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Rapid In Vivo Reactivation of Herpes Simplex Virus in Latently Infected Murine Ganglionic Neurons after Transient Hyperthermia

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A rapid and physiologically relevant hyperthermia-based induction procedure has been utilized to develop an in vivo model of induced herpes simplex virus (HSV) reactivation in outbred Swiss Webster mice. This procedure was found to efficiently reactivate latent virus from both trigeminal and lumbosacral ganglia. Examination of the time between hyperthermia and virus production demonstrated that detectable levels of infectious virus were present in ganglia as soon as 14 h posttreatment, with peak percent recoveries at 24 h. These data indicated that the switch from latent to active viral gene transcription occurred rapidly following treatment. Immunohistochemical staining for HSV type 1 antigens revealed rare antigen-positive ganglionic neurons 24 h postinduction. HSV antigens were not detected in any other cell type, and lateral spread of the infection was not observed. This is the first report of the detection of HSV antigens in vivo following induced reactivation in the intact nervous system and demonstrates that the neuron is the site of infectious virus production. In addition, our data strongly suggest that at least some neurons in which HSV antigens are detected during reactivation do not survive. Because the temporal and spatial characteristics of HSV reactivation have been clearly defined, this model is uniquely suited for the molecular dissection of the reactivation process.

Central to herpes simplex virus (HSV) pathogenesis in humans is the establishment and maintenance of its genome in a latent state. During primary infection at the body surface, the virus is transported through the axonal processes of innervating sensory neurons to the cell body, where it resides in a latent state. Periodic reactivation of this latent virus can result in a lifelong course of recurrent disease (see reference 22 for a review). This aspect of the natural history of HSV remains the fundamental challenge to the treatment and eventual elimination of this human pathogen.

Stimuli such as fever, sunburn, trauma, and other types of stress are commonly associated with the reactivation of latent virus in humans and are thus considered "induction triggers" (22, 29). However, the molecular mechanisms underlying the induction-reactivation pathway of HSV remain unknown. This is due primarily to the lack of a model system in which the timing of early reactivation events can be accurately predicted. In vivo reactivation models have been developed for a number of species (2, 7, 11, 12, 14, 15, 21, 26–28, 38). These models have proven valuable for addressing many aspects of HSV pathogenesis; however, the induction procedures most commonly require several days and reactivation occurs during a subsequent 3 to 7 days. In addition, the animals in which induction procedures have been most effective, namely, rabbits (2, 7, 10, 11, 14, 15) and guinea pigs (28), experience significant levels of spontaneous reactivation, which complicates the analysis of early molecular events.

Mice are not subject to significant spontaneous reactivation (12, 20, 26), but in them, virus has been notoriously difficult to reactivate by induction in any reasonable percentage (12, 21, 26). In this report we describe a practical and efficient model of HSV reactivation in outbred Swiss Webster mice. Reactivation from trigeminal and lumbosacral ganglia was induced following hyperthermia, a stress associated with reactivation of HSV in humans. Infectious virus was recovered from a high percentage of animals within 24 h posttreatment. Further, reactivated HSV type 1 (HSV-1) could be detected as early as 14 h postinduction, narrowly defining the temporal link between induction treatment and the initiation of the reactivation process.

It has been well documented that latency can be established in ganglionic neurons (30), but the number, type, and fate of reactivating cells is not known. It is assumed that the earliest reactivation events occur in neurons, and it has been suggested that they may survive reactivation through some unknown mechanism (15, 29). For the first time, HSV antigens were detected during a physiologically relevant reactivation event in vivo. These results offer compelling evidence that the ganglionic neuron is the site of reactivation and infectious virion production. Only 1 to 3 antigen-positive neurons per ganglion were detected, and histologic examination strongly indicated that at least some of these antigen-positive neurons did not survive.

This model is well suited to exploration of viral gene products involved in the establishment, maintenance, and in vivo reactivation of HSV latency. In the accompanying report (24a), we detail our findings utilizing this model to examine the role of the latency-associated transcript transcription unit of HSV-1 in both trigeminal and lumbosacral ganglionic neurons.

MATERIALS AND METHODS

Mice. Male Swiss Webster mice (Charles River), 4 to 5 weeks of age, were used throughout these studies. Animals were housed in American Association for Laboratory Animal Care-approved quarters with unlimited access to food and water.

Virus. HSV-1 17 syn$^+$ was obtained from J. Subak-Sharpe

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of the Medical Research Council Virology Unit in Glasgow, Scotland, and the derivation and history of this strain have been described previously (34). HSV-1 KOS(M) was obtained from M. Levine, Ann Arbor, Mich., and has been described previously (32). Viral stocks were generated by routine propagation in rabbit skin cells (RSC). Infected cells were harvested and sonicated, and the titer of the stock was determined by serial dilution plaque assay on RSC monolayers as previously described (33).

Inoculation. Mice were anesthetized by intraperitoneal injection of pentobarbital sodium (Nembutal [50 mg/kg of body weight]) prior to all inoculation procedures.

(i) Corneal scarification. Five microliters of inoculum containing 2.5×10^4 PFU of 17 syn^+ or 5×10^5 PFU of KOS(M) was applied to the cornea following scarification with a 26-gauge needle.

(ii) Snout abrasion. Twenty microliters of inoculum containing 1×10^5 PFU of 17 syn^+ or 2×10^6 PFU of KOS(M) was applied to a shaved and abraded 5-mm surface area on the side of the snout.

(iii) Footpad. Following saline pretreatment and abrasion of the footpads, 50 μl containing 2.5×10^3 PFU of 17 syn^+ and 5×10^6 PFU of KOS(M) was applied as previously described (32).

Detection of latent infection (cocultivation). Ganglia were removed aseptically and placed individually into wells of a 24-well tissue culture dish containing 0.5 ml of media and incubated at 37°C in 5% CO_2 for 3 days. Ganglia, along with media, were then transferred onto newly confluent monolayers of RSC, incubated at 37°C in 5% CO_2, and observed daily for 30 days for cytopathic effect.

In vivo reactivation of latent infection: elevation of core body temperature. (i) Heat source and thermometers. Animals were heated in a Haake A80 constant-temperature circulating water bath with digital readout which controls temperature to ±0.1°C. Temperature in the water bath was further monitored by using a mercury thermometer. Core body temperatures were monitored prior to, during, and after treatment by using a flexible rectal temperature probe (no. 402; Yellow Springs Instrument Co.) together with a temperature-indicating controller (model 73ATD; Yellow Springs Instrument Co.).

(ii) Procedure. The protocol used was adapted from one developed by Germain et al. to induce neural tube defects in mouse embryos (8). Mice were restrained, and the temperature probe was carefully inserted into the rectum to a depth of 3 cm. A piece of 3-mm latex tubing was slipped over the tail and temperature probe to keep the probe in place. The animal in the restrainer was then slowly lowered into a water bath equilibrated at 43°C. The rate of temperature increase was regulated by raising or lowering the animal in the water. After removal from the water bath, animals were gently blotted with paper toweling and placed in a 34°C dry incubator for 30 min to prevent hypothermia.

Detection of virus reactivated in vivo. (i) Isolation of infectious virus from ganglia. Ganglia were removed aseptically and homogenized in 0.5 ml of media. Homogenates were subsequently frozen and thawed to disrupt the cells and centrifuged briefly to remove cell debris, and the supernatants were layered onto newly confluent RSC monolayers and incubated for 45 min at 37°C in 5% CO_2. Monolayers were then rinsed and, after the addition of fresh media, were incubated at 37°C in 5% CO_2 and observed daily for cytopathic effect. The sensitivity of this assay procedure was estimated by reconstitution experiments in which ganglia were homogenized with 10-fold dilutions of viral stocks of known titers. The amount of input virus was compared with the number of PFU recovered after homogenization and plating on RSC monolayers.

(ii) In situ detection of viral antigens. Anesthetized animals were perfused with 4% paraformaldehyde, and ganglia were removed and postfixed for 1 h. Fixed tissues were dehydrated in a graded series of ethanols, embedded in paraffin, and sectioned at 5 μm. Localization of HSV antigens was performed by using a three-step biotin-avidin peroxidase system as previously described (16). Rabbit anti-HSV (type I) (Accurate Scientific) absorbed with normal mouse liver acetone powder was used at a dilution of 1:1,500. Specificity of this reagent was confirmed by using a series of known HSV-positive and -negative tissue specimens. Biotinylated goat anti-rabbit immunoglobulin G (Vector Laboratories, Burlingame, Calif.) absorbed with normal mouse serum was used at a dilution of 1:150. Details of the immunohistochemical procedure, including the avidin-peroxidase conjugate and substrate reaction, have been described previously (24). Negative controls consisting of serial or step sections incubated with a 1:1,000 dilution of normal rabbit serum in place of the anti-HSV antibody were included in each experiment.

Detection of heat shock proteins. Extract preparation and Western blot (immunoblot) analysis. Extracts of trigeminal and lumbosacral ganglia were prepared by homogenization in phosphate-buffered saline (PBS) containing 0.5% Nonidet P-40. Nuclei and insoluble material were removed by centrifugation, and extracts were boiled in sodium dodecyl sulfate (SDS), loaded onto 7% polyacrylamide gels, and electrophoresed according to the procedure of Laemmli (18). The electrophoresed proteins were transferred to nitrocellulose as described previously (35). The uniformity of transfer was evaluated by staining the nitrocellulose with Ponceau S, which was subsequently removed by washing in PBS. Western blot analysis of the transferred proteins was carried out with a 10-well blotting chamber (Hoefer Scientific) by using a three-step biotin-avidin-peroxidase system (16). The primary antibody C92F3A-5 (SPA-801) (StressGen, Victoria, British Columbia, Canada) was used at a 1:1,000 dilution. The secondary antibody, biotinylated horse anti-mouse immunoglobulin G (Vector Laboratories), was diluted 1:500; the peroxidase conjugate was diluted 1:200; and a 0.25% solution of 4-chloro-1-naphthol containing 0.05% H_2O_2 was used as the chromogenic substrate.

RESULTS

Hyperthermia. Our selection of hyperthermia or heat stress as a possible induction trigger was based on the strong correlation between fever and HSV reactivation in humans (3, 4, 22). Although this association is merely correlative, cellular responses to other known triggers of HSV reactivation, including UV irradiation, trauma, and certain types of hormonal stress, are related to the basic cellular stress response (for a review, see reference 36).

The procedure developed is based on inducing hyperthermia by immersion in warm water as described previously (8). This protocol has the advantage of being brief, reproducible, and well tolerated by the animals. The rate of rise in core body temperature could be controlled by the depth of immersion of the animal. This rate was maintained at approximately 1°C per minute. The core body temperature reached 43°C at 7 min and plateaued at 43°C for just a little more than 3 min. After this procedure, the core body temperature returned to normal within 10 min. It was essen-

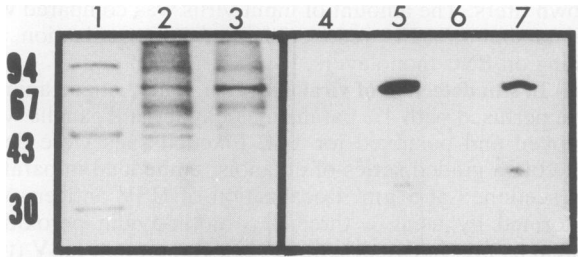

FIG. 1. Detection of HSP70 by Western blot analysis of trigeminal ganglionic homogenates prepared from mice pre- and posthyperthermia. Lanes 1 to 3, gel stained with Coomassie blue. Lanes: 1, Molecular mass standards (sizes, in kilodaltons, are listed at left); 2, extract from NIH 3T3 cells maintained at 37°C; 3, extract from NIH 3T3 cells harvested 12 h following a 30-min heat shock at 44°C. Lanes 4 to 7, Western blot probed with monoclonal antibody C92F3A-5 specific for the inducible form of HSP70. Lanes: 4, same as lane 2; 5, same as lane 3; 6, ganglionic extract from an untreated mouse; 7, ganglionic extract prepared from a mouse 12 h posthyperthermia. Note presence of a 70 kDa band in extracts from heat stressed but not unstressed cells and ganglia.

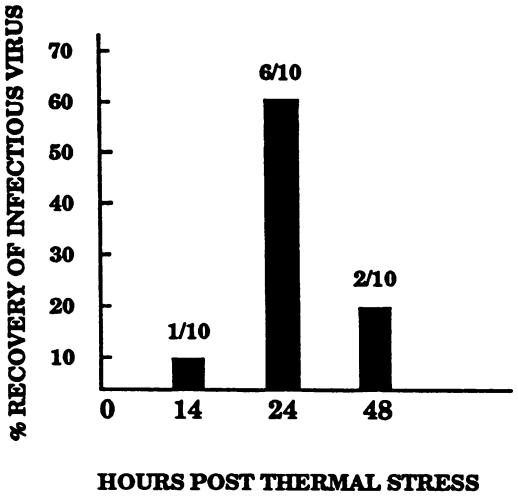

FIG. 2. Temporal profile of reactivation frequency from trigeminal ganglia of latently infected mice (17 syn$^+$) after hyperthermia. Trigeminal ganglia were harvested at 14, 24, and 48 h after treatment and assayed for infectious virus.

tial to dry the animals and place them in a warm (34°C) incubator for about 30 min to prevent hypothermia.

Heat shock protein synthesis. In order to determine whether this procedure induced a stress response in relevant target tissues, heat shock protein 70 (HSP70) synthesis in trigeminal and lumbosacral ganglia was examined. Tissues from uninfected mice were harvested pretreatment and 12 h posttreatment. Ganglionic protein extracts were produced as described above, and 10 μg per lane was electrophoresed by SDS-polyacrylamide gel electrophoresis (PAGE) and examined by Western blot analysis with monoclonal antibody C92F3A-5. This antibody has been characterized extensively and shown to be specific for the inducible form of HSP70 (37). This protein is not expressed at detectable levels under normal growth conditions but is expressed at high levels as part of the stress response (37). NIH 3T3 cell protein extracts from cells maintained at 37°C or heat shocked for 30 min at 44°C and maintained at 37°C for an additional 12 h served as negative and positive controls. As shown in Fig. 1, HSP70 was not detected in trigeminal ganglionic homogenates prior to treatment (lane 6) but was evident as a 70-kDa band at 12 h posthyperthermia (lane 7). Identical results were found with extracts from lumbosacral ganglia (data not shown). The detected protein migrated with an apparent molecular mass identical to that of a band detected in heat shocked but not normal NIH 3T3 cells (lanes 5 and 4, respectively). Therefore, this procedure induced a systemic stress response in agreement with those described in previous reports (for a review, see reference 9).

Evaluation of the protocol for induction of HSV reactivation. The following experiments were designed to determine (i) whether reactivation was induced by the treatment, (ii) at what time after treatment reactivation occurred, and (iii) the percentage of animals in which reactivation was induced. To control for any virus strain effect, two common laboratory HSV-1 strains, KOS(M) and 17 syn$^+$, were employed. The percentage of mice latently infected by the various inoculation procedures employed and the level, if any, of spontaneous reactivation were also assessed.

Preliminary experiments demonstrated that this procedure reactivated HSV in a significant number of animals (data not shown). To delineate the time posthyperthermia of greatest virus recovery, latently infected animals (17 syn$^+$; corneal scarification) were sacrificed at 14 h (n = 10), 24 h (n = 10), and 48 h (n = 10) after treatment and trigeminal ganglia were assayed for infectious virus. Infectious virus was recovered as soon as 14 h posttreatment, with peak percent recovery at 24 h (60%). By 48 h postinduction, the number of animals positive for infectious virus dropped significantly (Fig. 2). The replication cycle of strain 17 syn$^+$ in mouse embryo cells at 38.5°C (normal mouse temperature) is approximately 14 h (33). The fact that reactivated virus could be detected at 14 h postinduction indicates that viral replication must initiate very rapidly after hyperthermia.

The frequency of HSV reactivation in mice inoculated by three different routes was examined. Parallel experiments were performed with strains 17 syn$^+$ and KOS(M) for the purpose of comparing their reactivation phenotypes. Such a comparison was of interest because of the marked difference in the virulence of these two strains following peripheral routes of inoculation (32). KOS(M) effectively establishes latent infections in the ganglia (Table 1) but is completely avirulent. In addition, it has been reported that strain KOS cannot be induced to reactivate in the rabbit eye model

TABLE 1. Assessment of establishment of latency[a] and spontaneous reactivation[b]

Inoculation route	Viral strain	No. of mice with latent infection/no. tested (%)	No. of mice with spontaneous reactivations/no. tested (%)
Cornea	17 syn$^+$	10/10 (100)	0/12 (0)
Cornea	KOS(M)	10/10 (100)	0/10 (0)
Snout	17 syn$^+$	10/10 (100)	0/20 (0)
Snout	KOS(M)	17/18 (94)	0/19 (0)
Footpad	17 syn$^+$	10/10 (100)	0/20 (0)
Footpad	KOS(M)	10/10 (100)	0/40 (0)
Total		67/68 (98.5)	0/121 (0)

[a] Latency was evaluated by explant cocultivation.
[b] The frequency of spontaneous reactivation events in ganglia was evaluated by standard infectious virus assay of homogenates of latently infected ganglia.

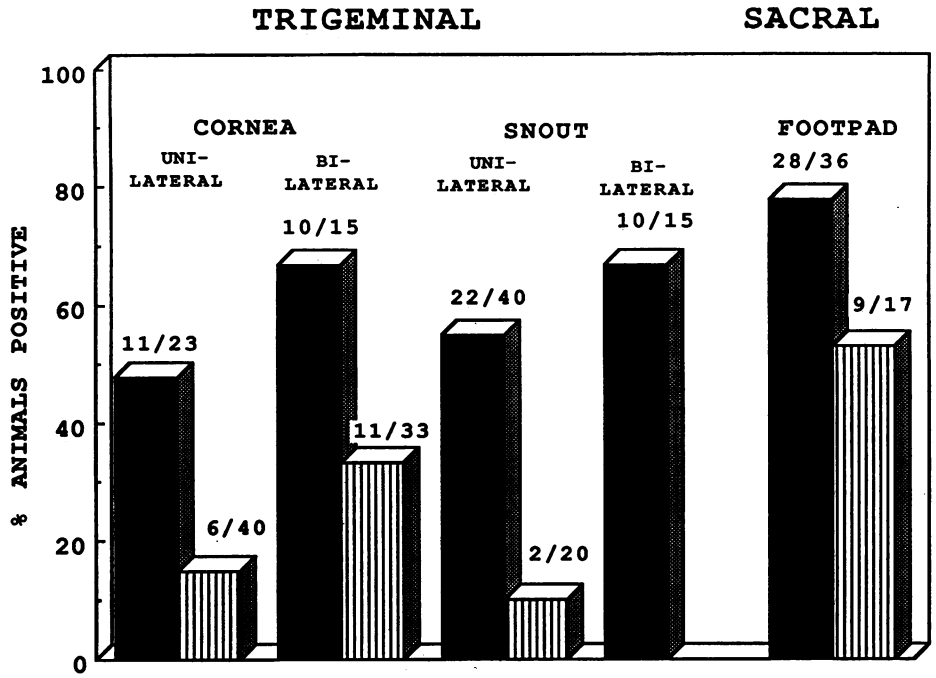

FIG. 3. Reactivation frequencies of 17 syn$^+$ (■) and KOS(M) (▥) following inoculation by various routes. The ganglia targeted by the various inoculation procedures are listed at the top. Bars represent percentages of animals positive for reactivation. Numbers above bars are the numbers of animals positive per the number of tested. Both unilateral and bilateral cornea and snout inoculations were performed. Footpad inoculations were bilateral. SACRAL, lumbosacral.

following ocular iontophoresis of epinephrine (10, 14). Therefore, KOS(M) may contain a defect in a gene or genes required for efficient establishment or reactivation.

Results from these studies are summarized in Fig. 3. It is clear from these data that the reactivation of HSV-1 24 h following hyperthermia is a consistent result and occurs in a significant percentage of animals. A unilateral inoculation with 17 syn$^+$ resulted in the recovery of reactivated virus in over 50% of mice, and virus was reactivated in 70% of the mice which had been inoculated bilaterally. In addition, latent virus reactivated in both trigeminal and lumbosacral ganglia, with similar percent recoveries. KOS(M) also reactivated from both lumbosacral and trigeminal ganglia; however, this strain was detected two- to threefold less frequently than 17 syn$^+$. The difference seen in reactivation frequencies between the viral strains was statistically significant ($P = 0.035$; Fisher's exact test). This was despite the fact that mice were inoculated with 20-fold more KOS(M) than 17 syn$^+$.

It was possible that not all animals were latently infected with strain KOS(M). By utilizing standard cocultivation techniques, reactivating virus was consistently recovered from ganglia of more than 98% of the animals at 30 days postinoculation (Table 1). This was true of all the routes of inoculation, indicating qualitatively the establishment of latent infection in essentially all animals by all inoculation procedures. In addition, spontaneous reactivation events in untreated mice were not detected (Table 1).

To eliminate the possibility that our assay procedure was biased against the detection of reactivated KOS(M) and to approximate the lower limits of our assay, reconstitution experiments were performed. Trigeminal ganglia from uninfected mice were homogenized as 10% suspensions in medium containing serial 10-fold dilutions of either strain 17 syn$^+$ or KOS(M) as described above. The resulting homogenate was plated on RSC monolayers, and a comparison of the input titers versus the amount of virus recovered was made. 17 syn$^+$ and KOS(M) were both detectable at approximately 50 PFU per ganglion. In cultured cells, both 17 syn$^+$ and KOS(M) produce between one and several hundred PFU per infected cell depending on the cell type tested (33). If this is also the case for neurons in vivo, this assay would be sensitive enough to detect the progeny virus from a single reactivating cell. The fact that antigen-positive cells are quite rare in reactivating ganglia (see below) suggests that this is the case.

Whether the difference seen in reactivation frequencies between these strains reflects a difference in the induction of reactivation, the inability of KOS(M) to replicate to detectable levels following reactivation, or the inefficient establishment of latency by KOS(M), was not determined. However, others have reported increased establishment efficiencies for strain KOS in mice (25), suggesting that this strain may indeed contain a defect in a function required for induced reactivation.

Reactivation occurs in neurons. The type of cell in which reactivation occurs in vivo has not been unambiguously identified (15, 29). The close temporal link between induction and reactivation in this model allowed the direct examination of this problem. Immunohistochemical staining demonstrated the restricted localization of viral antigens in large ganglionic neurons 24 h postinduction. Figure 4A shows a single HSV antigen-positive neuron in a section through a latently infected trigeminal ganglion 24 h posttreatment. Staining of this neuron was not present in the corresponding negative control section (data not shown). Cell identification was based on size, morphology, and location within the ganglion. The number of antigen-positive neurons detected

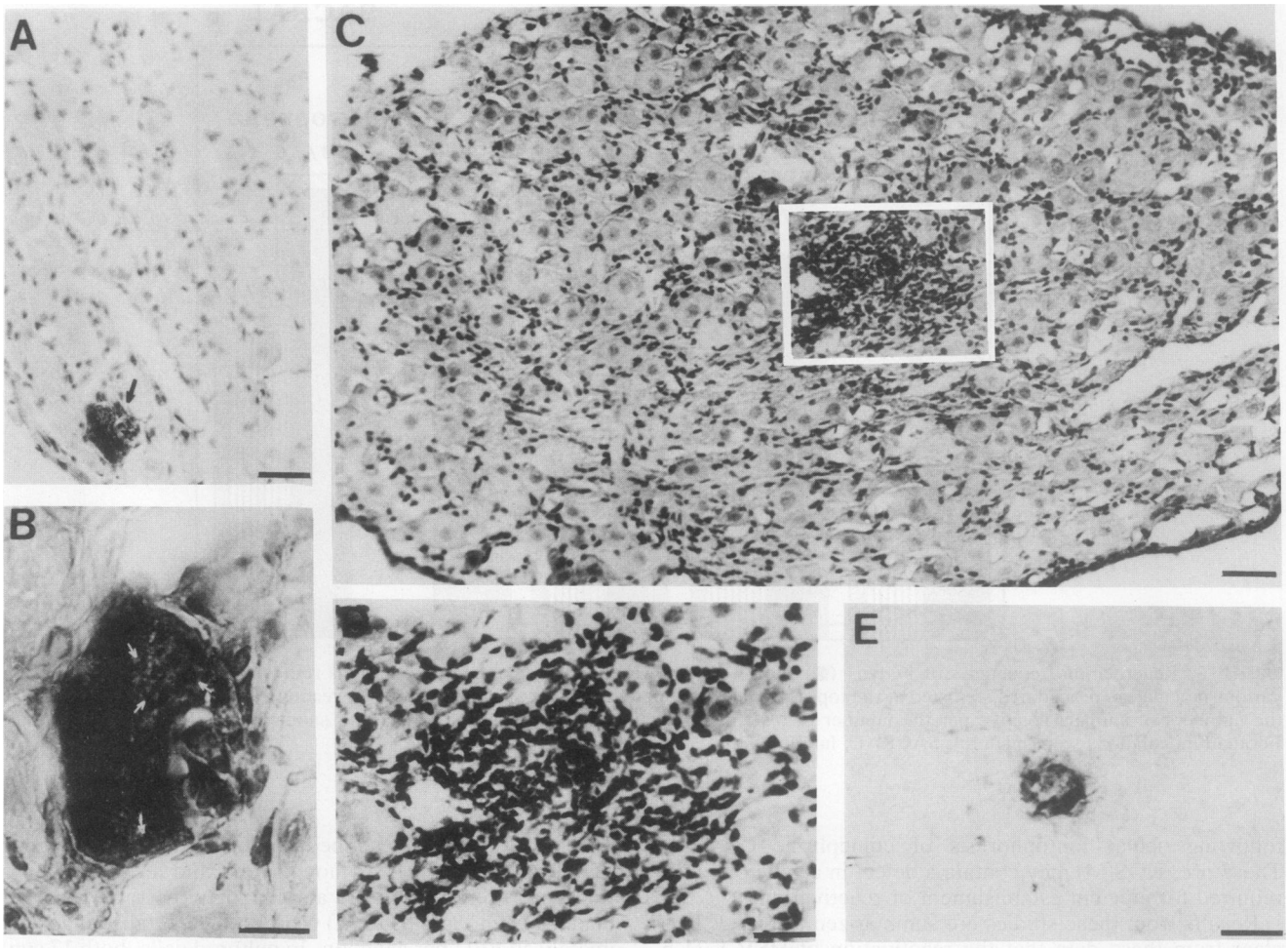

FIG. 4. Immunoperoxidase staining for HSV antigens in trigeminal ganglia from mice 24 h after hyperthermia. (A) Section of a trigeminal ganglion processed for the detection of HSV antigen and lightly counterstained with hematoxylin. A single isolated large antigen-positive neuron is present (arrow). (B) Higher magnification of the neuron shown in panel A. Vacuolar degeneration is evident (arrows). (C) Low-power view of a ganglionic section stained for HSV antigen and counterstained with hematoxylin. A mononuclear cellular infiltrate is seen in the center of the section (boxed). (D) The boxed area of panel C at a higher magnification. The cellular infiltrate is evident as many small darkly stained nuclei. (E) The same field as shown in panel D prior to counterstaining. A single large HSV antigen-positive cell is seen in the middle of the cellular infiltrate. Bars = 30, 10, 50, 30, and 30 μm for panels A, B, C, D, and E, respectively.

was low (1 to 3 neurons positive per reactivating ganglion). HSV antigens were not detected in any other cell type within the ganglion, and lateral spread of the virus was not seen. These data are the first definitive demonstration of active viral protein production in intact ganglionic neurons during reactivation. Coupled with the fact that infectious virus can be isolated at this time, these findings offer compelling evidence that infectious virion production occurred in neurons.

The reactivating neurons displayed degenerative changes in the nucleus and cytoplasm. When viewed at higher magnification (Fig. 4B), many large vacuoles (arrows) can be seen in the cytoplasm of the reactivating neuron shown in Fig. 4A. These degenerative changes are strong evidence that this neuron did not survive.

A mononuclear cell infiltrate localized to regions of reactivating neurons was observed in ganglia at 24 h posttreatment (Fig. 4C, counterstained with hematoxylin, boxed region). The region of inflammatory infiltrate demarcated by the box in panel C is shown at a higher magnification in panel D. The cellular infiltrate is evident as many small darkly stained nuclei. Panel E shows this same region after immunoperoxidase staining for HSV antigen but prior to hematoxylin counterstaining. A single antigen-positive neuron is present in the center of the infiltrate. This rapid and aggressive immune response directed at reactivating neurons is a likely explanation for the transient period in which infectious virus can be isolated.

DISCUSSION

It has been more than 50 years since Burnet postulated that HSV establishes a latent infection and can reactivate to cause recurrent disease. His conclusions were based on extensive serologic studies and anecdotal clinical evidence from the medical community (4). Virologists at that time considered the sensory neuron a likely candidate for harboring latency. Much later it was demonstrated that sensory ganglia did indeed harbor latent HSV (5, 30), and only recently has conclusive proof of latency in ganglionic neu-

rons been obtained (31). Despite the availability of animal models of induced reactivation of HSV, this event has not been previously observed in the intact nervous system, and many basic questions remain unresolved. Definitive evidence of the type, number, and fate of the cells in which reactivation occurs is lacking (29), and nothing is known about the precise molecular events which lead to the establishment of and reactivation from latency (1, 22, 29). Detailed understanding of these aspects of the pathobiology of HSV may lead to new strategies for the treatment and prevention of herpetic disease.

Our results clearly demonstrated HSV-specific antigen in isolated large ganglionic neurons within 24 h after hyperthermia induction. Previously, HSV antigens have been detected in explanted mouse dorsal root ganglia after 3 days in culture (5) and in vivo in neurectomized neurons 3 days postsurgery (19). The length of time between treatment and antigen detection in these studies precluded unambiguous assignment of the neuron as the reactivating cell. We have isolated infectious virus from the trigeminal ganglia of mice within 14 h post induction, the approximate time required for one round of viral replication in culture (33). This result, together with the fact that evidence of viral replication was observed exclusively in ganglionic neurons, makes it very unlikely that the viral antigen detected in ganglionic neurons was transported from some other site of reactivation. Thus, this is the first definitive demonstration that the neuron is the site of HSV reactivation.

The results of time course studies of reactivating ganglia demonstrated that the number of virus-positive ganglia at 48 h posttreatment was only half that at 24 h posttreatment. A mononuclear cell infiltrate was detected in ganglia 24 h postinduction. This infiltrate was localized to sites of reactivating neurons, and light microscopic examination strongly suggested that such neurons were destroyed. An immune response-modulated destruction of reactivating virus and/or neurons is a likely explanation for the decrease in virus-positive ganglia at 48 h.

These kinetics differ from those reported by others in which the peak virus production in trigeminal ganglia following reactivation in vivo (4 of 14 trigeminal ganglia positive of those tested) was found to be at 3 days postinduction. In that study, reactivation was induced by a 2-day administration of cyclophosphamide and dexamethasone followed by UV irradiation of the eye in a mouse model (26). The basis for this difference is not clear, but it is possible that the timing of reactivation varies between the induction protocols. There may be multiple pathways leading to the reactivation of this virus, and the variety of stimuli known to reactivate HSV supports this. Alternatively, the immunosuppressive drugs administered may have prolonged the period of active replication in the ganglia, although the lower percentage of ganglia positive for virus at earlier times argues against this possibility. It is possible that the virus obtained from ganglia at the latter points was a result of replication at a distal site and reinfection of the ganglia in this immunosuppressed model. With regard to this latter point, it is of interest that isolation of infectious virus from the eye peaked prior to isolation from the ganglia (26).

Only 1 to 3 antigen-positive neurons per ganglion were detected 24 h after stress. This is despite the facts that a systemic induction procedure was employed and that presumably all latently infected neurons in the animal received similar stress. There would be a strong selective pressure to restrict the number of reactivating neurons in any particular reactivation event in order to maintain a pool of latently infected neurons through time and to minimize the chance of spread to the central nervous system, with resulting fatal disease. Although there is currently no understanding of how such restricted reactivation is achieved, there are several possibilities. (i) The stoichiometry of the changes induced by stress in the neuron may be biased in favor of maintenance of latency. (ii) The physiological state of latently infected neurons at the time of the induction event may be important, resulting in just a few neurons being susceptible at any given time. (iii) Only a subpopulation of neurons may be capable of reactivating in response to this stress. (iv) There may be viral or host factors which counteract the induction event. Thus, many neurons may initiate reactivation in response to this stress, but only a minority proceed to virus production. (v) It is possible that other neurons in the ganglia produce small amounts of HSV during reactivation and that the antigen loads in such neurons are below the threshold of detection. Ongoing studies examining the early events in reactivation should provide some insight into this last possibility.

Fever is a well-recognized trigger of HSV reactivation in humans, thus the term "fever blister" (6). The relationship between fever and hyperthermia is not clear (17), but it has long been recognized that artificial pyrexia is a very efficient inducer of HSV reactivation in humans (3). Although we have clearly demonstrated that hyperthermia induces HSV reactivation, the physiological basis for this is currently not known. It has been well documented that essentially all eukaryotic cells respond to exposure to supraphysiologic temperatures in similar ways (17, 36). This heat shock response has been examined at length and includes a complex regulatory cascade resulting in the rapid accumulation of a specific set of proteins and a decrease in others. This extremely rapid alteration in the cellular protein profile is the result of regulatory events at many levels, including transcription (17, 36). Indeed, the *cis* elements involved in this transcriptional regulatory system comprise the most highly conserved genetic system known.

We have shown that the hyperthermia treatment induces heat shock proteins in trigeminal and lumbosacral ganglia. However, demonstrating that the heat shock response is induced does not indicate a causal connection to reactivation. Indeed, HSV infection of cells cultured at elevated temperatures has been found to repress viral replication, and this finding is the basis for several in vitro models of latency (13, 23). Certainly the cascade of events occurring at the organismal level which result in HSV reactivation are bound to be complex. The hyperthermia reactivation model is a system with which to address the molecular mechanisms underlying reactivation and to identify viral genes which contribute to the phenotypic variation in this function observed among different viral strains.

ACKNOWLEDGMENTS

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