Reactivation of Herpes Simplex Virus Infection by Ultraviolet Light and Possible Involvement of Prostaglandins

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SUMMARY

Herpes simplex infection in the mouse ear was used to investigate whether various treatments would reactivate the disease. Immunosuppressive drugs failed to induce clinical signs of reactivation but irradiation of the skin of the originally infected ear with ultraviolet light or injection of prostaglandin E\textsubscript{2} or PBSA into this site, caused reactivation of infection. This was detected by the appearance of infectious virus in the skin 2 to 3 days after these treatments. The results are discussed in relation to the mechanism of herpes reactivation in man.

Observations in humans and experimental studies in animals have proved that once infected, a host can harbour herpes simplex virus (HSV) in the nervous system throughout life (Stevens, 1975). During latency the virus can be recovered from the dorsal root ganglia of nerves associated with the site of recurrent lesions by culturing the whole ganglion in vitro.

In a previous experimental study (Hill, Field & Blyth, 1975) HSV was isolated from the cervical ganglia of 71% of mice that had recovered from disease following intradermal inoculation in the ear. We now report use of this experimental infection in efforts to stimulate recurrent disease as part of a study of the mechanisms of latency and recrudescence of herpes simplex virus.

Four week old female Swiss white mice from a closed colony were infected subcutaneously in the right ear with $6 \times 10^4$ p.f.u. of HSV type 1 strain SC16 (Hill et al. 1975). For experiments on reactivation only mice that had shown unequivocal signs of disease in the primary infection were used. All mice were clinically normal when reactivation was attempted; the interval between infection and stimulation varied from 4 to 14 weeks.

All attempts to isolate HSV were made on the right ear. The pinna was cut off and ground with 0.4 ml of maintenance medium (Hill et al. 1975) in a glass grinder. The whole suspension was used to infect cultures of Vero cells which were examined for c.p.e. daily for one week.

Clinically apparent reactivation of disease was not seen after administration of the following immunosuppressive drugs given intraperitoneally: prednisone 6 mg/dose, 2 doses/day for 5 days; cyclophosphamide 0.3 mg/dose, 1 dose/day for 3 days — these two treatments mixed together (the first combined dose was given at the same time); cortisone acetate 0.2 mg/dose, 1 dose/day for 10 days. When the effect of cortisone acetate was tested, attempts were also made to isolate virus from the ears of 10 mice on each of the 4th, 6th and 10th days of treatment; all results were negative. Stevens & Cook (1973) and Hurd & Robinson (1976) also failed to reactivate the disease by immunosuppression.

For irradiation with ultraviolet (u.v.) light mice were anaesthetized by intraperitoneal sodium pentobarbital and placed 22 cm vertically below a Hanovia model 1 u.v. lamp, usually for 50 s. This was just sufficient to cause erythema in most mice. Only the right (originally infected) ear was irradiated except in one experiment when either the left or the right ear was irradiated. It was not possible to detect reactivation of disease by induction of erythema since the erythema from irradiation alone lasted for up to 8 days. Therefore, at
Table 1. Reactivation of HSV infection in the skin after irradiation with u.v. light*

Virus isolated/number of mice tested.

<table>
<thead>
<tr>
<th>Days after irradiation</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/15</td>
<td>3/11</td>
<td></td>
<td></td>
<td>0/10</td>
</tr>
<tr>
<td>0/10</td>
<td>3/10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/10</td>
<td>2/8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/10 (0/4)†</td>
<td>3/10 (0/5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0/30)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/15 (0/15)</td>
<td></td>
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</tr>
</tbody>
</table>

* The right (originally infected) ear was irradiated for 50 s.
† Results in brackets are for mice anaesthetized but not irradiated.
‡ Virus was isolated from 2 of 15 mice irradiated for 100 s.

intervals after irradiation groups of 10 to 15 animals were killed and attempts were made to isolate virus from the irradiated ear (Table 1). On the second and third days after irradiation but not at other times, virus was isolated from the tissue of a proportion of the mice. This proportion was highest 3 days after irradiation when the mean isolation rate over 7 experiments involving 94 mice was 21%; the result was not affected by differences in the interval between infection and stimulation. In these and other experiments virus was isolated from the ear tissue of 2 of 60 mice tested 2 to 3 days after anaesthetization (but without irradiation) and from 1 of 45 untreated mice (Hill et al. 1975). In one experiment virus was not isolated from the originally infected ear of 4 mice after exposure of the other ear to u.v. light 3 days previously whereas virus was isolated from 3 of 14 mice in which the infected ear was irradiated. When the dose of u.v. irradiation was doubled (100 s) the number of mice from which virus was isolated was not increased.

Some of the effects of u.v. light on skin, particularly the late erythema (Solomon, Juhlin & Kirschenbaum, 1968), and increased number of S phase cells (Eaglstein & Weinstein, 1975) are probably mediated by prostaglandin E2 (PGE2). Therefore we tested whether injection of PGE2 subcutaneously into the originally infected ear caused reactivation of infection. PGE2 was a gift from Dr J. Pike, The Upjohn Co. Kalamazoo, Michigan, U.S.A. It was used at a concentration of 100 μg/ml in PBSA (Dulbecco & Vogt, 1954). Within 10 min of injection red/purple erythema developed throughout the ear and lasted for 3 to 5 h. Control mice injected with PBSA alone also frequently showed erythema but it was less severe and disappeared more quickly. On the 2nd, 3rd and 4th days after injection groups of 10 to 15 mice were killed and attempts were made to isolate HSV from the ear tissue (Table 2).

The common observation that in humans exposure to sunlight induces recurrent herpes labialis prompted our study of the effects of u.v. light on latency. The anecdotal evidence was recently supported by a study which showed that u.v. light caused recurrence of lesions in man (Wheeler, 1975) and our results show that this stimulus also causes the reappearance of virus in the original site of infection in the mouse.

Our experiments show that HSV can be isolated from only a proportion of animals after u.v. light irradiation. This proportion – about a quarter – is remarkably similar to that obtained in other studies where other reactivating stimuli were used. For instance, recurrent lesions were reported in 17% of mice treated with prednisone (Underwood & Weed, 1974) and by Hurd & Robinson (1976) in 30% of mice after plucking the hair from the original site of infection. The latter test was a control to others where cyclophosphamide and cortisone were also administered; use of the drugs increased the incidence of lesions to 60%.
Table 2. Reactivation of HSV infection in the skin after subcutaneous injection of prostaglandin E\textsubscript{2} (PGE\textsubscript{2})

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Virus isolated/total mice tested.</th>
<th>Days after injection of PGE\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>One injection { 2 \mu g PGE\textsubscript{2} in 0-02 ml PBSA</td>
<td>1/10</td>
<td>2/10</td>
</tr>
<tr>
<td>PBSA alone</td>
<td>1/10</td>
<td>0/10</td>
</tr>
<tr>
<td>PGE\textsubscript{2} as above</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>PBSA alone</td>
<td>1/10</td>
<td>0/10</td>
</tr>
<tr>
<td>3 injections at { 5 h intervals each of 2 \mu g PGE\textsubscript{2} in</td>
<td>0/9</td>
<td>2/10</td>
</tr>
<tr>
<td>0-02 ml PBSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 injections PBSA alone</td>
<td>0/9</td>
<td>0/9</td>
</tr>
</tbody>
</table>

The theory that reactivation follows a stimulus to the ganglion (Cook & Stevens, 1973; Merigan, 1974) does not easily provide an explanation for these results. Moreover, herpetic lesions can appear as little as 24 h after fever therapy in humans (Keddie, Rees & Epstein, 1940), and 36 h after u.v. irradiation of the eyes of rabbits (Spurney & Rosenthal, 1972). These intervals seem insufficient for skin lesions to form after stimulation in the ganglion. An alternative theory was therefore proposed (Hill & Blyth, 1976) that the ganglion frequently supplies the skin with virus thereby producing subclinical microfoci of infection which are usually eliminated by defence mechanisms. The development of lesions from these microfoci could then occur if alterations in the skin favoured virus multiplication. These alterations would exist for only a limited time after any given stimulus so that only in that proportion of mice which coincidentally carried virus in the skin during this period could reactivation of disease occur. The consistent finding that similar proportions of mice (20 to 30 \%) show recurrent disease after different stimuli would thus be explained.

The role of prostaglandins in reactivation of HSV infection is not yet clear (Hill & Blyth, 1976) but virus can be isolated after injection of PGE\textsubscript{2} into the skin. It is very probable that injection even of PBSA stimulates the release of many mediators of inflammation including prostaglandins so that, as seen in these results, the trauma from injection might be a sufficient stimulus to cause reactivation of infection. Work is now in progress to show whether prostaglandins are involved in HSV reactivation.

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REFERENCES


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