Neuropathology of Herpes Simplex Virus Encephalitis in a Rat Seizure Model

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Abstract. Herpes simplex virus type 1 (HSV-1) is the cause of a serious and often fatal encephalitis. Patients who survive herpes simplex encephalitis (HSE) experience behavioral abnormalities including profound cognitive dysfunctions. We have developed a rat model of acute HSE to investigate the cognitive impairments caused by HSV-1 central nervous system (CNS) infection. Following intranasal inoculation of Lewis rats with a neurovirulent strain of HSV-1, animals shed virus in both ocular and nasal secretions and developed clinical signs of infection, including partial complex motor seizures that eventually generalized. Homogenization assays demonstrated infectious virus in the trigeminal ganglia, olfactory bulbs, and the piriform and entorhinal cortices. Histopathological assessment revealed inflammatory and hemorrhagic lesions in the trigeminal ganglia, olfactory bulbs, amygdala, hippocampus, the piriform and entorhinal cortices, and the spinal trigeminal nuclei. Viral antigens and nucleic acids were also detected within these structures by immunofluorescence microscopy and in situ hybridization, respectively. Viral-induced astrocytic hypertrophy in the CNS was demonstrated by glial fibrillary acidic protein immunoreactivity. Together, these results indicate that HSV-1 has the ability to invade, replicate, and induce site-specific CNS damage in the Lewis rat.

Key Words: Encephalitis; Herpes simplex virus; Histopathology; HSV-1; Neuroanatomy; Rat; Seizures.

INTRODUCTION

Several families of viruses can cross the blood-brain barrier and replicate within the central nervous system (CNS) (1–3). Other viruses can replicate at the periphery and are transported to the CNS via nervous tissues innervating those sites (1–3). Neuroinvasive viruses may be further categorized by their ability to infect different classes of cells within the CNS or particular subpopulations of neurons (4). The selective differential specificity and sensitivity of neuronal populations to infection by neurotropic viruses is theorized to relate to the route of virus entry into the CNS and/or to the presence or absence of specific receptors (2, 5–7). Although the physiologic is largely unknown, certain populations of neurons are more permissive to viral replication (8, 9). This permissiveness may in part be due to specific signals contained within the viral genome and in part due to host machinery within the infected cell (8, 9).

Progression of CNS viral infection has significant deleterious consequences including viral or immune lysis of the infected cell, and intracellular or cytotoxic effects from specific viral or host factors (2, 3). The pathological changes that result may produce abnormalities of behavior, gross histological damage, and death of the animal. Interestingly, the deleterious effects of infection may be limited to the disruption of normal cellular chemistry without the destruction of the cell (3, 10–12).

Herpes simplex virus type 1 (HSV-1) is a prototype neurotropic virus. HSV-1 infection is the major cause of sporadic fatal encephalitis among adults in the United States (13, 14). Each year herpes simplex encephalitis (HSE) accounts for up to 2,000 cases of viral infections involving the CNS (14, 15) and, until recently, culminated with death in approximately 70% of afflicted patients (16). Herpes simplex encephalitis begins with the acute onset of fever, headache and altered consciousness with focal neurological signs (17). It may progress to produce anosmia, aphasia, hemiparesis and, in 38% of patients, seizures (18). Most of the signs and symptoms of the disease are indicative of temporal and frontal lobe involvement (18, 19).

Pathologically, HSE is most notably characterized by hemorrhagic or necrotizing lesions of the posterior orbital cortex, and the inferior and middle temporal gyri. Lesions are most commonly found in the amygdala, hippocampus, and related limbic structures, and may extend through the superior temporal gyrus to become continuous with the insular cortex (20–22). The nature and extent of HSV-1 infections is likely to be the result of multiple factors influenced by both virus and host.

A distinctive property of HSV-1 is its ability to become latent with limited gene expression within the nervous system of the host (23, 24). A variety of external or systemic stimuli may reactivate HSV-1 from latency by
mechanisms that remain to be elucidated (25, 26). When reactivated from trigeminal ganglionic neurons, the virus is transported back to the primary site of infection and causes characteristic recurrent orofacial or ocular lesions (25). In experimental systems, HSV reactivation within the CNS has been shown to produce focal necrotizing encephalitis of the inferior temporal lobes (27).

Although several attempts have been made to produce an animal model of HSE (27–33), an adequate reflection of both the acute and chronic characteristics of the disease has not been developed. We chose to use the +GC strain of HSV-1 because it has been shown in other systems to be capable of infecting temporal lobe structures (32). Based on these models (27, 29–33), it was likely that +GC would infect the temporal lobes and related limbic structures following intranasal inoculation of the rat. However, because neither the strain of virus nor the route of infection has been studied in the Lewis rat, it was important to determine whether animals intranasally inoculated with HSV-1 developed CNS pathology reminiscent of that reported in human HSE (17, 20–22). Therefore, in order to later assess whether behavioral dysfunctions observed in human HSE (34–41) could be reproduced in an animal model, we developed the Lewis rat model of the acute and latent disease. Rats were chosen because the neuroanatomy has been described in detail and numerous studies have been performed assessing experimentally induced behavioral impairments. These factors will be invaluable in future studies designed to correlate HSV-1 lesion location and severity with memory impairments. This report describes the development of a rat model of HSE that reflects acute and latent features observed in the human disease.

MATERIALS AND METHODS

Cells and Media

African green monkey kidney (VERO) cells and baby hamster kidney-21 (BHK) cells were grown in Eagle’s minimal essential medium (MEM) supplemented with 5% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 25 mM glutamine.

Infection of Rats

The HSV-1 strain +GC was used to intranasally infect rats. +GC (42) is a strain of the Miyaya HSV-1 strain originally isolated from a case of herpes labialis in 1958 (32, 43, 44), causes syncytial formation in cell culture and is neurovirulent for mice and rabbits (32, 42, 44). +GC was obtained from Dr. Robert R. McKendall (Galveston, TX) and was propagated by low multiplicity passage on confluent monolayers of BHK cells to a final titer of 7 × 10^6 tissue culture infectious doses (TCID₅₀) per ml (45).

Twenty-six (18 in group I and 8 in group II) individually housed female Lewis rats (Harlan Sprague Dawley Inc., Indianapolis, IN) weighing approximately 225 grams were anesthetized by an intraperitoneal (i.p.) injection of 35 mg/kg of sodium pentobarbital (Sodium Nembutal®, 50 mg/ml, Abbott Laboratories, North Chicago, IL), and intranasally inoculated with 1.4 × 10^6 TCID₅₀ of +GC. Preliminary studies showed that this dose produced low mortality. Three uninfected rates were used as controls. All studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Determination of Peripheral Viral Shedding

Viral shedding in ocular and nasal secretions was monitored in 18 animals (group I) between 0 and 14 days postinfection (dpi) as described (32). To insure adequate detection of intranasal viral shedding, the nasal passages of each animal were lavaged with culture media prior to sacrifice, and the fluid was assayed on VERO cells. All negative cultures were frozen at −70°C, thawed, and repassed on fresh VERO cells to confirm the absence of infectious HSV-1.

Preparation of Tissue

Three animals from group I were sacrificed at each of 3, 5, 7, 14 and 30 dpi, and in a separate study, four animals were sacrificed from group II at each of 76 dpi and 160 dpi by a lethal i.p. injection of sodium pentobarbital. Subsequent to thoracotomy and intracardiac perfusion with either phosphate buffered saline (PBS) or paraformaldehyde-lysine-paraformate (PLP) solutions, the trigeminal ganglia, eyes, and brains, including the olfactory bulbs, cerebellum and brain stem, were carefully removed and processed for viral isolation, routine histopathology, immunofluorescence, and in situ hybridization.

Isolation of +GC from Tissues

Following perfusion with PBS, the brain of one animal at each time point postinfection from group I was removed and the cerebrum was cut into three coronal pieces. The right trigeminal ganglion, cerebellum plus brain stem, and each of the three coronal pieces were separately homogenized in MEM (10% [v/v]) supplemented with 2% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and immediately frozen at −70°C. The suspensions were then thawed, clarified by centrifugation at 400 × g, and the supernatants titered for the presence of HSV-1 infectivity by TCID₅₀ assays using VERO cells. Titration cultures were observed for the presence of viral cytopathic effect for up to 7 days. All negative cultures were frozen at −70°C, thawed, and repassed to confirm the absence of infectious HSV-1.

Histology

Two animals at each time point in group I and four animals at both time points in group II were perfused with PLP solution. Tissues were further immersion fixed in PLP for 24 hours at 4°C, cut into 5 mm blocks and paraffin embedded. Each block was cut into six 6 μm sections and stained with Luxol fast blue (LFB) for myelin, cresyl violet (CV), or with Gill’s hematoxilin and eosin (H & E) for routine pathological examination.

Immunofluorescence for Viral Antigen

Six 6 μm sections taken from four levels separated by 100–150 μm were deparaffinized, rehydrated through graded solutions of ethanol, digested with trypsin to enhance immunofluorescence (32), and processed for the detection of HSV-1 antigens using rabbit anti-HSV-1 hyperimmune serum as the
primary antiserum (also obtained from Dr. Robert R. Mackay, Galveston, TX) and goat anti-rabbit serum conjugated to fluorescein isothiocyanate (FITC) (Cappel Laboratories, Inc., Cochranville, PA) as the secondary serum, as previously described (32). Controls from both uninfected and infected animals, as well as uninfected and infected VERO cells, were incubated with: (i) PBS instead of primary antiserum; (ii) normal rabbit serum as the primary serum; and (iii) rat anti-HSV-1 as a primary blocking serum, followed by rabbit anti-HSV-1 serum. Finally, all sections were incubated with FITC-anti-rabbit serum.

**Immunofluorescence for Astrocytic Glial Fibrillary Acidic Protein**

Six 6 μm sections were cut from each block and processed for the detection of glial fibrillary acidic protein (GFAP) as described above, but without protease treatment. Lyophilized monoclonal GFAP antibody (Boehringer Mannheim Biochemicals, Indianapolis, IN) was rehydrated and diluted with distilled water according to the manufacturer. Nonspecific binding was blocked by overlaying sections with PBS for 30 minutes prior to incubation with anti-GFAP antibody (32). Secondary antibody was sheep anti-mouse hyperimmune serum IgG conjugated to FITC (Cappel Laboratories, Inc.). Controls from both uninfected and infected animals were incubated with only secondary FITC serum without the primary antiserum and with normal mouse serum as the primary serum.

**In Situ Hybridization**

Six 6 μm sections from tissue blocks of animals sacrificed at 7, 14, and 160 dpi were placed onto treated slides and pretreated for the detection of HSV-1 DNA plus RNA (29). The probes for in situ hybridization consisted of: (i) whole genomic HSV-1 strain F DNA which was purified as described (29), digested to completion with EcoRI endonuclease (Promega, Madison, WI), and assessed for purity by agarose electrophoresis (46); and (ii) the EcoRI E/K fragment from the KOS strain of HSV-1 (pSG28 obtained from Dr. Myron Levine, Ann Arbor, MI) which was digested with EcoRI, electrophoresed, cut from the agarose gel and purified with a GeneClean II® kit (Bio 101, La Jolla, CA). Both the F strain viral DNA and KOS E/K fragment were radiolabeled with 3H-deCTP and 3H-deUTP to a specific activity of 10^6 dpm/ng using either the nick translation (29) or the random primer method (Boehringer Mannheim Biochemicals). Hybridization was performed essentially as described (29), except that 0.5-1.0 mg/ml of rat kidney fibroblast nucleic acids were included in the prehybridization step. Following hybridization, the slides were extensively washed, dehydrated, dipped in nuclear track emulsion (Kodak NTB-2), exposed at 4°C for 3 weeks, developed, and stained with H & E. Controls used for in situ hybridization included uninfected rat CNS tissue, infected and uninfected VERO cells, and rabbit CNS tissue previously shown to be positive for HSV-1 nucleic acids (47).

**RESULTS**

**Clinical Course**

Initially, 18 female Lewis rats (group I, Fig. 1), and in a second study, another eight female Lewis rats (group II) were intranasally inoculated with 1.4 × 10^6 TCID_{50} of the +GC strain of HSV-1. Three uninfected rats were used as negative controls. All animals appeared completely healthy through 3 dpi. At 4 to 5 dpi, all HSV-1 infected animals displayed signs of illness which included ruffled fur, hunched posture, loss of appetite, hypoexcitability, and dried oral and nasal secretions on their fur. A total of three animals from group I were found dead on 6 and 7 dpi (Fig. 1). Nine of the remaining 23 rats (39%; 6 from group I, Fig. 1, and 3 from group II) developed partial complex seizures that eventually generalized between 7 and 10 dpi. The seizures occurred at about 30 minute intervals and lasted for 30 to 60 seconds.

**Virology**

Viral infection at the periphery was monitored in group I by assaying ocular and nasal secretions obtained from each animal for 14 dpi. Thirteen of 18 (72%) and five of 18 (28%) rats were positive for active viral production from their noses and eyes, respectively (Fig. 1). Although the difference between ocular and nasal shedding was statistically significant (p < 0.01 by chi^2), the failure to
**TABLE 1**  
Isolation of Herpes Simplex Virus from Peripheral and Central Nervous System Tissues following Intranasal Inoculation in the Lewis Rat

<table>
<thead>
<tr>
<th>DPI</th>
<th>Olfactory bulb</th>
<th>Entorhinal cortex*</th>
<th>Trigeminal ganglion</th>
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<tbody>
<tr>
<td>3</td>
<td>BDL</td>
<td>BDL</td>
<td>$1.9 \times 10^3$</td>
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<tr>
<td>5</td>
<td>$4.6 \times 10^3$</td>
<td>$1.2 \times 10^3$</td>
<td>$1.8 \times 10^3$</td>
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<tr>
<td>7</td>
<td>$7.3 \times 10^3$</td>
<td>$1.2 \times 10^3$</td>
<td>BDL</td>
</tr>
<tr>
<td>14</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
</tbody>
</table>

DPI = days postinfection.  
* = entorhinal cortex included neocortical and hippocampal tissue.  
BDL = below detectable limits. The lower limit of the assay was $\leq 10^3$ TCID/gram.  
Titer were calculated from homogenates of four rats.

Isolate infectious virus from nasal secretions was most likely due to the technical difficulty in adequately swabbing the interior of the nasal passage.

Virus was easily isolated from homogenates of both peripheral and CNS tissues. Table 1 demonstrates that +GC was first detected in the trigeminal ganglion at 3 dpi at a titer of $1.9 \times 10^3$ TCID/gram of tissue. At 5 dpi, the virus was recovered from the olfactory bulbs and rostral and caudal brain portions with titers between $1.2 \times 10^3$ and $1.8 \times 10^3$ TCID/gram. By 7 dpi, infectious virus was below detectable limits in the ganglia but was recovered from both rostral and caudal brain regions. We were unable to recover virus from any portion of the nervous system at 14 or 30 dpi.

**Histopathology**

In order to examine the pathological changes that occurred in HSV-1-infected rats, tissues were processed for histological inspection. Tissue from the three animals found dead in group 1 were not processed for histological examination (Fig. 1). The brains and trigeminal ganglia of two animals examined at 3 dpi were normal, except for mild inflammation in the olfactory bulb and piriform cortex of one animal. Tissues examined at 5 dpi revealed mild leptomenigitis in the olfactory bulb, and piriform and entorhinal cortices in two of two animals. +GC produced severe leptomenigitis of the olfactory bulbs, olfactory tract, and piriform and entorhinal cortices in two animals sacrificed at 7 dpi (Fig. 2). Bilateral inflammatory infiltrates and hemorrhagic lesions in the piriform and entorhinal cortices (Fig. 2) and dentate gyrus of the hippocampus (Fig. 3) were also observed in these animals (Table 2). Intranuclear viral inclusion bodies were noted within cellular nuclei of the entorhinal cortex at 7 dpi. Sparse trigeminal system inflammation was observed in four animals, two at each of 5 and 7 dpi (Table 2). At 5 dpi, two animals had inflammation in the trigeminal ganglia and inflammation was observed in one of the two animals in the mesencephalic nucleus (Fig. 2). By 7 dpi, two of two animals had inflammation in the trigeminal ganglia, mesencephalic nuclei, and the spinal nuclei of the trigeminal system. In three of the nine animals that developed seizures, the CA3 region of the hippocampus contained hypoxic neurons (Fig. 3). By 14 dpi, the inflammatory response was unremarkable in all structures examined, except for mild inflammation in the entorhinal cortex and spinal trigeminal tract of two animals. By 30 dpi and later at both 76 and 160 dpi, we were unable to demonstrate any pathological indication of an antecedent viral infection. These data suggest that although the +GC strain of HSV-1 is capable of invading the CNS of the rat and inducing significant pathological damage, the histologic response apparently resolves between 14 and 30 dpi.

Because HSV-1 can cause corneal blindness (48), the eyes of each animal were examined for histological evidence of +GC-induced pathology. The eyes from the three animals in group 1 that were found dead on 6 and 7 dpi were not examined. Histological examination of the eyes from the remaining 23 infected animals (15 animals from group I and 8 animals from group II) were normal (data not shown).

**Detection of Viral Antigens**

Tissues assayed by immunofluorescent microscopy at 3 dpi were negative. However, +GC antigens were found within the CNS of four rats, two at each of 5 and 7 dpi (Fig. 4). Overall, antigens at 5 and 7 dpi were sparse in whole coronal sections, and low power microscopy was insufficient to photographically document their distribution. However, denser accumulations of viral antigens within adjacent cells in the olfactory bulb, amygdala, piriform and entorhinal cortices, cingulate cortex, and at the

Fig. 2. Representative peripheral and central nervous system histopathology in Lewis rats following intranasal infection with HSV-1, strain +GC. A. +GC-induced mild inflammation in the trigeminal ganglion 5 dpi ($\times 480$). B. Severe inflammation of the olfactory bulb 7 dpi ($\times 225$). C. Hemorrhagic lesion located in the piriform cortex 7 dpi ($\times 130$). Extravasation of red blood cells can be seen (arrowheads). D. Large hemorrhagic lesion in the entorhinal cortex (center) 7 dpi. Mild leptomenigitis can also be seen (arrowhead) ($\times 125$). E. Mild inflammation of the spinal trigeminal tract 14 dpi. Plasma cell can be seen (arrowhead) ($\times 350$). Stained with H & E.
TABLE 2
Inflammation in the Nervous System of Lewis Rats Intranasally Inoculated with HSV-1, Strain +GC*

<table>
<thead>
<tr>
<th>DPI</th>
<th>Olfactory system</th>
<th>Trigeminal system</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Olfac. bulb</td>
<td>Piriform cortex</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
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<tr>
<td>3.5</td>
<td>1+</td>
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<td>14</td>
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<td>14.5</td>
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<tr>
<td>30</td>
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*Inflammatory lesions were graded as follows: 1+ = Mild inflammation; 2+ = more severe inflammation, with mild perivascular cuffing; 3+ = more severe inflammation with mild necrosis; 4+ = severe inflammation with necrosis; - = no inflammation.

DPI = days postinfection.

CA1-CA2 boundary of the hippocampus were detected at higher magnification. Although only mild inflammation was observed in the trigeminal ganglia at 5 and 7 dpi, +GC antigens were detected within ganglionic neurons on both dpi. At 14 and 30 dpi, and later at 76 and 160 dpi, viral antigens were not detected in any nervous system structure. All uninfected control tissue was negative.

Detection of Astrocytic Gliarial Fibrillary Acidic Protein

Viral-induced astrocytic changes within the CNS of +GC intranasally inoculated rats were detected by immunofluorescence using a monoclonal antibody specific for GFAP in normal as well as reactive astrocytes and Bergmann glia of the cerebellum (49, 50). Reactive astrocytes, characterized by hypertrophy and hyperplasia (50), were observed in the amygdala, hippocampus, piriform and entorhinal cortices, and cingulate cortex in six rats, two at each of 5, 7 and 14 dpi (Fig. 5). Animals sacrificed at 3 and 30 dpi were not examined. Hypertrophic astrocytes were not detected at 76 and 160 dpi. The GFAP antibody stained uninfected tissue; however, there was no evidence of astrocytic hypertrophy or hyperplasia.

In Situ Hybridization

In situ hybridization was used to localize HSV-1 DNA and RNA in nervous system tissues of infected rats sacrificed during acute and latent infections. +GC nucleic acids were detected during the acute phase (7 dpi) in neurons and occasional inflammatory cells in the parenchyma. The majority of these cells were located in the olfactory bulb, piriform and entorhinal cortices, and in the dentate gyrus and the CA1-CA2 region of the hippocampus (Fig. 6). However, positive hybridization was also detected in the amygdala and the medial frontal cortex at 7 dpi (Table 3). +GC nucleic acids were also detected within trigeminal ganglionic neurons at 7 dpi.

Because histological evidence of inflammation, gliotic scarring, or necrosis in the CNS or ganglia was not de-

Fig. 3. Histopathology of the hippocampus of +GC-infected Lewis rats. A. Hemorrhagic lesion in the dentate gyrus 7 dpi. Extravasation of red blood cells is shown (arrowhead) (x300). B. CA3 region of the hippocampus from a +GC-infected rat showing darkly staining hypoxic neurons 7 dpi (x225). Stained with H & E.

detected in four animals examined at each of 76 and 160 dpi, *in situ* hybridization was used to determine whether +GC established ganglionic or CNS latency. Tissue was probed at 30, 76, and 160 dpi to insure that any viral nucleic acids would most likely represent latent rather than acute infections. Positive hybridization was detected in the trigeminal ganglia at 30 dpi. +GC nucleic acids were found in the hippocampus and the piriform and entorhinal cortices in four rats at 76 dpi and four rats at 160 dpi (Fig. 6). All +GC nucleic acids detected were in cellular nuclei, which is characteristic of HSV-1 latency (47, 52). Using the *Eco*RI E/K fragment as a probe specific for the HSV-1 latency associated transcripts (LAT), viral nucleic acids were detected in the same CNS structures and also within the olfactory bulb and spinal trigeminal nucleus (Fig. 6). It was difficult to locate latently infected cells by *in situ* hybridization because they were rare. Collectively, the virologic, histopathologic and *in situ* hybridization data indicate that +GC is neuroinvasive and neurotropic, and can establish ganglionic and CNS latency in the rat.

**DISCUSSION**

We undertook these studies to develop an animal model of encephalitis that would mimic the pathologic features of human HSE, with the primary goal that such a model would allow us to later correlate histopathologic damage with behavioral abnormalities. A rat model of HSE is highly desirable because it allows the unique opportunity to simultaneously address HSV-1 pathogenesis.
and behavioral dysfunction in the same animal. We chose the intranasal route of infection because the olfactory pathway offers a direct, non-invasive access for virus entry into the brain and because it is thought to be an important route of infection in human HSE (1, 51, 53). Further, necrotizing lesions of CNS structures associated with the olfactory pathway are characteristically damaged during HSE (13, 17, 20–22). Although lethal in mice and rabbits, previous studies have shown that the +GC strain of HSV-1 infects temporal lobe structures, produces hypoxic changes within the hippocampus, and induces seizures in rabbits (32). Therefore, we chose the +GC strain of HSV-1 to maximize our chances of producing the pathologic features of human HSE. We chose to use rats for these studies because they are among the most suitable animals for behavioral studies and because the neuroanatomical connections in the rat CNS have been thoroughly described. We believe this is the first report describing both the clinical and pathologic features of rat HSE in which seizures are a prominent feature.

Our studies reveal that the rat model is a reasonable reflection of human HSE. The histopathologic evidence demonstrated in intranasally inoculated rats parallel those described in patients that succumb to HSV-1 infection. Specifically, we observed bilateral inflammatory infiltrates and hemorrhagic lesions in the hippocampus and piriform and entorhinal cortices which correspond to the posterior orbital and inferior temporal cortices of the human. Indicative of herpes simplex infection, we observed intranuclear viral inclusion bodies within the nuclei of cells of the entorhinal cortex at 7 dpi. Consistent with the clinical observations seen in human HSV-1 infections (11), 9/23 (39%) animals developed motor seizures. Three of the nine animals that developed seizures contained hypoxic neurons in the hippocampus. The remaining six animals that developed seizures were sacrificed at 30, 76 and 160 dpi, making the detection of hypoxia difficult.

There have been four studies of +GC CNS infection in animals (41–43, 51). +GC causes seizures in 10/17 (59%) intranasally inoculated rabbits (41) and produces death in a large number (80–100%) of animals (Stroop, unpublished data). In contrast to intranasal infection of rabbits (41), only 3/26 (12%) rats died following intranasal inoculation. Footpad inoculation with +GC in mice is non-lethal below 10⁵ plaque forming units (PFU) and only produces mortality in 3/8 (38%) of mice when inoculated with 10⁷ PFU (42). However, following intracerebral inoculation, +GC produces mortality in 32/32

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**Fig. 5.** +GC-induced astrocytic changes within the central nervous system at 7 dpi by immunofluorescent antibody staining of GFAP. A. Hippocampus (×350). B. Amygdala (×350). C. Entorhinal cortex (×350).
(100%) of mice with titers ranging as low as 10 and up to 10^3 PFU, the highest titer tested (42). Seizure activity has not been reported in mice.

In contrast to human HSE, we observed inflammation of the olfactory bulbs. Although not reported in humans, a mild inflammatory response was detected within the trigeminal system which is consistent with +GC infection in intranasally inoculated rabbits. Additionally, another distinction between rat and human HSE is that Lewis rats appear to resolve HSE without readily apparent microscopic evidence of an antecedent infection.

The virologic and immunofluorescent data indicate that +GC is capable of invading and replicating within olfactory structures of the adult rat CNS. This is in contrast to many murine models of HSV infection, which more closely resemble the panencephalitis seen in neonatally acquired HSE. The results of the homogenization assays suggest that +GC was transported from the nasal mucosa to the piriform and entorhinal cortices via the olfactory pathway. +GC also infects the trigeminal system during the acute phase as evidenced by the isolation of infectious virus at 3 and 5 dpi, detection of viral antigens in the ganglia at 5 and 7 dpi, and the presence of inflammatory lesions in the mesencephalic nuclei at 7 dpi. The immunofluorescence data demonstrate that these structures were also capable of sustained at least one round of viral replication. Additionally, anti-GFAP immunofluorescence studies showed that +GC induced astrocytic hypertrophy within these same sites. Astrocytic changes may be observed in response to almost every type of injury or disease in the CNS (50). However, the immunofluorescence data conclusively show that these astrocytes responded to injury and were not themselves infected with +GC, in that they were hypertrophic, but viral antigen-negative.

The in situ hybridization experiments substantiate the results of the homogenization and immunofluorescence studies, and document the transition between the acute and latent phases of the infection. During the acute phase of the nervous system infection, which ranged from 3 through 7 dpi, both viral antigens and nucleic acids were detected. At 14 dpi, viral antigens were no longer detectable, indicating that the virus was no longer actively replicating and had likely established latency. +GC nucleic acids were detected in trigeminal ganglia and trigeminal brain stem nuclei at 30 dpi. At 76 and 160 dpi, the sites of viral latency within the CNS of the rat, as demonstrated by in situ hybridization using the latency specific E/K fragment as the probe, include the trigeminal ganglia, hippocampus, and the piriform and entorhinal cortices. It should be noted that this is the first report documenting the sites of +GC CNS latency in vivo. Other animal studies have also shown that other strains of HSV-1 are capable of invading the CNS during the acute phase of infection (30, 32), but only a few have demonstrated

### Table 3

Presence and Location of HSV-1 Nucleic Acids within the Rat Central Nervous System following Intranasal Inoculation

<table>
<thead>
<tr>
<th>Olfactory system</th>
<th>Trigeminal system</th>
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<tbody>
<tr>
<td>DPI</td>
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<td>7</td>
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<tr>
<td>7***</td>
<td>4+</td>
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<tr>
<td>30</td>
<td>1+</td>
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<td>76</td>
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<td>160</td>
<td>1+</td>
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<tr>
<td>160§</td>
<td>1+</td>
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</table>

* Positive nucleic acid hybridization signal was graded as follows: 1+ = one to two cells found positive per tissue section; 2+ = three to five cells found positive per tissue section; 3+ = five to ten cells found positive per tissue section; 4+ = more than ten cells found positive per tissue section.

DPI = days postinfection.

** = entorhinal cortex included neocortical and hippocampal tissue.

*** = positive nucleic acid hybridization was found in the medial frontal cortex.

† = positive nucleic acid hybridization was found in the amygdala.

§ = positive nucleic acid hybridization was found using the EcoRI E/K fragment specific for HSV-1 latency.

ND = hybridization was not performed on this tissue.

The neuroanatomical location of HSV-1 latency within specific structures of the CNS (29, 47).

Clinical and histopathologic examination suggest the Lewis rat model of HSE is suitable for study of the disease process as it presents in adult humans, with the exception that the disease in rats apparently resolves without histopathologic evidence of antecedent infection. It is unclear why rats resolve the acute infection without gial scarring or residual inflammation, which are often associated with human HSE. However, differences between the immune response of the rat and the human may provide a partial explanation for this finding. Weinstein et al (54) have shown that major histocompatibility complex (MHC) class I and class II expression is only detected on microglia, endothelial cells, and some leukocytes following HSV-1 infection of the rat CNS. In that study, MHC glycoproteins were not detected on neurons, astrocytes, or oligodendroglia, even under the circumstances of severe viral infection, and disappeared by 30 days postinfection. Although we found a large increase in GFAP-positive astrocytes during the acute infection, we do not believe that these cells were involved in virus antigen
processing, since they were viral antigen-negative, and because Weinstein et al. (54) have reported that GFAP-positive astrocytes fail to express MHC antigens under circumstances that cause vigorous expression in microglia following HSV infection. Therefore, microglial cells are likely to be the first CNS cells in the rat to process viral antigen in conjunction with MHC antigens, which is the first step in immune recruitment. Because our immunofluorescent and in situ hybridization data indicated that relatively few CNS cells were infected with +GC during the acute phase of infection and since viral titers in the entorhinal cortex were low (Table 1), it is possible that a low level of infection in conjunction with a low degree of antigen processing by microglia limited the severity of CNS damage. Later injury may have also been lessened by further reduction of microglial MHC antigen expression (54). Although tissue injury must have occurred in +GC infected rats, we believe that it was below the threshold necessary to have been detected by the methods employed in this study.

Another possible explanation for the absence of residual tissue damage in the rat relates to the roles of cellular and humoral immunity to HSV-1 clearance from the CNS. Humoral immunity is important for protection against viral infection and neutralization of extracellular virus, but clearance of virus from infected tissues is thought to be mediated solely by cellular immunity. However, McKendall et al. (55) demonstrated that HSV-1 antiserum is highly protective when given to mice prior to, or shortly after, HSV-1 infection. In addition, Levine et al. (56) have recently reported that in a SCID mouse model of alphavirus encephalomyelitis, adoptive transfer of hyperimmune serum resulted in clearance of infectious virus from the nervous system, whereas adoptive transfer of sensitized T lymphocytes had no effect. Thus, antibody alone can mediate the clearance of virus from the CNS. Therefore, clearance of HSV within the Lewis rat CNS may have involved a non-antibody-dependent cell-mediated cytotoxicity (non-ADCC) mechanism.

These studies document the sites of HSV-1 replication and spread within the CNS of the rat. We further demonstrate that +GC is capable of establishing latency within the CNS structures which were also involved in the acute infection. Because structures of the olfactory and related limbic systems are critical for the storage and recall of information, these studies provide an animal model that will allow evaluation of potential behavioral abnormalities that may arise from HSE. This model of HSE may conceivably lead to the development of non-invasive techniques for the diagnosis of HSE during its early clinical stages and the ability to differentiate HSE from other infectious and noninfectious encephalitic conditions. Finally, this model may provide a means for testing the effectiveness of newly synthesized antiviral drugs and for the development of drugs that enhance memory in HSE patients.

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Fig. 6. Representative localization of +GC nucleic acids by in situ hybridization of acutely (A–C) and latently (D–F) infected peripheral and central nervous system tissues. A. Trigeminal ganglion 7 dpi (×515). B. Piriform cortex 7 dpi (×545). C. Entorhinal cortex 7 dpi (×515). D. Hippocampus 160 dpi (×545). E. Piriform cortex 160 dpi (×545). F. Spinal trigeminal nucleus 160 dpi (×545). Counterstained with H & E.
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