

# Photochemistry and Photobiology

Published by the American Society for Photobiology

Subscriptions &  
Membership

Editorial  
Board

Issue  
Alert

Help &  
FAQ

Advertising

Society  
Homepage

Current  
Issue

Archived  
Issues

[\[PDF Version\]](#) [\[Create Reference\]](#)

[\[PubMed Citation\]](#) [\[Related Articles in PubMed\]](#)

## TABLE OF CONTENTS

[\[INTRODUCTION\]](#) [\[MATERIALS AND...\]](#) [\[RESULTS\]](#) [\[DISCUSSION\]](#) [\[REFERENCES\]](#) [\[TABLES\]](#) [\[FIGURES\]](#)

American Society for Photobiology Journal 74:108–114 (2001)

doi: 10.1562/0031-8655(2001)074<0108:ULIRIA>2.0.CO;2  
*Photochemistry and Photobiology*: Vol. 74, No. 1, pp. 108–114.

## Ultraviolet Light Induces Reactivation in a Murine Model of Cutaneous Herpes Simplex Virus-1 Infection<sup>¶</sup>

Diane E. Goade,<sup>1</sup> Robert A. Nofchissey,<sup>1</sup> Donna F. Kusewitt,<sup>2</sup> Brian Hjelle,<sup>3</sup> John Kreisel,<sup>4</sup> Julene Moore,<sup>1</sup> and C. Richard Lyons<sup>1</sup>

<sup>1</sup>Departments of Medicine, Health Sciences Center, University of New Mexico, Albuquerque, NM

<sup>2</sup>Cell Biology and Physiology, Health Sciences Center, University of New Mexico, Albuquerque, NM

<sup>3</sup>Pathology, Health Sciences Center, University of New Mexico, Albuquerque, NM

<sup>4</sup>Department of Medicine, University of Utah School of Medicine, Salt Lake City, UT

Received April 17, 2001; accepted May 5, 2001

### ABSTRACT

**We have developed a model of cutaneous herpes simplex virus-1 (HSV-1) reactivation in SKH-1 hairless mice which closely mimics the condition in humans. Sixty plaque-forming units of HSV-1 strain 17 *syn+* were applied to a superficially abraded area on the lateral body wall. More than 85% of**

**mice developed primary HSV-1 infection characterized by a zosteriform pattern of cutaneous vesiculation and ulceration. Approximately one-third of mice with primary skin lesions succumbed to neurologic disease and in the remaining mice cutaneous lesions healed completely. Subsequent exposure of healed areas to two minimal inflammatory doses of UV resulted in recrudescence of skin lesions in the irradiated areas in almost 60% of mice. Lesions appeared approximately 4 days after irradiation, persisted for 3–5 days and then resolved completely. Reactivation rarely resulted in death due to neurologic disease. Primary lesions had a histologic appearance typical of cutaneous HSV-1 infection with vesicles and focal epithelial necrosis accompanied by the formation of epithelial syncytial cells and the presence of herpetic intranuclear inclusion bodies. In primary lesions HSV-1 was demonstrated by immunohistochemistry, polymerase chain reaction and culture. In reactivated lesions epithelial syncytia and inclusion bodies were not seen; however, virus was demonstrable by polymerase chain reaction and culture. Exposure of the uninfected side to UV did not stimulate disease recurrence suggesting that local effects of UV rather than systemic immunosuppression were responsible for reactivation. Reactivation could also be obtained with two minimal inflammatory doses of UV from a UV-340 light source which emits light approximating the solar spectrum.**

## **INTRODUCTION** [Return to TOC](#)

Herpes simplex viruses (HSV-1 and HSV-2)<sup>†</sup> are among the most common and troublesome human viral pathogens. HSV-1 infects approximately 80% of the world's population while HSV-2 affects 30% (1). Primary HSV infections are initiated by cutaneous or mucous membrane exposure to live virus (1–4). After a 2–8 day incubation period characteristic vesicular lesions on an erythematous base develop. These lesions progress to form sharply demarcated crusted ulcers. After primary infection lifelong latency is established, with persistence of the viral genome in spinal ganglia (5). Latent virus can be reactivated by environmental or physiologic stimuli, including fever, mechanical trauma and UV (1,6). For many individuals HSV reactivation is asymptomatic or results in only mild clinical signs; however, even those with clinically nonapparent reactivated disease shed infectious virus (7–9). Furthermore, in immunosuppressed patients such as those infected with acquired immune deficiency syndrome or transplant recipients persistent reactivation of HSV-1 or HSV-2 can be debilitating or life-threatening (10,11).

Animal models of primary cutaneous and ocular HSV infection and viral reactivation have been developed in mouse, rat, guinea pig and rabbit (reviewed in Wagner and Bloom [12]). Stimuli for reactivation have included a variety of chemical and physical agents. Although each of these models is valuable for studying particular aspects of reactivation, the general usefulness of many is limited by factors such as difficulty in establishing latent infection, nonphysiologic nature of the reactivation stimulus, significant spontaneous reactivation and low or inconsistent reactivation frequency.

We have developed a very useful murine model of cutaneous HSV-1 reactivation that closely resembles the disease in humans. SKH-1 mice that recover from primary HSV-1 disease develop latent infection that can be reactivated by exposure to two minimal

inflammatory doses (MID) of UV. Because the model employs hairless mice and reactivation is triggered by UV it is particularly suitable for photobiology studies. Preliminary studies with this model indicate that local rather than systemic effects of UV are responsible for reactivation. The success of these initial studies suggests that our model will be valuable in examining the pathophysiology of HSV latency and reactivation and for testing strategies to prevent and treat HSV-1 reactivation.

## **MATERIALS AND METHODS** [Return to TOC](#)

*Animals.* SHK-1 hairless mice were obtained from Charles Rivers (Wilmington, MA) and maintained in isolation in Biosafety Level 2 housing. Pelleted feed and water were available *ad libitum*. Rooms were maintained at 20–25°C and 33% humidity with a 12 h light/dark cycle. Both male and female mice were used for these studies; groups of four to five mice of the same sex were housed together. Before potentially painful procedures mice were anesthetized with a mixture of ketamine and xylazine (80 mg/kg ketamine and 16 mg/kg xylazine) or Avertin (4 M 2,2,2-tribromoethanol in 7% 2-methyl-2-butanol; 250 mg/kg) given intraperitoneally. Mice that developed neurologic disease, as evidenced by abdominal distension or hindlimb paralysis, were euthanized promptly by CO<sub>2</sub> inhalation. All protocols employed were approved by the University of New Mexico Health Sciences Center Animal Care and Use Committee.

*Virus.* HSV-1 viral stocks, strain 17 *syn+* (provided by Dr. Kreisel), were collected from the cell culture medium of HSV-1–infected confluent VERO-E6 cell cultures. Supernatants were centrifuged at 350 *g* for 15 min to remove cell debris. The plaque-forming unit (PFU) concentration was determined by standard Vero cell culture of a dilution series of virus stock (13). Viral stocks were stored at –80°C. The dose of virus that established grossly apparent primary infection in 50% of inoculated mice was determined by challenging three to four groups of SKH-1 mice with serial dilutions of viral stock.

*Primary infection.* A 5–10 × 2 mm linear dermabrasion was created on the lateral body wall using a small wire brush attached to a cordless rotary tool (Dremel Minimite Model 750, Racine, WI) set at 5000 rpm. Dermabrasion barely penetrated the epidermis; the resultant lesion was markedly reddened but did not bleed. A 50 µL inoculum containing 60 PFU of virus (1200 PFU/mL) was applied to the abraded area, rubbed into the wound and allowed to dry. This dose was chosen based on preliminary studies indicating that it gave the best balance of survival and susceptibility to reactivation. At higher doses the number of animals that survived infection was too low to be useful, while at lower doses too few animals developed primary infection to yield reasonable numbers of mice with latent infection. Control mice underwent dermabrasion and application of suspension medium without virus. Mice were observed daily for signs of infection. Skin lesions were graded as mild (discrete, noncoalescing lesions in a zosteriform pattern), moderate (partially coalescing lesions) or severe (coalescing, hemicircumferential ulcers). For these studies ‘reactivation’ was defined as the appearance of gross skin lesions compatible with HSV-1, including macules, vesicles and ulcers. Reactivated lesions were graded like primary lesions; however, reactivated lesions were never hemicircumferential. Additional controls for reactivation studies included mice that had recovered from primary infection but were not exposed to UV.

*Light sources.* Most studies utilized a transilluminator (Ultra-Lum, Model UVB-15,

Carson, CA) covered with an UV-transparent glass protector (UviClear, Fotodyne Inc., Hartland, WI) that provided 70 J/m<sup>2</sup>/s UV. This apparatus emitted wavelengths of light between 250 and 400 nm with a peak emission at 312 nm. Approximately 12% of the energy was in the UV-C range (250–290 nm), 49% was in the UV-B range (290–320 nm) and 39% was in the UV-A range. A UV-340 light source (Q-Panel, Cleveland, OH) that emitted a UV spectrum nearly identical to that of a solar simulator (14) was employed for one study. This light source produced light in the range of 290–400 nm and the peak energy output was at 342 nm. The emitted light contained no UV-C wavelengths, 8% of the energy was in the UV-B range and 92% was in the UV-A range. Separate studies indicated that the MID for SKH-1 mice, as determined by development of edema (15,16), was approximately 1000 J/m<sup>2</sup> for the transilluminator light source; exposure to 15 min of irradiation from the UV-340 light source yielded edema comparable to one MID from the transilluminator. The development of edema is a more sensitive indicator of UV-induced inflammation than visual detection of erythema (15). For disease reactivation mice were anesthetized and exposed to UV on only one side of the body. Except for a single study the side exposed was the originally infected side. During UV exposure the eyes were protected from UV damage by shielding with an aluminum foil.

*Sample collection.* Material were collected in a laminar flow hood, instruments were cleaned and disinfected and fresh gloves were donned between mice. Blood was collected from the retro-orbital sinus or heart. Skin samples were taken on days 1–14 following viral inoculation and on days 0–8 following UV exposure. Skin samples were fixed in 10% neutral zinc-buffered formalin (for histology and immunohistochemistry) or frozen in liquid nitrogen (for polymerase chain reaction (PCR), immunohistochemistry and viral isolation). Skin samples for virus culture were approximately 5 × 5 mm in size; they were placed in sterile tubes and immediately frozen in liquid nitrogen for storage at –80°C. For viral isolation 1.5 mL culture medium and 1 g of 2.5 µm zirconia/silica beads (Bio Spec Products, Bartlesville, OK) were added to each frozen sample and the sample was processed in a minibeadbeater apparatus (Bio Spec Products) at 2500 rpm for 30 s. Supernatants were collected and plated immediately as described below.

*Serology.* Recombinant proteins encoded by the carboxy terminal regions of glycoprotein B (gB-SS1, amino acids 228–903) and glycoprotein D (gD-PB1, amino acids 306–462) were generated using pATH expression vectors (American Type Culture Collection, Manassas, VA), separated by SDS-PAGE and transferred to 0.2 µm nitrocellulose. To detect antibodies to these viral proteins in blood and serum, samples to be tested were diluted 1:200 in a buffered milk solution and incubated with blots of recombinant proteins overnight at 4°C. Blots were developed with goat anti-mouse alkaline phosphatase–conjugated secondary antibody (Southern Biotechnology, Birmingham, AL) using standard techniques.

*Histology and immunohistochemistry.* Formalin-fixed skin was embedded in paraffin, sectioned at 6 µm and stained with hematoxylin and eosin. For immunohistochemistry deparaffinized slides or frozen sections were stained for HSV-1 by standard techniques using a rabbit primary antibody (Dako #B0114, Dako, Carpinteria, CA) at dilutions of 1:1000–1:10 000, a horseradish peroxidase–conjugated goat anti-rabbit secondary antibody as recommended (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA) and a diaminobenzidine color detection system (Vector Laboratories). As a negative control a similarly prepared rabbit polyclonal antibody against human secretory factor (Dako) was used at the same protein concentration.

*Polymerase chain reaction.* DNA was extracted from frozen skin samples by grinding the skin with a liquid nitrogen-cooled mortar and pestle and processing the resultant powder with a DNA minicolumn kit (Qiagen, Valencia, CA). The yield was approximately 40 µg of DNA per 20 mg skin sample. PCR primers (5'-CATGACCCTTGTGAAGTACG-3', 5'-GCTCGAGAGCTTGATCTT-GTCG-3') were designed to amplify a region of the HSV-1 *pol* gene from nucleotides 1930–2191. A single cycle of 95°C for 4 min was followed by 30 cycles of 95, 64 and 72°C for 45 s at each temperature with a 10 min extension at 72°C after the last of the 30 cycles. Positive controls included DNA prepared from HSV-1 17 *syn+* and MacIntyre virus stocks.

*Virus culture.* Culture medium consisted of minimal essential medium with Earle's balanced salt solution (HyClone, Logan, UT) containing 10% heat-inactivated fetal calf serum, 2 mM glutamine, 40 U/mL penicillin, 40 µg/mL streptomycin and 40 mg/mL fungizone. Samples were plated at varying dilutions on confluent Vero cell monolayers and observed for virus cytopathic effect (CPE) (13).


## RESULTS [Return to TOC](#)

Four studies performed to characterize primary and UV-reactivated HSV-1 disease in SKH-1 mice are summarized in [Table 1](#) (14). Overall, an inoculum of 60 PFU established grossly apparent primary disease in 86% of mice. About one-third of mice with primary skin lesions developed neurologic signs (hindlimb paralysis or intestinal stasis) and were humanely killed. In later studies mice with severe skin lesions were euthanized early, and remaining mice did not develop nervous disease. In all studies zosteriform lesions of primary infection appeared 4–6 days after inoculation. Initial lesions were erythematous macules that progressed to small vesicles each on an erythematous base. Vesicles developed into sharply demarcated ulcers over a 1–2 day period. Lesions spread dorsally but did not cross the midline ([Fig. 1a](#) (14)). In mice surviving the initial infection skin healing was complete in a week with little or no visible scarring.


Upon treatment with two MID of UV from a transilluminator ([Table 1](#) (14), Groups 1–3) or a UV-340 light source ([Table 1](#) (14), Group 4) approximately 60% of mice that had completely recovered from primary infection developed skin lesions within 4 days ([Table 1](#) (14)). Skin lesions appeared somewhat less severe and healed more quickly than those seen in primary disease. Lesions consisted of macules that progressed to vesicles; vesicles resolved or evolved into shallow ulcers. Lesions of reactivated disease were more widespread than in primary disease but remained restricted to the side of the body initially infected ([Fig. 1b](#) (14)). Fewer than 10% of animals developed neurologic signs following HSV-1 reactivation.



Sixteen mice with healed lesions of primary HSV-1 disease ([Table 1](#) (14), Group 2) were first exposed to two MID of UV from a transilluminator on the side of the body opposite the infected side. Twice daily examination of the mice for 2 weeks did not reveal evidence of reactivated disease. Subsequent exposure of the infected side gave the expected reactivation frequency. Twenty mice that had recovered from primary infection, exposed to two MID of UV, and allowed to recover from reactivated disease were used to test the ability of a second exposure to UV to stimulate reactivation. Mice were exposed to UV for a second time after periods ranging from 1 week to 3 months following the initial UV exposure. Of the 20 mice tested, 12 had reactivated upon initial UV exposure. Of these 12 ten developed reactivated disease following a second exposure to UV; thus, two mice did not reactivate a second time. One mouse refractory to HSV-1 reactivation on first UV exposure developed cutaneous disease after a second UV exposure. These results indicated that mice latently


infected with HSV-1 could undergo at least two episodes of reactivation and that the pattern of susceptibility to reactivation shown by individual animals was largely reproducible.

Eighteen mice that survived primary HSV-1 infection (Group 4, [Table 1](#) ) were exposed to two MID from a UV-340 light source that simulated the solar spectrum. Fifty-six percent of these mice (10/18) developed reactivated HSV-1 disease. One of the 10 mice with reactivated cutaneous lesions also developed neurologic signs. Thus, both UV that closely simulates the solar spectrum and UV from a transilluminator emitting mostly UV-B wavelengths can stimulate reactivation.

An IgM serum antibody response to glycoprotein B was detectable by Western blot by 7 days after virus inoculation in 23% (3/13) of mice tested (data not shown). Forty-six mice that developed clinical disease were tested, and all had IgG antibodies to glycoproteins B and D by 21 days after inoculation (data not shown). Occasional mice with very mild or inapparent disease developed specific antibodies to the glycoproteins and were able to reactivate disease upon exposure to UV.

Histologically, primary HSV-1 disease was similar to cutaneous HSV infection in man ([1,6](#)). In the earliest grossly apparent primary lesions there were foci of pale and swollen keratinocytes within which epithelial syncytia and eosinophilic intranuclear inclusion bodies could be identified ([Fig. 2a,b](#) ). Lesions progressed first to intraepidermal vesicles and then to ulcers covered by crusts of necrotic debris. The underlying dermis became heavily infiltrated with mixed inflammatory cells. Lesions remained localized and were sharply demarcated from surrounding normal skin. A mouse with flaccid paralysis of the hindlimbs had an extensive area of malacia in the spinal cord; this necrotizing myelitis was consistent with an HSV etiology ([17–19](#)). The histologic lesions of reactivated disease were similar to those of primary disease, except that neither epithelial syncytia nor intranuclear inclusion bodies were seen either in vesicles or in ulcers.

Immunohistochemical staining localized HSV-1 antigen to the epidermal lesions of primary disease. Surface epithelium and hair follicles in vesicles and foci of epidermal necrosis were strongly immunoreactive with antibodies against HSV-1 ([Fig. 3a](#) ). Immunoreactivity did not extend into the surrounding normal skin. Both cytoplasm and nuclei of epithelial cells were immunopositive, with nuclear staining often more prominent than cytoplasmic. With the development of ulceration immunoreactivity disappeared, except in hair follicles. Occasional cutaneous nerve fibers in proximity to the initial lesions exhibited HSV-1 immunoreactivity ([Fig. 3b](#) ). HSV-1 immunoreactivity in reactivated lesions was detectable using frozen sections of skin; however, the intensity of the staining in reactivated lesions was markedly reduced compared to primary lesions and the high concentration of antibody required resulted in appreciable background staining in negative control samples.

HSV-1 was cultured from all four samples taken from cutaneous lesions at 5–7 days following primary infection. In these cultures high levels of virus and distinct CPE were evident within 24–48 h (not shown). HSV-1 was cultured from 10 of 15 (67%) samples taken from animals with reactivated disease 3–6 days after UV exposure. Up to 7 days of incubation were required for the development of CPE in samples from reactivated lesions suggesting that the amount of virus present was very low ([13,20](#)). HSV *pol* gene segments were demonstrated by PCR in all eight lesions tested on days 5–7 of primary infection and in five of six (83%) reactivated lesions tested 3–6 days after UV exposure ([Fig. 4](#) ). HSV-1 could not be demonstrated by culture or PCR in crusted lesions sampled late in the course

of primary or reactivated disease. The virus could not be detected by PCR in samples taken on days 0–2 after UV exposure given to stimulate reactivation.

Sham-infected mice did not develop grossly or microscopically evident lesions of HSV-1 following primary infection or UV treatment. UV exposure caused slight reddening and flaking of the skin with microscopic evidence of epidermal hyperplasia. HSV-1 was not detected in these mice by immunohistochemistry, culture or PCR. HSV-1 was also not detected by these methods in mice that had recovered from primary infection when these mice were tested at 3–4 weeks after primary disease.

## DISCUSSION [Return to TOC](#)

The histologic changes seen in primary HSV-1 infection in this model, vesiculation and ulceration with epithelial syncytia and intranuclear inclusion bodies, were entirely consistent with HSV-1 disease in man (1,6). Primary infection was verified by immunohistochemistry, culture and PCR. All observations on primary disease suggested that large numbers of infective viral particles were present. However, in reactivated disease there appeared to be considerably fewer viruses. Viral inclusion bodies were not seen histologically, and viral antigen was difficult to demonstrate by immunohistochemistry. Furthermore, the appearance of viral CPE in culture was delayed relative to primary disease. However, virus was readily demonstrable in reactivated lesions by PCR. Similarly, in reactivated HSV disease in humans the amount of virus is significantly lower than in primary disease and virus is present only in early macular and vesicular lesions leading to difficulties in diagnosis (1,20). In human HSV reactivation multinucleated epithelial cells and intranuclear inclusions are seen in very early macular and vesicular lesions but not in ulcers (1,6). These indications of viral reactivation might have been observed in our study had we sampled lesions at sufficiently early stages of lesions development, perhaps at the stage of macules or very early vesicles.

Experimental reactivation of HSV-1 or HSV-2 in latently infected humans can be stimulated by UV-B irradiation of the site of primary infection, the lip or peri-genital region, respectively (21,22). Three minimal erythematous doses of UV-B directed to a site of previous reactivation induces reactivation about 60% of the time with lesions appearing 1–6 days after exposure (23,24). Lesions arise within the UV-exposed areas and extend up to 1 cm beyond the margin of the exposed areas but are generally restricted to regions sharing local innervation. Virus can be recovered from early vesicles in approximately 75% of cases (24). Reactivation is difficult to study in humans because primary disease or very early reactivation may be indistinguishable and the kinetics of reactivation are unpredictable. Thus, animal models such as the one we describe are required for thorough investigation of the disease process.

Mice can be infected with HSV by inoculation of the skin, footpad, eye or vaginal mucosa (25–36). Mortality from systemic disease in these studies is usually reported to be about 30% (31,32,35) even when passive immunization precedes infection (33). Reactivation has been elicited in other cutaneous and ocular models of HSV infection by a variety of stimuli, including mechanical trauma, chemical irritation, hyperthermia, prednisone administration and UV exposure (25,26,28,30,31,33–36). Reactivation rates using these models are variable ranging from 10–90%; in some of the models spontaneous reactivation of disease is a complicating factor (25,30,31,34,36).

The HSV-1 reactivation model that we describe is a reproducible and clinically relevant model of human HSV-1 reactivation. It fulfills the criteria suggested as appropriate for an animal model of human HSV-1 reactivation (12): the host is immunocompetent; the initial infection is sufficiently mild so that most animals survive; reactivation occurs in response to physiologically relevant stimuli; recovery from reactivation is complete; reactivation occurs *in vivo* and in a large proportion of infected animals and the strain of virus employed (17 *syn+*) is appropriate for generating mutations to examine the function of critical viral genes. Our model has distinct advantages compared to other HSV reactivation models. Death from primary disease does not exceed 40%, and passive immunization to prevent excessive mortality is not required. Reactivation is seen consistently in almost 60% of mice in response to relatively low doses of UV, a common stimulus for HSV-1 reactivation in man. Preliminary studies indicate that reactivation in our model occurs with similar frequency in response to irradiation with both UV-B and UV-340 light sources. This makes the model particularly relevant to human disease because the energy output of the UV-340 light source closely approximates the solar spectrum. The lack of spontaneous reactivation in our model further enhances its power. In addition, because the mice we employ are hairless shaving is not required; thus a confounding stimulant to reactivation is eliminated from the model. Our model will be useful for studies examining the pathophysiology of primary and reactivated HSV-1 disease, for testing therapeutic approaches and for testing vaccination strategies.

There are several distinct mechanisms by which UV might stimulate reactivation of HSV-1 but these mechanisms are not mutually exclusive. First, there may be continual low-level production of virus even when disease is clinically inactive, and overt disease may be prevented by an effective immune response against the virus. UV may cause local or systemic immunosuppression that allows escape of the virus from immune surveillance and recrudescence of disease; the precipitating immune suppression might well be mediated by cytokines released from UV-exposed epidermis. Secondly, UV may stimulate the release of cytokines from the UV-exposed skin that interact directly with receptors on sensory nerves to initiate signaling cascades that culminate in a burst of virus replication. Finally, UV may directly activate virus present in the skin at very low levels during latency resulting in active disease.

There is evidence to support all mechanisms for reactivation. Although the cellular immune response to primary HSV-1 infection of the ganglia develops slowly (7–10 days after infection) immune cells rapidly infiltrate latently infected neurons during reactivation, as early as 12 h after viral antigen appears (37). Viral antigen is present in these immune cells and is rapidly cleared from the dorsal root ganglia. This suggests that immune cells limit the spread of reactivated virus, perhaps thereby preventing disease or reducing its severity. On the other hand, peripheral neurectomy, presumably resulting in the loss of trophic factors to neurons in dorsal root ganglia, can reduce the incidence of HSV-1 reactivation (38). This indicates that non-immune-mediated alterations in the neuronal milieu can have profound effects on latency. In a recent study of cutaneous HSV infection in rats it was demonstrated that the cellular immune response to HSV was suppressed by UV exposure prior to primary infection while the severity of the subsequent skin lesions was not altered (39). On the other hand, UV exposure after primary HSV infection increased the severity of skin lesions without changing the immune response. This suggests that UV might stimulate viral replication directly if small amounts of virus persist in the skin during latency. It should be noted, however, that we were unable to demonstrate persistent virus in healed lesions in the present study.

Based on *in vitro* and *in vivo* studies it appears that a variety of local signaling molecules

can influence HSV-1 reactivation. Factors that have been implicated include nerve growth factor, interleukin 6, interleukin 1 $\alpha$ , epinephrine, tumor necrosis factor- $\alpha$ , interferon  $\alpha/\beta$  and interferon  $\gamma$  (40–48). Neither the cellular origin nor the cellular target of these modulating factors has been clearly identified *in vivo*. Signaling molecules may originate from skin innervated by latently infected neurons, inflammatory cells that infiltrate the skin or inflammatory cells or satellite cells within latently infected ganglia. Furthermore, signals may impinge on the soma, the axon or the dendrites of the reactivating neurons.

In our studies mice exposed to UV on the uninfected side failed to reactivate clinically apparent disease. The mice subsequently reactivated at the expected frequency and with the expected pattern of lesions upon exposure of the infected side indicating that successful primary infection and latency had been achieved. These findings suggested that local rather than systemic UV effects were responsible for reactivation. Our results are in keeping with studies in mice and humans showing that, to be effective, stimuli for reactivation must be applied to the site of original infection thus directly affecting processes of nerves in latently infected ganglia (21,23,49). We hypothesize that these local effects are mediated by signaling molecules released from UV-exposed epidermis, and we anticipate that further studies employing this murine model of HSV-1 reactivation will yield significant insights into the pathophysiology of HSV-1 reactivation.

## REFERENCES [Return to TOC](#)

1. Whitley, R. J. (1996) Herpes simplex viruses. In *Fields Virology*, 3rd Ed. (Edited by B. N. Fields and D. Knipe), pp. 2297–2341. Raven Press, New York.
2. Nahmias, A. J. and B. Roizman (1973) Infection with herpes simplex virus 1 and 2.1. *N. Engl. J. Med.* **289**, 667–674. [Links](#) ►
3. Nahmias, A. J. and B. Roizman (1973) Infection with herpes simplex virus 1 and 2.2. *N. Engl. J. Med.* **289**, 719–725. [Links](#) ►
4. Nahmias, A. J. and B. Roizman (1973) Infection with herpes simplex virus 1 and 2.3. *N. Engl. J. Med.* **289**, 781–789. [Links](#) ►
5. Roizman, B. and A. Sears (1996) Herpes simplex viruses and their replication. In *Fields Virology*, 3rd Ed. (Edited by B. N. Fields and D. Knipe), pp. 2231–2295. Raven Press, New York.
6. Hirsch, M. S. (1995) Herpes simplex virus. In *Principles and Practice of Infectious Diseases* (Edited by G. L. Mandell, J. E. Bennett and R. Dolan), pp. 1336–1344. Churchill Livingstone, New York.
7. Koelle, D. M., J. Benedetti, A. Langenberg and L. Corey (1992) Asymptomatic reactivation of herpes simplex virus in women after the first episode of genital herpes. *Ann. Intern. Med.* **116**, 433–437. [Links](#) ►
8. Mertz, G. J., R. W. Coombs, R. Ashley, J. Jourden, M. Remington, C. Winter, A. Fahlander, M. Guinana, H. Ducey and L. Corey (1988) Transmission of genital herpes in couples with one symptomatic and one asymptomatic partner: a prospective study. *J. Infect.*

*Dis.* **157**, 1169–1177. [Links ▶](#)

9. Wald, A., J. Zeh, S. Selke, R. L. Ashley and L. Corey (1995) Virologic characteristics of subclinical and symptomatic genital herpes infections. *N. Engl. J. Med.* **333**, 770–775.

[Links ▶](#)

10. Levin, M. J. (1993) Impact of herpesviruses in the future. *J. Med. Virol.* **1**(Suppl.), 158–164. [Links ▶](#)

11. Peterslund, N. A. (1991) Herpesvirus infection: an overview of the clinical manifestations. *Scand. J. Infect. Dis.* **80**(Suppl.), 15–20. [Links ▶](#)

12. Wagner, E. K. and D. C. Bloom (1997) Experimental investigation of herpes simplex virus latency. *Clin. Microbiol. Rev.* **10**, 419–443. [Links ▶](#)

13. Rawls, W. E. (1979) Herpes simplex virus types 1 and 2 and herpesvirus simiae. In *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections* (Edited by E. H. Lennette and N. J. Schmidt), pp. 309–360. American Public Health Association, Washington.

14. Beasley, D. G., J. Beard, J. W. Stanfield and L. K. Roberts (1996) Evaluation of an economical sunlamp that emits a near solar UV power spectrum for conducting photoimmunological and sunscreen immune protection studies. *Photochem. Photobiol.* **64**, 303–309. [Links ▶](#)

15. Wolf, P., C. K. Donawho and M. L. Kripke (1993) Analysis of the protective effect of different sunscreens on ultraviolet radiation–induced local and systemic suppression of contact hypersensitivity and inflammatory responses in mice. *J. Investig. Dermatol.* **100**, 254–259. [Links ▶](#)

16. Reeve, V. E., C. Boehm-Wilcox, M. Bosnic and W. G. Reilly (1994) Differential photoimmunoprotection by sunscreen ingredients is unrelated to epidermal cis urocanic acid formation in hairless mice. *J. Investig. Dermatol.* **103**, 801–806. [Links ▶](#)

17. Folpe, A., L. W. Lapham and H. C. Smith (1994) Herpes simplex myelitis as a cause of acute necrotizing myelitis syndrome. *Neurology.* **44**, 1955–1957. [Links ▶](#)

18. Kinjo, T., K. Tshako, W. Siriungsi, K. Sunagawa, I. Nakazato and T. Iwamasa (1997) Experimental myelitis caused by herpes simplex virus type 2 in C57BL/6N and BALB/cN mice. *Int. J. Exp. Pathol.* **78**, 401–409. [Links ▶](#)

19. Townsend, J. J. and P. K. Collins (1986) Peripheral nervous system demyelination with herpes simplex virus. *J. Neuropathol. Exp. Neurol.* **45**, 419–425. [Links ▶](#)

20. Ashley, R. (1993) Laboratory techniques in the diagnosis of herpes simplex infection. *Genitourin. Med.* **69**, 174–183. [Links ▶](#)

21. Rooney, J. F., S. E. Straus, M. L. Mannix, C. R. Wohlenberg, S. Banks, S. Jagannath, J. E. Brauer and A. L. Notkins (1992) UV light–induced reactivation of herpes simplex virus type-2 and prevention by acyclovir. *J. Infect. Dis.* **166**, 500–506. [Links ▶](#)

22. Spruance, S. L., D. J. Freeman, J. C. Stewart, M. B. McKeough, L. G. Wenerstrom, G. G. Krueger, M. W. Piepkorn, W. G. Stroop and N. H. Rowe (1991) The natural history of ultraviolet radiation–induced herpes simplex labialis and response to therapy with peroral and topical formulations of acyclovir. *J. Infect. Dis.* **163**, 728–734. [Links ▶](#)
23. Perna, J. J., M. L. Mannix, J. F. Rooney, A. L. Notkins and S. E. Straus (1987) Reactivation of latent herpes simplex virus infection by ultraviolet light: a human model. *J. Am. Acad. Dermatol.* **17**, 473–478. [Links ▶](#)
24. Spruance, S. L. (1985) Pathogenesis of herpes simplex labialis: experimental induction of lesions with UV light. *J. Clin. Microbiol.* **22**, 366–368. [Links ▶](#)
25. Blatt, A. N., K. A. Laycock, R. H. Brady, P. Traynor, D. J. Krogstad and J. S. Pepose (1993) Prophylactic acyclovir effectively reduces herpes simplex virus type 1 reactivation after exposure of latently infected mice to ultraviolet B. *Investig. Ophthalmol. Vis. Sci.* **34**, 3459–3465. [Links ▶](#)
26. Blyth, W. A., T. J. Hill, H. J. Field and D. A. Harbour (1976) Reactivation of herpes simplex virus infection by ultraviolet light and possible involvement of prostaglandins. *J. Gen. Virol.* **33**, 547–550. [Links ▶](#)
27. Ecob-Prince, M. and K. Hassan (1994) Reactivation of latent herpes simplex virus from explanted dorsal root ganglia. *J. Gen. Virol.* **75**, 2017–2028. [Links ▶](#)
28. Harbour, D. A., T. J. Hill and W. A. Blyth (1983) Recurrent herpes simplex in the mouse: inflammation in the skin and activation of virus in the ganglia following peripheral stimulation. *J. Gen. Virol.* **64**, 1491–1498. [Links ▶](#)
29. Junejo, F. and S. M. Brown (1995) Latent phenotype analysis of three deletion variants of herpes simplex virus type 1 (HSV-1) in mouse model. *J. Pakistan Med. Assn.* **45**, 99–104. [Links ▶](#)
30. Miller, J. K., K. A. Laycock, J. A. Umphress, K. K. Hook, P. M. Stuart and J. S. Pepose (1996) A comparison of recurrent and primary herpes simplex keratitis in NIH inbred mice. *Cornea.* **15**, 497–504. [Links ▶](#)
31. Norval, M., S. E. M. Howie, J. A. Rossand and J. P. Maingay (1987) A murine model of herpes simplex virus recrudescence. *J. Gen. Virol.* **68**, 2693–2698. [Links ▶](#)
32. Podlech, J., F. Hengerer, M. Fleck, K. Eray and D. Flake (1996) Asymptomatic vaginal herpes simplex virus infections in mice: virology and pathohistology. *Arch. Virol.* **141**, 263–274. [Links ▶](#)
33. Sawtell, N. M. and R. L. Thompson (1992) Rapid in vivo reactivation of herpes simplex virus in latently infected murine ganglionic neurons after transient hyperthermia. *J. Virol.* **66**, 2150–2156. [Links ▶](#)
34. Shimeld, C., T. Hill, B. Blyth and D. Easty (1989) An improved model of recurrent herpetic eye disease in mice. *Curr. Eye Res.* **8**, 1193–1205. [Links ▶](#)

35. Underwood, G. E. and S. D. Weed (1974) Recurrent cutaneous herpes simplex in hairless mice. *Infect. Immun.* **10**, 471–474. [Links ▶](#)
36. Willey, D. E., M. D. Trousdale and A. B. Nesburn (1984) Reactivation of murine latent HSV infection by epinephrine iontophoresis. *Investig. Ophthalmol. Vis. Sci.* **25**, 945–950. [Links ▶](#)
37. Shimeld, C., J. L. Whiteland, N. A. Williams, D. L. Easty and T. J. Hill (1996) Reactivation of herpes simplex virus type 1 in the mouse trigeminal ganglion: an in vivo study of virus antigen and immune cell infiltration. *J. Gen. Virol.* **77**, 2583–2590. [Links ▶](#)
38. Tenser, R. B., W. A. Edris and K. A. Hay (1993) Neuronal control of herpes simplex virus latency. *Virology.* **195**, 337–347. [Links ▶](#)
39. Garssen, J., R. van der Molen, A. de Klerk, M. Norval and H. van Loveren (2000) Effects of UV irradiation on skin and nonskin-associated herpes simplex virus infections in rats. *Photochem. Photobiol.* **72**, 645–651. [Links ▶](#)
40. Millhouse, S. and B. Wigdahl (2000) Molecular circuitry regulating herpes simplex virus type 1 latency in neurons. *J. Neurovirol.* **6**, 6–24. [Links ▶](#)
41. Cantin, E., B. Tanamachi and H. Openshaw (1999) Role for gamma interferon in control of herpes simplex virus type 1 reactivation. *J. Virol.* **73**, 3418–3423. [Links ▶](#)
42. Walev, I., J. Podlech and D. Falke (1995) Enhancement by TNF- $\alpha$  of reactivation and replication of latent herpes simplex virus from trigeminal ganglia of mice. *Arch. Virol.* **140**, 987–992. [Links ▶](#)
43. Carr, D. J. J., S. Noisakran, W. P. Halford, N. Lukacs, V. Asensio and I. L. Campbell (1998) Cytokine and chemokine production in HSV-1 latently infected trigeminal ganglion cell cultures: effects of hyperthermic stress. *J. Neuroimmunol.* **85**, 111–121. [Links ▶](#)
44. Hill, J. M., H. H. Garza, M. F. Helmy, S. D. Cook, P. A. Osborne, E. M. Johnson, H. W. Thompson, L. C. Green, R. J. O'Callaghan and B. M. Gebhardt (1997) Nerve growth factor antibody stimulates reactivation of ocular herpes simplex virus type 1 in latently infected rabbits. *J. Neurovirol.* **3**, 206–211. [Links ▶](#)
45. Kreisel, J. D., B. M. Gebhardt, J. M. Hill, S. A. Maulden, I. P. Hwang, T. E. Clinch, X. Cao, S. L. Spruance and B. A. Araneo (1997) Anti-interleukin-6 antibodies inhibit herpes simplex virus reactivation. *J. Infect. Dis.* **175**, 821–827. [Links ▶](#)
46. LeBlanc, R. A., L. Pesnicak, E. S. Cabral, M. Godleski and S. E. Straus (1999) Lack of interleukin-6 (IL-6) enhances susceptibility to infection but does not alter latency or reactivation of herpes simplex type 1 in IL-6 knockout mice. *J. Virol.* **73**, 8145–8151. [Links ▶](#)
47. Keadel, T. L., N. Usui, K. A. Laycock, J. K. Miller, J. S. Pepose and P. M. Stuart (2000) IL-1 and TNF- $\alpha$  in the pathogenesis of murine recurrent herpetic stromal keratitis. *Investig. Ophthalmol. Vis. Sci.* **41**, 96–102. [Links ▶](#)

48. Kaufman, H. E., E. D. Varnell, B. M. Gebhardt, H. W. Thompson and J. M. Hill (1996) Propanolol suppression of ocular HSV-1 recurrence and associated corneal lesions following spontaneous reactivation in the rabbit. *Curr. Eye Res.* **15**, 680–684. [Links ▶](#)

49. Sekizawa, T., H. Openshaw and A. L. Notkins (1985) Site specificity of epithelial irritants in the reactivation of herpes simplex virus. *J. Infect. Dis.* **152**, 841–842. [Links ▶](#)

## TABLES [Return to TOC](#)

**Table 1.** Primary and reactivated HSV-1 disease in SKH-1 mice

	Group 1	Group 2	C
<i>Primary infection</i>			
Mice inoculated	24	16	16
Sex	F	F	9
Age at primary infection (weeks)	8–11	7	5
Mice with primary disease	19/24 (79%)	13/16 (81%)	14
Mice with neurologic signs	7/19 (37%)	5/13 (39%)	—
Mice surviving primary infection	9/19 (47%)	7/13 (54%)	11
<i>Reactivation</i>			
Age at reactivation (weeks)	30–52	11	9
Infection–reactivation interval (weeks)	20–40	4	3
UV source	UV-B†	UV-B	UV
Mice with reactivated disease	6/9 (75%)	3/7 (43%)	7
Mice with neurologic signs	1/6 (17%)	0/3 (0%)	0

\*Mice with severe skin lesions were removed from study before neurologic signs dev

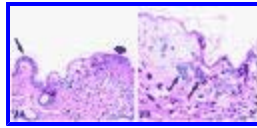
†Transilluminator.

## FIGURES [Return to TOC](#)



[Click on thumbnail for full-sized image.](#)

**Figure 1.** Gross lesions of (A) primary and (B) reactivated HSV-1 disease in hairless mice. Lesions of primary disease were linear and deeply ulcerated while lesions of reactivated disease were more widespread but generally shallower



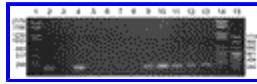
Click on thumbnail for full-sized image.

**Figure 2.** Microscopic lesions of primary HSV-1 disease. (A) Normal skin (long arrow) can be seen adjacent to an area of necrosis (short arrow). (B) In the region adjacent to the area of necrosis there are HSV-induced syncytia (arrows)



Click on thumbnail for full-sized image.

**Figure 3.** Immunohistochemical detection of HSV-1 in lesions of primary disease. Viral antigen was readily detectable in (A) skin and (B) sensory nerves underlying affected skin during active primary infection



Click on thumbnail for full-sized image.

**Figure 4.** HSV-1 PCR. Lane 1, markers; Lane 2, HSV-1 MacIntyre strain; Lane 3, water blank; Lane 4, HSV-1 17 *syn+*; Lane 5, uninfected control mouse skin; Lane 6, skin from the unexposed side of an infected mouse; Lane 7, unexposed skin from an infected mouse; Lane 8, late reactivation lesion; Lane 9, early primary lesion; Lane 10, HSV 17 *syn+*; Lanes 11 and 12, early reactivated lesions from two mice; Lane 13, fatal primary infection; Lanes 14 and 15, markers. A 262 bp *pol* gene segment was recovered by PCR from DNA prepared from infected mouse skin samples and from viral DNA stock preparations. HSV was demonstrated in early primary lesions and in early reactivated lesions only

¶Posted on the website on 14 May 2001.

\*To whom correspondence should be addressed at: Department of Veterinary Biosciences, The Ohio State University, 1925 Coffey Road, Columbus, OH 43210, USA. Fax: 614-292-6473; E-mail: [kusewitt.1@osu.edu](mailto:kusewitt.1@osu.edu)

†*Abbreviations:* CPE, cytopathic effect; HSV, herpes simplex virus; MID, minimal inflammatory dose; PCR, polymerase chain reaction; PFU, plaque-forming units.