sears, 1990; Fraser
et al., 1991) that a smaller copy number of HSV-1 genomes within latently infected nervous tissue cells is associated with inability of the virus to initiate replication within the cell nucleus, whereas a larger copy number/cell will facilitate the ability of the virus to reactivate. This hypothesis is an extrapolation from the in vitro situation, where inability of HSV-1 to replicate in cell culture induced by mutagenesis within essential viral genes can be overcome by increasing the m.o.i. (Sears et al., 1985; Ace et al., 1989). Thus, the number of viral copies/cell and copies/PSG may determine ability of HSV-1 to reactivate under favourable conditions, such as reversible cell damage. This possibility was further supported by a recent study that showed a direct correlation between the number of latently infected cells and the ability of the HSV-1 to reactivate from mice trigeminal and sacral ganglia (Sawetell & Thompson, 1992).

Based on these considerations, the following mechanism of HSV-1 reactivation in the mouse eye model of latent infection is proposed. Suppression of primary infection of a viral strain, which under normal conditions is replication-competent, by administration of hyper-immune serum and pre-immunization, prevents destruction of cells and increases both the number of neuronal cells available for HSV-1 to establish latency as well as the copy number of viral genomes/cell. This may facilitate in vivo reactivation under conditions that favour reactivation.

Lack of viral replication and absence of clinical diseases in this model might be relevant to the human situation. Primary infection and seroconversion take place in most human individuals without clinical mucocutaneous disease (Whitley, 1990). In addition, primary infection is probably not associated with significant viral replication within the TG because it does not culminate in a sensory deficit within the respective dermatom, which would be the outcome of neuronal cell loss within the PSG (Gominak et al., 1990). This model might also explain the observation that there is an age-dependent decrease of reactivation rates of HSV-1 and HSV-2 in human individuals. If each reactivation is associated with depletion of the reservoir of HSV-1 genomes transported from the PSG to the periphery during reactivation, it may eventually reduce both the copy number per latently infected cell and the number of latently infected cells in the ganglia to a level below the threshold required for reactivation.

The present model may provide a useful tool for the study of the molecular phenomena associated with in vivo reactivation of HSV-1 and may help to understand how reactivation is initiated within the human peripheral nervous system from the viral genome without the prior presence of immediate early genes of Vmw65 expression. We are greatly indebted to Drs T. Brenner and D. Softer for critical reviewing of the manuscript and helpful suggestions. This work was supported in part by grant no. 88-00184 from the U.S.-Israel Binational Science Foundation, Jerusalem, Israel, grants by the Chief Scientist, Ministry of Health and the Israel Cancer Association, and by the Lena P. Harvey Fund for Neurological Research.

References


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