A Murine Model of Herpes Simplex Virus Recrudescence

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SUMMARY

A murine model is described in which recrudescence of herpes simplex virus (HSV) type 1 was achieved. C3H mice were shaved and irradiated with u.v. B light 3 days before being infected epidermally with a clinical isolate of HSV. Seven weeks or longer following the primary infection, the survivors were again shaved, irradiated with u.v. and mildly tape-stripped. Recrudescent lesions occurred in up to 80% of mice at the site of the original lesion in most cases, but also occasionally at other sites. Skin painting with u.v.-irradiated urocanic acid (a substance suggested to be a photomediator of the immunosuppressive effects of u.v.) in place of u.v.-irradiation induced some recrudescence but was not as efficient as u.v.-irradiation. Antibody titres to HSV had no value in predicting whether recrudescence would occur but lymphoproliferative responses in draining lymph nodes may provide some indication of viral activity at the epidermal site. A hypothesis is developed that u.v.-irradiation before primary infection with HSV induces a suppressive immune response to the virus which affects the virus–host interaction and accounts for a high incidence of recrudescent lesions on subsequent stimulus.

Once infected with herpes simplex virus (HSV), a host harbours the virus in a latent form in the dorsal root ganglia of nerves associated with the site of the original lesion (Stevens & Cook, 1971). At intervals there may be reactivation of virus in the nervous tissue; the virus then moves to a peripheral site, either initiating a lesion in the neurodermatome relating to the particular ganglion (recrudescence) or not causing an observable clinical lesion (recurrence) (for review, see Wildy et al., 1982). Certain common triggering factors for recrudescent lesions are recognized in man including exposure to u.v. light (Wheeler, 1975), fever, skin trauma and immunosuppression.

Attempts have been made to develop animal models to examine the circumstances surrounding the control of latency, recurrence and recrudescence. Using mice, one of the most detailed studies has been by Hill and his co-workers (Hill et al., 1975, 1978; Blyth et al., 1976, 1984). The strain of mouse was found to be critical to the success of inducing recurrence and recrudescence, with outbred strains showing repeated recurrence on stimulation (Harbour et al., 1981).

In order to study various immunological parameters of recrudescent HSV infection in detail, it was of obvious interest to develop a model in a genetically defined (at the major histocompatibility complex locus) inbred strain of mouse such as the C3H strain we had used previously for syngeneic cell transfer experiments which are not possible using outbred mice. If these mice were u.v.-irradiated before infection with a clinical isolate of HSV, then an alteration in the antigen presentation of the virus to the immune system was found to induce the generation of a different type of immune response from normal (Howie et al., 1986a, b, d, 1987). This led to the hypothesis that the way in which HSV is first presented to the immune system may determine not only the severity of the primary lesion, but perhaps also the extent of neuronal
involvement during latency and the frequency of recurrence and recrudescence. In studies on HSV infections in mice we have demonstrated specific T lymphocyte-mediated immunosuppression of delayed type hypersensitivity (DTH) to HSV following irradiation with u.v. at a sub-erythemaal dose (Howie et al., 1986a) or following skin painting with u.v.-irradiated urocanic acid (u.v.-UCA) (Ross et al., 1986). Urocanic acid (UCA) has been suggested as a possible photomediator of the immunosuppressive effects of u.v. (De Fabo & Noonan, 1983). It is located preferentially in the stratum corneum and, on u.v.-irradiation, is isomerized from the trans to the cis form. Our model was examined to determine the effect of these two treatments on attempts to recrudesce herpes simplex lesions following epidermal primary infection with HSV.

Female C3HfBu/Kam mice, bred and maintained in the departmental animal house, were infected at 6 to 8 weeks of age with a clinical isolate of HSV type 1 (HSV-1) (Howie et al., 1986a). Mice were shaved dorsally, anaesthetized using ether and tape-stripped (four times using Sellotape) over the right mid-dorsal surface before being infected with 10^4 p.f.u. HSV rubbed gently over the same area, or mock-infected. Mice were observed daily for 3 weeks using an illuminated magnifying lens. Visible lesions formed after 2 to 3 days. When the infecting dose of virus was greater than 10^3 p.f.u. zosteriform spread, paralysis and death of 90% mice occurred after 7 to 10 days. When infected with 10^4 p.f.u., 60% mice developed lesions with half becoming zosteriform. An infecting dose of 2 × 10^4 p.f.u. per mouse was used in all the following experiments.

Two preliminary studies ascertained that u.v.-irradiation before the primary HSV infection affected the way in which the mouse responded to the virus such that, on subsequent u.v.-irradiation, recrudescence of lesions would occur in a proportion of mice.

In the first experiment, 42 mice were irradiated with a sub-erythemaal dose of u.v. (96 mJ/cm^2; Howie et al., 1986a) and infected epidermally with HSV 3 days later. Three mice died and after 2 months the survivors were shaved, u.v.-irradiated and tape-stripped. Thirty-two per cent developed clinical lesions beginning 24 h after tape-stripping. Eighteen mice were retained for a further 3 months and exposed again to u.v. with tape-stripping; 14 (80%) developed lesions which began 1 day after tape-stripping; five mice had lesions on the original site, four on a high mid-dorsal site and five on both sites. Using the method of Mackenzie & Squier (1975) epidermal skin sheets were prepared from visibly affected areas of four mice 2 days after tape-stripping. They were positive for HSV antigens by indirect immunofluorescence (Howie et al., 1986c).

In the second experiment 40 mice were irradiated with u.v. and infected epidermally with HSV. Twenty-five mice developed lesions of which four died. After 10 weeks, the survivors were shaved, u.v.-irradiated and tape-stripped. Twenty-one developed lesions (59%), 14 on the original site, five on a mid-dorsal site of which two were multiple and two on both sites. HSV was isolated from two mice, 1 and 3 days after tape-stripping by mincing pieces of skin from sites of lesions and co-cultivating with Vero cell monolayers. Any culture showing development of a cytopathic effect was checked for HSV antigens using a monoclonal antibody to HSV-1 labelled directly with fluorescein isothiocyanate (Microtrak, Syva Co.). Skin sheets prepared from another mouse 2 days after tape-stripping were positive for HSV antigens by indirect immunofluorescence.

Using another group of 102 mice, more information was obtained on the effects of u.v.-irradiation before HSV infection and the triggering factors of u.v. and tape-stripping on recrudescence. In addition to noting lesion development, antibody titres to HSV were measured at intervals, and the lymphoproliferative activity of draining lymph node cells to HSV antigens was assayed. To prepare the antigens for ELISA, Vero cells were infected with HSV at a multiplicity of infection of 5 or mock-infected. They were harvested 20 h later, washed and resuspended in a small volume of 0.1 M-glycine and 0.1 M-NaOH (5:3 by vol.). After freezing and thawing three times and ultra-sonicating for 1 min, suspensions were centrifuged at 1000 g for 10 min and the clarified supernatants used as antigens. Protein content was estimated by Lowry's method. The ELISA was based on that described by Booth et al. (1979) with HSV or control antigen being used to coat immunoplates (Nunc) with 15 μg/ml protein at 4 °C overnight. Sera were pooled from at least three mice (five normally). The titre was estimated as
Table 1. The outcome of epidermal HSV infection of mice 3 days after u.v.-irradiation

<table>
<thead>
<tr>
<th>Number of mice</th>
<th>U.v.-irradiation</th>
<th>HSV (2 × 10^4 p.f.u.)</th>
<th>Number with lesions</th>
<th>Number of survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Yes</td>
<td>No</td>
<td>0</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>33</td>
<td>No</td>
<td>Yes</td>
<td>25</td>
<td>28 (85%)</td>
</tr>
<tr>
<td>64</td>
<td>Yes</td>
<td>Yes</td>
<td>49</td>
<td>44 (69%)</td>
</tr>
</tbody>
</table>

Table 2. Response of groups of mice to irradiation with u.v. before infection with HSV on day 0 and to triggering factors for HSV recrudescence

<table>
<thead>
<tr>
<th>Day 59 lympho-proliferation</th>
<th>Day 60 Antibody titre</th>
<th>Day 63 Lympho-proliferation</th>
<th>Day 65 IF* (one mouse)</th>
<th>Day 70 Antibody titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day -3 u.v.</td>
<td>Yes</td>
<td>&lt;1</td>
<td>Lesions†</td>
<td>1:3200</td>
</tr>
<tr>
<td>S.I.*</td>
<td>3/21</td>
<td>4:60</td>
<td>Yes</td>
<td>4:21</td>
</tr>
<tr>
<td></td>
<td>4/20</td>
<td>1:15</td>
<td>No</td>
<td>2:43</td>
</tr>
<tr>
<td>No</td>
<td>1/11</td>
<td>1:600</td>
<td>Yes</td>
<td>1:20</td>
</tr>
<tr>
<td></td>
<td>0/12</td>
<td>1:800</td>
<td>No</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* S.I., stimulation index.
† Number with lesions/number tested.
‡ IF, HSV-positive immunofluorescence.

that dilution of serum which gave an absorbance > the mean ± 3 S.D. of four wells of a negative serum at 1:100 dilution. For measurement of lymphocyte proliferation, lymph nodes draining the sites of clinical lesions were collected aseptically, usually from three mice, homogenized, and the cell suspension was washed three times in 0.01 M-phosphate-buffered saline pH 7.2, before being resuspended at 10^6 cells/ml in RPMI 1640 (Northumbria Biologicals) supplemented with 5% horse serum, 2 mM-L-glutamine, 5 × 10^{-3} M-2-mercaptoethanol, 100 units/ml penicillin, 200 µg/ml streptomycin, 100 µg/ml gentamicin and 20 µg/ml fungizone. Cells were plated out in quintuplicate wells in 96-well flat-bottomed tissue culture plates (Falcon) at 2 × 10^5 cells per well with either 2 µg HSV antigen (prepared as for ELISA except the cells were extracted 6 h after infection) or control antigen per well and incubated at 37 °C in 5% CO₂ and 95% humidified air for 3 or 5 days. Eighteen h before harvesting, 0.75 µCi [Me-3H]thymidine (Amersham) was added to each well. Cells were harvested onto glass fibre discs using an automatic harvester. When dry the discs were counted in a toluene-based liquid scintillator for 1 min. Lymphocyte proliferation was expressed as the stimulation index (S.I.) where: S.I. = (mean c.p.m. with HSV antigen)/(mean c.p.m. with control antigen). Standard errors of the means were all <10%. An S.I. value of 2.0 or greater was considered positive.

Initially all mice were shaved, some were u.v.-irradiated and all were infected with HSV 3 days later except for five control mice. The outcome of the primary infection is indicated in Table 1. Antibody titres were 1:1600 28 days post-infection in both infected groups whether pre-irradiated or not. Thus no suppression of antibody synthesis after u.v.-irradiation was found. The antibody titre of the uninfected mice was <1:100 and remained at this level throughout. Just before recrudescence procedures, no infectious virus was isolated from the epidermis of three mice in either infected group. Sixty days after primary infection, all mice were re-shaved and 34 were irradiated of which six developed recurrences 3 days later (Table 2). At this time recrudescence was also induced in four of 20 pre-irradiated mice and none of 12 non-irradiated mice. Perhaps this suggests a tendency to recrudescence after u.v. which may be exacerbated by
Table 3. Response of groups of mice to UCA and u.v.-UCA before infecting with HSV and to triggering factors for HSV recrudescence

<table>
<thead>
<tr>
<th>Day 0</th>
<th>Number with primary lesions*</th>
<th>Number of survivors</th>
<th>Day 44 antibody titre</th>
<th>Day 49 triggering factors</th>
<th>Day 51 lesions</th>
<th>Day 59 antibody titre</th>
<th>Day 72 antibody titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCA + HSV</td>
<td>9/15</td>
<td>11/15 (73%)</td>
<td>1:400</td>
<td>Tape strip + UCA</td>
<td>0/5</td>
<td>1:200</td>
<td>1:100</td>
</tr>
<tr>
<td></td>
<td>u.v.-UCA + HSV</td>
<td>22/25</td>
<td>11/25 (44%)</td>
<td>1:800</td>
<td>Tape strip + UCA</td>
<td>0/5</td>
<td>1:800</td>
</tr>
<tr>
<td></td>
<td>HSV alone</td>
<td>33/40</td>
<td>23/40 (57%)</td>
<td>1:800</td>
<td>Ethanol</td>
<td>1/5</td>
<td>1:1600</td>
</tr>
</tbody>
</table>

* Number with lesions/number tested.

shaving the mice. Spontaneous recrudescent lesions were never seen in any group. Antibody titres rose in all groups except in the non-irradiated mice. By 5 days (day 65), recrudescence of clinical lesions had occurred in 14 of 18 mice which had been pre-irradiated with u.v. before infection and re-irradiated. The number was increased from six of nine to eight of nine by tape-stripping. In five mice, the lesion occurred on the site of the primary lesion, in eight on this site plus a high mid-dorsal site with three being multiple, and in one case there was a lesion on the other flank in addition to the original flank. Recrudescence also occurred in seven of 16 mice pre-irradiated with u.v. but not re-exposed and this number was increased from two of eight to five of eight by tape-stripping. In all cases the lesions occurred at the site of the primary lesion. Two of ten mice not irradiated before HSV infection showed recrudescent lesions when irradiated subsequently, but none of nine where u.v. was not used. All lesions were undetectable by day 71. Recrudescence procedures increased the antibody titre fourfold in some instances but the titre did not correlate with the number of recrudescent lesions in each group. Three groups which had been tape-stripped showed a low HSV titre. This result was unexpected and was confirmed by a virus neutralization test. No lymphoproliferative activity of draining lymph node cells to HSV antigens was found by day 59 post-infection. The S.I. became positive on day 63 in the group pre-irradiated with u.v. and re-irradiated. By day 66 both groups irradiated on day 60 had positive S.I.s, whereas both groups not irradiated at that time remained negative. The uninfected control mice were negative throughout.

We have already shown that u.v.-UCA, containing 24% cis isomer, when applied to the shaved skin has an immunosuppressive effect on the DTH response to HSV infection (Ross et al., 1986). In the following experiment, 200 μg u.v.-UCA or UCA (dissolved in DMSO and diluted in ethanol) was applied 5 h before epidermal infection with HSV. Seven weeks later the same solutions were used after mild tape-stripping as triggering factors for recrudescence. The results are given in Table 3. In a few cases recrudescence occurred. In the group of six pretreated with u.v.-UCA and triggered with u.v.-UCA, two (33%) developed recrudescent lesions and similarly for the group not pretreated but triggered with u.v.-UCA. Lesions were always very small. No recrudescent lesions occurred in the UCA-treated groups. Antibody titre differences between groups were usually no more than four-fold.

A final study used various pretreatments before HSV infection followed by various triggering factors applied approximately 10 weeks later. Only those mice which developed primary lesions were kept for attempted recrudescence. The results are shown in Table 4. Where u.v.-UCA or...
Table 4. Response of groups of mice to u.v.-irradiation or u.v.-UCA before infection with HSV and to triggering factors for HSV recrudescence

<table>
<thead>
<tr>
<th>Treatment before HSV infection</th>
<th>Tape-strip + u.v.-UCA</th>
<th>U.v. + tape-strip</th>
<th>Tape-strip</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0/8*</td>
<td>0/8</td>
<td>0/3</td>
</tr>
<tr>
<td>U.v.-UCA</td>
<td>0/8</td>
<td>3/8</td>
<td>0/5</td>
</tr>
<tr>
<td>U.v. B</td>
<td>1/7</td>
<td>4/7</td>
<td>0/4</td>
</tr>
</tbody>
</table>

* Number with lesions/number tested.

u.v.-irradiation was used as pretreatment before infecting with HSV, and u.v.-irradiation was given as a triggering factor; recrudescent lesions developed in approximately half the mice.

This paper describes a reproducible murine model of recrudescence which involves pre-exposure of animals to an external immunosuppressive signal before infection with HSV. It provides evidence that pretreatment of mice resulting in antigen-presenting cell alteration before infection with the virus (Howie et al., 1987) leads to the generation of a suppressive immune response and to the establishment of latency with an increased chance of recrudescence after further stimuli. The dose of u.v. used was a small one, not sufficient to cause erythema and was the same as that which gave maximum immunosuppression of DTH to the virus (Howie et al., 1986a). Similarly u.v.-UCA was used at the concentration that gave maximum immunosuppression (Ross et al., 1986). Mice were infected epidermally after mild tape-stripping as it was thought that this method could mimic best a natural human infection. For the same reason a clinical isolate of HSV-1 was preferred to a laboratory strain of virus. The use of an inbred strain of mice allows us to investigate in detail the interactions between the various components of the immune system during recrudescent infection. The results of these studies will be reported elsewhere.

In a hypothesis outlined by Isawaka et al. (1983) from results obtained in a guinea-pig model of HSV-2 infection, development of recrudescent lesions was thought to be controlled at more than one level. One control would be at the level of the persistently infected cells where increased viral replication (due to an unknown mechanism) would be checked by stimulation of the immune response, already primed by the primary infection. If this control was not immediate or was inefficient, then virus replication would occur and recrudescent lesions would tend to develop. Suppressor cell factors could induce such a delay in an immunological response, and evidence for such factors released from the spleen and peripheral blood lymphocytes during recrudescence was found. These factors were not present during latency. In a different model, Altmann & Blyth (1985) showed that mice, which had been infected under conditions favouring activation of T suppressor cells (T_S) by injecting HSV intravenously, demonstrated a more limited immunopathology of the central nervous system. However, they also noted an increased frequency of recrudescence in such mice after tape-stripping.

Irradiation with u.v. is known to have many effects on the immune system; in essence, there is induction of immunosuppression, both local, at the site of irradiation, and systemic (for review, see Kripke & Morison, 1985). Antigen-presenting cell function at the local site and systemically is defective, some antigen-presenting cells may be destroyed, interleukin-1-like factor production is reduced and prostaglandins PGE_2 and PGF_2 are released from keratinocytes. There is generation of T_S, probably both locally and systemically, and recent evidence for circulating suppressive factors in serum 3 to 5 days after irradiation (Harriot-Smith & Halliday, 1986). Skin-painting of mice with u.v.-UCA mediates immunosuppression with generation of two T_S subsets, just as happens with u.v.-irradiation (Ross et al., 1986, 1987). It is not known how u.v.-UCA induces immunosuppression but it is of interest to note that it is structurally similar to histamine and may block the effect of this inflammation mediator.

From the data in Table 2, it is clear that the greatest incidence of recrudescence was found in the group irradiated with u.v. before infection and irradiated again as a triggering stimulus; tape-stripping increased this number. In some cases recrudescent lesions occurred at sites other than the original one, indicating a spread of the virus in the central nervous system presumably...
from the lumbar to the thoracic region during the primary infection. Pretreatment with u.v.-UCA followed by u.v.-UCA as a triggering stimulus was not as efficient as u.v. in inducing recrudescence (Table 3). It is possible that its immunosuppressive effects are not so great as that of u.v.-irradiation or that u.v.-irradiation may have additional effects on components other than the immune system, such as the central nervous system or on the virus itself. The triggering factors for recrudescence, u.v.-irradiation and u.v.-UCA together with tape-stripping, would produce a combination of transient immunosuppression and inflammation. These may act as 'skin triggers' for the virus to be reactivated in the ganglion and/or start replicating in the skin. If the memory immune response is one of suppression, then the virus may replicate freely producing a recrudescent lesion before being contained by other T cell subsets.

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