Reactivation of herpes simplex virus from latently infected mice after administration of cadmium is mouse-strain-dependent

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It was previously reported that administration of cadmium (Cd) to CBA mice latently infected with herpes simplex virus (HSV) results in a high incidence of virus reactivation in vivo. In the present study, Cd-inducible reactivation was used to compare CBA with four other laboratory mouse strains. HSV reactivation, as measured by the recovery of infectious particles from latently infected trigeminal ganglia following Cd treatment, occurred predominantly in the CBA strain and was almost entirely absent from other strains tested. There was no correlation of strain-dependent Cd toxicity with the recovery of infectious virus. In situ examination of Cd-treated ganglia from latently infected CBA and BALB/c mice revealed that viral antigens were expressed exclusively in CBA specimens, but that viral replicative transcripts were expressed in both strains, although more strongly in CBA than in BALB/c specimens. We conclude that Cd treatment had induced reactivation of HSV from both mouse strains, and that the reactivation process was completed in CBA but not in BALB/c mice.

Herpes simplex virus (HSV) is a ubiquitous human pathogen prototypic of a subfamily of herpesviruses which colonize and remain latent primarily in sensory ganglia of the peripheral nervous system (for reviews, see Fraser et al., 1990; Roizman & Sears, 1993). Ocular inoculation of animals leads to an acute phase of HSV infection, characterized by virus replication in corneal epithelium and surrounding tissues. During this time, infectious particles are adsorbed by sensory nerve endings and transported intra-axonally to neuronal nuclei located in the associated trigeminal ganglion (TG). Virus replication occurs in ganglionic neurons and infectious particles can be detected in homogenates of acutely infected ganglia from approximately 2 to 10 days post-inoculation. Within 2 weeks after inoculation, immune clearance operationally defines the end of the acute phase and the beginning of the latent infection phase, after which infectious virus is no longer detectable in ganglion homogenates. Subsequently, latent HSV infection can be demonstrated by explanting ganglia into culture medium. Within a few days of incubation in culture medium, explant-reactivated virus can be detected by plaque formation in cell monolayers.

In animal models during latent infection, viral proteins are not expressed and transcriptional activity is substantially restricted to a single region of the HSV genome. Infectious virus may reappear episodically in the latently infected host in response to local or systemic stimuli. Each reactivation event is transient and limited to a subset of latently infected neurons. Experimentally induced reactivation is conclusively demonstrated by recovery of infectious particles from ganglion homogenates. The mouse model of HSV reactivation differs from the rabbit model in that infectious particles can be recovered from mouse ganglia, but not from rabbit ganglia (Bloom et al., 1994). The mouse model offers the added advantages of being immunologically defined and more economically maintained over the long periods necessary to study HSV latency. However, it has a disadvantage in that reactivation is less readily inducible in mice than in rabbits, as measured by the detection of virus shedding from peripheral tissues. At present, four different methods of induction have claimed to produce high rates (> 50%) of reactivation from ganglia of mice latently infected with HSV-1. Ocular iontophoresis of epinephrine induced reactivation from BALB/c mice infected with HSV-1(McKrae) (Willey et al., 1984). Ultraviolet irradiation of corneal epithelium induced reactivation of McKrae strain from NIH mice (Laycock et al., 1991). Hyperthermic shock induced reactivation from Swiss Webster mice infected with HSV-1(KOS) (Sawtell & Thompson, 1992).
Lastly, cadmium (Cd) administration induced reactivation from CBA mice infected with HSV-1(F) (Fawl & Roizman, 1993). Notably, each of these methods has been applied to only one mouse strain. The purpose of this study was to compare the efficacy of Cd administration for reactivation of latent HSV from several laboratory mouse strains. Cd is a toxic heavy metal which strongly induces metallothioneins and heat-shock proteins. When administered to mice, Cd ions rapidly accumulate in the mouse peripheral nervous system and remain at high levels in these tissues for at least 1 week (Arvidson & Tjalve, 1986). Daily administration of Cd to latently infected CBA mice leads to reactivation of HSV from TG within 2 to 4 days of treatment (Fawl & Roizman, 1993). Surprisingly, CBA mice were found to be much more efficient than BALB/c in this regard.

Four- to six-week-old female mice (obtained from Jackson Laboratories, Bar Harbor, Me., USA) were anaesthetized with methoxyflurane for corneal scarification prior to inoculation with virus. We selected five inbred mouse strains for which relative sensitivity to Cd had been determined in earlier toximetric studies (Liu et al., 1992; Maitani & Suzuki, 1986; Nolan & Shaikh, 1986; Quaife et al., 1984; Shaikh et al., 1993). BALB/cBy (H-2a) and C57BL/6 (H-2b) strains are derived from unrelated dealer stocks; CBA and C3H/He strains (H-2k) are derived from a cross of BALB and DBA stocks; A strain (H-2~) is derived from a cross of BALB and Cold Spring Harbor albinos. All mice were inoculated with wild-type HSV-1(F) at 3 x 10⁸ p.f.u. per eye, except for strain A mice, which were inoculated at 5 x 10⁶ p.f.u. per eye in order to offset a higher susceptibility to HSV-1 mortality (Lopez, 1975; Kastrukoff et al., 1986). Beginning at 30 or more days after inoculation, latently infected mice were given a footpad injection of 50 μl Cd solution (2 mg CdSO₄·H₂O/ml sterile saline) daily for 3 days. Within 18 to 24 h after the third Cd injection, the mice were sacrificed and each TG was removed to a culture tube containing 1-2 ml Minimal Essential Medium supplemented with 5% newborn calf serum. For assay of latent virus, each TG explant was cultured for 5 days in 5% CO₂ at 37 °C, then homogenized and plated onto subconfluent CV-1 monolayers (one 25 cm² culture per ganglion). For assay of infectious virus, each TG upon removal was immediately frozen in a dry ice–ethanol bath and stored at −80 °C until thawed, homogenized and plated onto CV-1 monolayers. After 2 h, homogenates were replaced with fresh medium, and cultures were scored as positive if viral plaques appeared within 1 week of incubation. For examination by in situ hybridization (ISH) or immunohistochemistry, each TG upon removal was fixed overnight in 2% periodate–lysine–paraformaldehyde (PLP), then embedded in paraffin and sectioned at 5 μm.

Post-mortem weights were determined for Cd-treated mice, and the cumulative dose for each mouse was calculated in μmol Cd ions/kg body weight. Cd mortality was observed in two of the five mouse strains used for this study; there was 11% loss of BALB/c mice and 52% loss of A mice during Cd treatment. Cd mortality had previously been reported in BALB/c mice but not in A mice during treatment with CdCl₂ (Hata et al., 1980). The markedly high Cd mortality of A mice in this study was likely a result of the higher cumulative body burden of Cd in this study; 74 μmol Cd/kg body weight over 3 days, as compared to 30 μmol Cd/kg body weight over 1 day (Maitani & Suzuki, 1986; Shaikh et al., 1993). No mortality followed Cd treatment of C3H/He mice, although this strain had been reported to be most hepatosensitive to Cd (Hata et al., 1980; Maitani & Suzuki, 1986; Nolan & Shaikh, 1986; Quaife et al., 1986; Tsunoo et al., 1979). There was no correlation of Cd mortality with post-mortem body weight in this study. Following administration of Cd for 3 days, infectious virus was detected in homogenates of latently infected TG from the CBA strain in each of four separate experiments. However, the overall frequency of infectious virus recovery was considerably less in the current study than that which was previously obtained (Fawl & Roizman, 1993), and each reactivating TG yielded fewer than 10 plaques in this study, whereas homogenates of individual TG in the earlier study had yielded a range of 1 to 150 plaques. In both of these studies, TG were assayed for the presence of infectious virus within 18 to 24 h after the third injection of CdSO₄, and recovery of infectious virus was invariably unilateral. Because of the high rate of Cd mortality observed in both BALB/c and A mice, we assayed TG from BALB/c, A and CBA strains for infectious virus after 2 days of CdSO₄ treatment. Infectious virus was not recovered from any of six BALB/c mice, but was detected in 2 of 24 TG from A mice. The incidence of infectious virus in CBA mice after a second Cd injection (detected in 4 of 11 TG) was higher than that which occurred after a third Cd injection (detected in 7 of 53 TG).

In our hands, the recovery of latent virus from TG explants has been nearly 100% for both CBA and BALB/c mice. To determine the relative efficiency with which latent HSV infection was established in the other mouse strains used in this study, TG from ten mice of each strain were taken at the indicated times post-infection and assayed for the presence of explant-reactivated virus. Nearly all of the TG explants tested positive for latent virus, irrespective of mouse strain, with the single exception of a TG from one of the CBA mice (Table 1). Therefore, all of the mouse strains in this study sustained an equally high rate of latent infection, and an absence of infectious particles in some strains of Cd-treated mice did not reflect failure to establish latency. Nevertheless, variance in the number of latently infected cells in the TG may account for differences in the incidence of reactivation in vivo (Sawtell & Thompson, 1992). To address this question, and to examine the possibility of partial reactivation producing infectious particles in amounts below our level of detection, we used ISH to compare TG from CBA and BALB/c mice following Cd treatment. Ganglion sections were probed with sequences coding either for the immediate-early viral transactivator (ICP4), or for the major viral latency-associated transcript.
Table 1. Administration of Cd during latent HSV-1 infection is associated with strain-dependent morbidity and induction of virus reactivation in vivo

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>H-2 locus</th>
<th>Acute infection</th>
<th>Cd toxicity</th>
<th>No. of virus-positive TG/no. of TG total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Latent virus†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2Cd</td>
</tr>
<tr>
<td>CBA</td>
<td>k</td>
<td>6/48</td>
<td>0/42</td>
<td>9/10</td>
</tr>
<tr>
<td>C3H/He</td>
<td>k</td>
<td>18/50</td>
<td>0/22</td>
<td>20/20</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>b</td>
<td>0/40</td>
<td>0/30</td>
<td>20/20</td>
</tr>
<tr>
<td>BALB/cBy</td>
<td>d</td>
<td>1/46</td>
<td>5/45</td>
<td>20/20</td>
</tr>
<tr>
<td>A</td>
<td>a</td>
<td>4/35</td>
<td>11/21</td>
<td>20/20</td>
</tr>
</tbody>
</table>

* For each mouse strain, 10 mice which survived acute infection were sacrificed without Cd treatment in order to assay for latent virus in ganglion explants.
† Recovery of ex vivo reactivated virus from untreated ganglion explants.
‡ Recovery of in vivo reactivated virus from ganglion homogenates following administration of Cd for 2 days (2Cd) or 3 days (3Cd).

Table 2. Summary of ISH analyses of TG from BALB/c and CBA mice after 3 days of Cd treatment, using probes for latency-associated transcript (LAT) or for virus replication transcript (ICP4)

The LAT dsDNA probe was a 35S-labelled BseII fragment containing sequences which make up the 2 kb LAT. The ICP4 dsDNA probe was a 35S-labelled BamHI N fragment containing the coding sequences for the main viral transactivator.

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Cd treatment</th>
<th>No. of LAT-positive cells/no. of sections*</th>
<th>No. of ICP4-positive cells/no. of sections*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c, uninfected</td>
<td>No</td>
<td>0/12</td>
<td>1/27</td>
</tr>
<tr>
<td>BALB/c, acute-infected</td>
<td>No</td>
<td>93/6</td>
<td>217/22</td>
</tr>
<tr>
<td>BALB/c, latent-infected</td>
<td>Yes</td>
<td>3705/176</td>
<td>24/137</td>
</tr>
<tr>
<td>CBA, latent-infected</td>
<td>Yes</td>
<td>2693/137</td>
<td>34/121</td>
</tr>
<tr>
<td>CBA, latent-infected</td>
<td>No</td>
<td>1350/128</td>
<td>5/112</td>
</tr>
</tbody>
</table>

* No. of positive cells per section is shown in parentheses. A rough average of 200 neurons per section was obtained from counts of 100 representative sections.

(LAT) as previously described (Dearly et al., 1987). The number of LAT-expressing neurons was nearly equal in TG from Cd-treated CBA and BALB/c mice (Table 2), suggesting that both strains of mice contained roughly the same number of latently infected cells. However, LAT-positive signals were detected twice as frequently in both strains of Cd-treated mice relative to CBA saline controls. ICP4 transcripts were expressed in a small number of cells in latently infected TG following Cd treatment, and were rarely detected in latently infected TG from saline-treated controls. These ICP4-positive signals were generally stronger in CBA than in BALB/c TG (Fig. 1F, H). Whereas LAT expression was confined to neuronal nuclei, ICP4 expression occurred predominantly in non-neuronal cells (or possibly neuronal fibres) in Cd-treated CBA and BALB/c mice. These same PLP-fixed specimens were also examined immunohistochemically with HSV-specific polyvalent antiserum as previously described (Gesser et al., 1994). Only Cd-treated CBA mice subsequently exhibited HSV-1 immunostaining in their TG (data not shown), consisting primarily of disintegrating small diameter neurons with surrounding antigen-positive satellite cells, or less commonly, isolated immuno-stained satellite cells. Overall, the percentage of CBA TG containing foci of HSV-1 protein expression (19%) was in agreement with the percentage of CBA TG positive by virus.
Fig. 1. For legend see facing page.
culture (22%), and confirmed that recovery of infectious virus did not under-represent the incidence of reactivation.

Our results show that in mice latently infected with HSV-1, Cd treatment induced release of infectious particles frequently in strain CBA, infrequently in strain A and had no detectable effect in four other strains. This difference in response did not correlate with relative susceptibility to HSV-1 infection observed in this study, which was high for strain A, moderate for CBA and C3H/He strains and low for BALB/c and C57BL/6 strains. Cd-induced reactivation did not coincide with Cd mortality in this study, and also did not match any previously reported strain-dependent responses to Cd, such as tissue deposition (Maitani & Suzuki, 1986; Shaikh et al., 1993), relative nephro- or hepato-sensitivity (Maitani & Suzuki, 1986), immunotoxicity (Quaife et al., 1993) or production of metallo-thionines (Nolan & Shaikh, 1986; Shaikh et al., 1993). Nor was there any apparent correlation with the major H-2 loci of these five mouse strains, since CBA and C3H/He mice (the latter did not reactivate virus) share the same H-2 haplotype, which differs from that of strain A mice at several H-2 loci. It is possible that undefined differences within the minor H-2 loci of these mouse strains influenced our ability to detect Cd-induced reactivation. However, in vivo reactivation of HSV from latently infected BALB/c mice has previously been reported in response to induction by epithelial trauma (Openshaw et al., 1979a), neurectomy (Price & Schmitz, 1978) or immunosuppression (Openshaw et al., 1979b). This indicates that virus reactivation under these conditions can be detected as readily in BALB/c as in the other mouse strains tested. Relative to these other strains, latently infected BALB/c ganglia do not generally appear to be either less inducible or more rapidly cleared. Consequently, strain-dependent immune responses are unlikely to account for the different reactivation rates observed in this study.

We conclude that Cd treatment of CBA mice induces HSV reactivation in a small number of latently infected cells, as shown by expression of ICP4 mRNA and viral antigens in situ. In the absence of viral antigen expression in BALB/c specimens, our detection of weak ICP4 hybridization signals in BALB/c TG sections may at most indicate an incipient or abortive reactivation in Cd-treated BALB/c mice. Considering the various reports of strain-dependent differences in Cd toxicity, it is likely that CBA and A differ from BALB/c and other mouse strains in some unidentified aspect of Cd metabolism that leads to virus reactivation. Whether induced by Cd or by other means, in vivo reactivation at the primary site of HSV latency appears to vary considerably between mouse strains.

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References


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