Recurrent Cutaneous Herpes Simplex in Hairless Mice

GERALD E. UNDERWOOD AND SHELDON D. WEED

Experimental Biology Research, The Upjohn Company, Kalamazoo, Michigan 49001

Received for publication 17 April 1974

Passively immunized hairless mice were inoculated cutaneously with herpes simplex virus. Thirty-nine days later, when the primary cutaneous lesions had completely healed, the mice were treated subcutaneously with prednisone. Within 12 to 30 days after starting prednisone treatment, herpesvirus was recovered by skin swabs from 12 of 71 (17%) of the treated mice. This new model has potential application for understanding and treating recurrent cutaneous herpes infections.

Cutaneous infections with herpes simplex virus (HSV) on hairless mice have been described (3, 6, 9). This animal model has been used for evaluation of potential antiviral agents (6, 9, 10). Clinically, cutaneous herpes simplex infections often recur at the same or nearby sites. This has led to speculation that the virus may persist in a latent form between clinical episodes and, in fact, herpesvirus has been recovered from human trigeminal ganglia (1, 2). Latent herpes simplex also has been recovered from nervous tissue of experimentally infected mice (8) and rabbits (5). A laboratory animal model, in which recurrences could be induced at the site of the original cutaneous lesion, would be valuable both for testing antiviral agents and for understanding the mechanism of lesion recurrence. Accordingly, we attempted to induce recurrent skin lesions in hairless mice which had recovered from primary cutaneous herpes infections. This report describes a procedure which gave us infrequent, variable, but significant recurrent herpes simplex lesions in our model system.

MATERIALS AND METHODS

Virus. The HWC strain of HSV was isolated originally from a herpetic lip lesion and was passed three times in rabbit kidney cell cultures. It had a titer of 10⁷ plaque-forming units (PFU) per ml when assayed on primary rabbit kidney cells.

Mice. Four-week-old male hairless mice of the HRS/J strain were obtained from the Jackson Laboratories in Bar Harbor, Me.

Herpes immune serum. Two-kilogram rabbits were hyperimmunized by a series of three intramuscular inoculations, each containing 2 × 10⁷ PFU of virus, given at weekly intervals. Twice weekly bleedings were begun 10 days after the final virus inoculation and were continued for a total of 10 bleedings. Serum was collected and pooled. Twofold dilutions of the serum were mixed with an equal volume (0.5 ml) of virus, diluted to contain 500 PFU/ml. The serum-virus mixtures were held overnight at 4 C, and then 0.4 ml of each mixture was added to duplicate petri plates containing primary rabbit kidney cell cultures. After 2 h of incubation at 37 C, the cells were overlayed with agar, and plaques were counted 4 days later. Half of the virus was neutralized by a 1:256 dilution of serum; i.e., the immune serum had a titer of 256.

Method of infection. Each mouse was injected intraperitoneally with 0.5 ml of undiluted immune serum. Forty-eight hours later, the mice were inoculated with virus: the lumbar area was lightly scratched with a hypodermic needle and the undiluted virus suspension was gently applied to the scratched area with a cotton swab.

Scoring of lesions. Development of cutaneous herpesvirus lesions in nonimmunized mice has been described (3, 9). A similar but delayed course occurred in passively immunized mice. Lesions were scored on a 0 to 4 scale: 0, no lesion; 0.5, lesion <2 mm in length; 1, lesion 2 to 4 mm; 2, lesion 5 to 8 mm; 3, lesion 9 to 15 mm; 4, lesion >15 mm.

Steroid treatment. Thirty-nine days after primary infection with herpesvirus, mice were treated subcutaneously at the nape of the neck with prednisone or hydrocortisone at a dose of 200 mg/kg, twice daily, for five consecutive days. The steroids were prepared as suspensions in saline at a concentration of 16 mg/ml.

Virus isolations. Suspect recurrent lesions were gently swabbed with sterile cotton moistened with cell culture medium. The swab was broken off into a small serum bottle containing medium and stored at −20 C. Each sample was tested for HSV by inoculating 0.2 ml into each of two tubes of rabbit kidney cells. One blind passage, after the cells were frozen and thawed, was made 5 to 7 days postinoculation from tubes showing no cytopathology. Fluids were harvested from positive tubes after disrupting the cells by freezing, and a sample was mixed with an equal volume of undiluted HSV-immune serum. An equivalent sample was also mixed with normal rabbit serum. Both mixtures were held overnight at 4 C and then titrated by plaque assay on rabbit kidney cells. Titer of the
sample containing immune serum must be at least 2 logs lower than the sample containing normal serum in order to classify the isolated virus as herpes simplex.

RESULTS

Infection of passively immunized hairless mice gave skin lesions, followed by healing, on 38% of the mice (Table 1). Thirty-four percent of passively immunized mice succumbed to the primary infection, compared with our average experience of 95% death with nonimmunized controls.

After complete resolution of lesions, except for a slight residual scar in some cases, mice were caged and marked so that each mouse could be studied individually. The recovered mice then were treated with either hydrocortisone or prednisone in an attempt to induce recurrence of the cutaneous lesions. Skin swabs for virus isolation were collected whenever there was evidence of a lesion.

No virus was obtained from recovered mice that were treated with hydrocortisone (Table 1). An additional 19 mice from experiment 1, which were inoculated with virus but showed no primary lesion, were also treated with hydrocortisone, but again no lesions developed. Based on these observations, no additional studies were done with hydrocortisone. More encouraging results were obtained with prednisone; i.e., 12% of mice which had recovered from primary lesions yielded demonstrable herpesvirus after prednisone treatment (Table 1, experiment 1). Prednisone, at the level used, is tolerated during the treatment period but will produce sporadic deaths thereafter; about 50% of normal hairless mice treated in this way succumbed when held for 3 weeks.

Results obtained with prednisone in experiment 1 were confirmed in a second experiment in which virus was recovered from 22% of the mice with healed lesions (Table 1, experiment 2). In this experiment, 69 other mice which had received virus but developed no primary lesions were also treated with prednisone; no virus was recovered from these.

A third experiment was run to determine whether virus could be recovered in the absence of prednisone treatment. Of 168 mice inoculated with virus in this experiment, 38 developed no lesions, whereas 65 developed lesions and recovered. At 39 days postinfection, the same interval at which steroid treatment was initiated in experiments 1 and 2, each of these 103 mice was swabbed at the site of the original virus inoculation; no virus was recovered from any of these swabs.

Course of the infection is shown diagrammatically (Fig. 1) for each of the 12 mice treated with prednisone in which lesions recurred and virus was isolated. The mouse in experiment 1, which was still alive on day 80, showed no lesion after day 97. On day 286, another 5-day course of prednisone treatment was begun with this mouse. No lesions developed, but the mouse died on day 294. The surviving mouse from experiment 2 showed no residual lesion by day 106. It was rechallenged with herpes on day 119, with the virus applied cutaneously at the same site as the original lesion. No new lesion developed. On day 195, a second course of prednisone was started; again, no lesion appeared, but the mouse died on day 206.

DISCUSSION

We noted previously (9) that pretreatment

| Table 1. Primary and recurrent cutaneous herpes on passively immunized hairless mice |
|--------------------------------------|------------------|-------------------|-----------------|
| Expt no. | Age of mice (weeks)* | Inoculated with virus* | Developed lesion* | Died with lesion | Lesion healed | Treated with |
|          |                  |                   |                 |               |              | Hydrocortisone | Prednisone |
| 1        | 7                 | 45                | 42              | 13             | 29            | 10 (0)*       | 19 (1)     |
| 1        | 9                 | 50                | 35              | 7              | 28            | 18 (0)       | 10 (2)     |
| 1        | 11                | 40                | 37              | 17             | 20            | 9 (0)        | 11 (2)     |
| 1        | 13                | 8                 | 5               | 3              | 2             | 2 (0)        |            |
| 2        | 5                 | 50                | 30              | 24             | 5             | 5 (1)        |            |
| 2        | 7                 | 50                | 29              | 12             | 17            | 17 (4)       |            |
| 2        | 11                | 48                | 20              | 11             | 9             | 9 (2)        |            |
| Total    |                   | 291               | 198             | 87             | 110           | 39 (0)       | 71 (12)    |

* Age at time of immune serum inoculation, i.e., day 0.
* Pretreated with immune serum.
* Lesion score ≥ 1.
* Number of mice with confirmed recurrent cutaneous herpes shown in parentheses.
Fig. 1. Course of cutaneous infection of mice with recurrent herpes. Each curve depicts the response of one of the 12 mice treated with prednisone which showed recurrent skin infection with herpesvirus. Range of scores indicated during the primary lesion represents highest and lowest scores for individual mice in that cage on the designated day. Each mouse was followed individually after starting prednisone with immune serum permitted lesion development and recovery in a higher percentage of mice than was the case when no antibodies were present. For this reason, and because the presence of circulating antibodies at the time of the initial cutaneous infection more nearly simulates the usual human experience, we adopted this procedure. Preliminary experiments indicated that optimal time for pretreatment, to obtain the highest percentage of lesions followed by healing, was 48 h before inoculation of virus.

Limited attempts to reactivate healed lesions by means of heat, ultraviolet light, or mechanical trauma were unsuccessful. The conclusion that true reactivation has been achieved with prednisone treatment is supported by the fact that the time between primary inoculation of virus and first recovery of recurrent virus ranged from 7 to 10 weeks (see Fig. 1). It is of interest that reactivation of another herpesvirus, infec-
tious bovine rhinotracheitis, occurred after treating cattle with a corticosteroid (7, 4).

In the mouse experiments, little difference due to age was noted either in response to the primary infection or in the rate of recurrent virus recovery when animals ranged from 1 to 3 months old at time of initial infection (see Table 1). Nor was there any apparent pattern with respect to age of mice and severity of the recurrent lesion (see Fig. 1). Indeed, this lack of consistency in lesion severity makes difficult an accurate estimate of rate of recurrence; it can be seen that some of these lesions were barely detectable even though virus was demonstrated in skin swabs. When a consistent response can be achieved, this model should prove valuable for learning more about the biology and therapy of recurrent cutaneous herpes.

LITERATURE CITED