JOURNAL OF VIROLOGY, Mar. 1988, p. 749–756
0022-538X/88/030749-08$02.00/0
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Vol. 62, No. 3

Latent Herpes Simplex Virus Type 1 Transcripts in Peripheral and Central Nervous System Tissues of Mice Map to Similar Regions of the Viral Genome

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Received 24 September 1987/Accepted 24 November 1987

Herpes simplex virus type 1 (HSV-1) DNA and RNA have been detected in peripheral nervous system (PNS) and central nervous system (CNS) tissues of latently infected mice. However, explant methods are successful in reactivating HSV-1 only from latently infected PNS tissues. In this report, latent herpesvirus infections in mouse PNS and CNS tissues were compared by in situ hybridization to determine whether the difference in reactivation was at the level of the virus or the host tissue. It was demonstrated that the HSV-1 transcripts present during latency in the mouse PNS and CNS originated from the same region of the genome, the repeats which bracket the long unique sequence. Therefore, the difference in reactivation with PNS and CNS tissues cannot be accounted for by differences in the extent of the HSV-1 genome transcribed during herpesvirus latency. Latent HSV-1 RNA was detected in the trigeminal ganglia (PNS) and the trigeminal system in the CNS from the mesencephalon to the spinal cord as well as other regions of the CNS not noted previously. Latent HSV-1 RNA was found predominantly in neurons but also in a small number of cells which could not be identified as neuronal cells. It is suggested that host differences in CNS and PNS tissues, rather than differences in latent virus transcription, may be important determinants in the HSV-1 reactivation process in explanted tissues.

Goodpasture postulated that latent herpes simplex virus type 1 (HSV-1) infections are established in the trigeminal ganglia of the peripheral nervous system (PNS), since these ganglia supply sensory innervation to the ophthalmic and orofacial areas in which herpetic lesions recur (19). Latent HSV-1 has been demonstrated in PNS tissue by organ culture (42), by explant cocultivation (7, 26, 49, 50), and by in vivo reactivation following neurectomy (48). HSV-1 has also been shown to spread to the central nervous system (CNS), where latent infections can be established (1, 2, 7, 24, 26, 33, 45, 47, 51). Although infectious viral particles are not detectable in latently infected cells of the PNS and CNS, viral DNA has been detected by reassociation kinetics (4, 36) and Southern blot analysis (11, 14, 37), and limited transcription of the genome has been demonstrated in PNS tissues (10, 13, 35, 43).

A characteristic of latent herpesvirus infections is that the virus can be reactivated following appropriate stimuli (for review, see Hill et al. [22]). Reactivation, as studied by explant cocultivation and explant homogenization with mouse tissue, occurs consistently from PNS but not from CNS tissue (4, 7, 22, 47). Differences in reactivation with PNS and CNS tissues could be due to differences in latent HSV-1 gene expression, the latent cell type, cellular factors, or differences in the ability of the tissues to survive in explant culture. To investigate potential differences in latent viral gene expression, the regions of the viral genome that encode latent HSV-1 RNA in the PNS and CNS tissues were compared. To study potential differences inherent in the host PNS and CNS tissues, the pathology of both acute and latent HSV-1 infections in the mouse PNS and CNS was studied. The data presented demonstrate that latent HSV-1 RNA in both PNS and CNS tissue of mice is encoded by the same region of the genome. The pathology of the acute and latent HSV-1 infections appears predominantly in the trigeminal system of the CNS; however, infected cells were also detected in other systems of the CNS not noted previously. The infected cells in latently infected PNS and CNS tissues were predominantly neurons, but latent HSV-1 RNA was detected in a small percentage of non-neuronal cells, possibly glial cells in the CNS and satellite cells in the PNS. Therefore, we suggest that the inherent differences between PNS and CNS tissues (such as neuroanatomical features, ability to repair nerve processes, cell density, and cellular factors) affect their ability to support the explant reactivation of latent HSV-1.

MATERIALS AND METHODS

Preparation of HSV-1 (F) stocks. Confluent CV-1 cell monolayers, grown in Eagle minimal essential medium plus 5% fetal calf serum, 50 U of penicillin per ml, and 50 mg of streptomycin per ml at 37°C, were infected with HSV-1 (F) at a multiplicity of infection of 0.01. Virus harvested from these cells was used to infect CV-1 cells at an multiplicity of 1 to produce the stocks for animal inoculation. Infected cells were maintained in culture until maximum cytopathic effect was observed. The cells were removed by shaking, the suspension was sonicated for 15 s, and cell debris was removed by low-speed centrifugation. Virus was concentrated by centrifugation in a Beckman type 19 rotor for 2 h at 18,000 rpm at 4°C. The pellets were suspended in serum-free minimal essential medium at a concentration of 10^6 to 10^7 PFU per 5 μl and stored in portions at −70°C.

Infection of mice. Female BALB/c mice (cBYJ; Jackson Laboratories), 4 to 6 weeks old, were anesthetized and inoculated with 10^6 to 10^7 PFU of HSV-1 (F) in each eye

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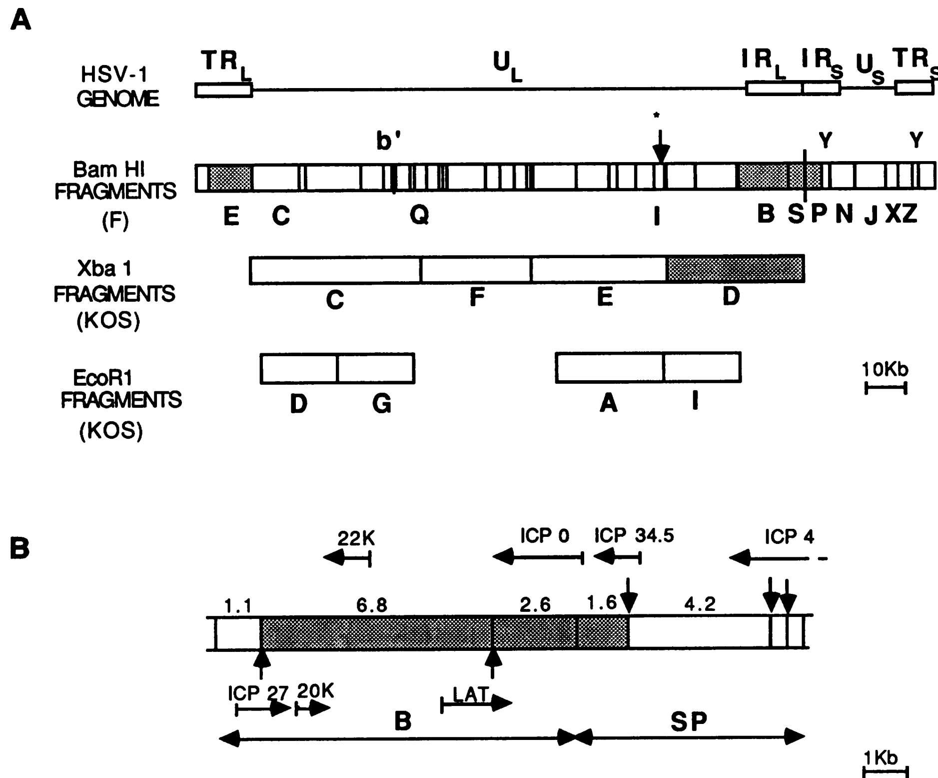

FIG. 1. (A) Structure of the HSV-1 DNA, including the long unique (U_L) region, which is bounded by the terminal repeat (TR_L) and the internal repeat (IR_L), and the short unique (U_S) region which is bounded by the terminal repeat (TR_S) and the internal repeat (IR_S). The *Bam*HI restriction map of HSV-1 (F) DNA (34) and the map positions of the *Xba*I (KOS) and *Eco*RI (KOS) fragments which were used are also pictured. (B) The IR_L region is enlarged to show the transcripts that map to the region of *Bam*HI-B and *Bam*HI-SP. The arrows below the line indicate the *Sal*I restriction sites of *Bam*HI-B and above the line indicate the *Sac*I restriction sites of *Bam*HI-SP. The numbers above the line are the size (in kilobases) of the subfragments. The arrow in A indicates the location of the *Eco*RI-*Bam*HI-I-I fragment. LAT shows the approximate map location of the recently detected latent transcripts (39, 43). 20K and 22K are approximate locations of open reading frames (29, 39). The shaded areas indicate the regions of the genome showing positive hybridization.

after corneal scarification, as described previously (37). Mock-infected animals were inoculated with an extract prepared from uninfected CV-1 cells.

Explant reactivation assays. HSV-1 was reactivated from the trigeminal ganglia of latently infected mice by two techniques: explant cocultivation (8) and explant incubation and titration (25, 40). For cocultivation, the trigeminal ganglia or brain stems were rapidly dissected and incubated with subconfluent monolayers of CV-1 cells in minimal essential medium supplemented with 5% fetal calf serum at 37°C in 5% CO_2. The cultures were inspected daily for cytopathic effect in the CV-1 cells. Every 4 to 5 days the tissues were transferred to fresh monolayers of cells. For explant incubation and titration, the ganglia and brain stems were incubated, as above, in the absence of CV-1 cells. At each assay time, typically 4 days postexplant, the tissues were Dounce homogenized with a type A pestle. Serial 10-fold dilutions of trigeminal ganglia homogenates in minimal essential medium were adsorbed at 37°C to subconfluent monolayers of CV-1 cells for 1 to 2 h with periodic agitation. At the end of the adsorption period, fresh medium containing human immune serum globulin (0.5 mg/ml; Armour) was added (41). Two days later, the plaques were stained with 1% methylene blue and counted.

Probes for in situ hybridization. HSV-1 DNA was isolated from virions and purified by CsCl gradient centrifugation. pBR322 and adenovirus DNA (purified as described previously [15, 30]) were used as controls to demonstrate HSV-1-specific hybridization. Plasmid pBR322 containing *Bam*-HI-a' (KOS) (9), which corresponds to *Bam*HI-b' (F), was obtained from E. K. Wagner and R. H. Costa, University of California, Irvine; plasmid pBR322 containing *Eco*RI-*Bam*HI fragment I-I (KOS) was obtained from E. K. Wagner and R. J. Frink, University of California, Irvine (16); plasmids pRB112 (*Bam*HI-B), pRB113 (*Bam*HI-Y), pRB114 (*Bam*HI-N), pRB115 (*Bam*HI-SP), pRB122 (*Bam*HI-Z), pRB123 (*Bam*HI-J), pRB124 (*Bam*HI-X), and pRB134 (*Bam*HI-E) were obtained from B. Roizman, University of Chicago (34). Plasmid LE578, obtained from L. Enquist, Du Pont Co., contains a 3.4-kilobase (kb) *Bam*HI fragment of the Patton strain of HSV-1 (12), which encodes the thymidine kinase gene (*tk*) and corresponds to the *Bam*HI Q fragment in HSV-1 strain F. pUC19 plasmids containing KOS strain *Xba*-C (pMC121), *Xba*-D (pMC124), *Xba*-E (pMC123), and *Xba*-F (pMC122) were supplied by M. Challberg (5). KOS strain *Eco*RI restriction fragments A, I, D, and G were used with the permission of R. Sandri-Goldin, University of California, Irvine (18). Refer to Fig. 1 for location of probes on the HSV-1 genome. Plasmid Δ-2globin was obtained from C. Lo, University of Pennsylvania, and used as a control to demonstrate that the hybridization was to RNA and not to DNA (27).

HSV-1 DNA fragments were gel purified before use. Samples (0.25 to 0.50 μg) were nick-translated at 14°C for 2

TABLE 1. In situ hybridization with probes specific for different temporal classes of HSV-1 RNA

Probe[c] (strain)	Gene	Class	Presence of HSV-1 RNA during infection[a]			
			PNS		CNS	
			Latent	Acute	Latent	Acute
2.6-kb *Sal*I-BamHI-B (F)	ICP0	Immediate early	+	+	+	+
*Bam*HI-Y (F)	ICP4	Immediate early	−	+	−	+
*Bam*HI-Q (Patton)	*tk*	Early	−	+	−	+
*Bam*HI-a' (KOS)	Vp5[b]	Early late	−	+	−	+
*Eco*RI-*Bam*HI-I-I (KOS)	Glycoprotein C	Late	−	+	−	+

[a] +, Present; −, not detected.
[b] Major capsid protein.
[c] See Fig. 1, panel B.

to 3 h with 100 μCi of [α-^{35}S]dATP and 100 μCi of [α-^{35}S]dCTP (>1,000 Ci/mmol; Amersham). Unincorporated radionucleotides were separated from nick-translated DNA by passage through Sephadex G-50 mini-spin columns (Boehringer Mannheim) or by phenol extraction and ethanol precipitation. The specific activities of the ^{35}S-labeled probes were 1×10^8 to 2×10^8 cpm/μg.

In situ hybridization. For in situ hybridization experiments, uninfected or mock-infected (control), acutely infected (3 or 5 days postinoculation), and latently infected (>1 month postinoculation) mice were injected intraperitoneally with Nembutal (10 mg in 200 μl) and perfused transcardially with 10 ml of phosphate-buffered saline (0.15 M NaCl plus 15 mM sodium phosphate, pH 7.6), followed by 10 to 20 ml of fresh PLP fixative (2% paraformaldehyde, 0.1 M lysine hydrochloride, 0.5 M sodium phosphate buffer, pH 7.4, and 0.1 mM sodium *m*-periodate) (31). PNS and CNS tissues were removed aseptically and fixed overnight in PLP at 4°C. Blocks of tissue were prepared for paraffin embedding as described (44). Tissue sections (5 to 6 μm) were cut from paraffin-embedded blocks of trigeminal ganglia and brain stem, deparaffinized with xylenes, rehydrated, and treated as previously described (21, 44). Sections used for RNase controls were treated as described (21, 44).

Prior to hybridization, ^{35}S-labeled nick-translated DNA probes were denatured in a boiling water bath for 5 min, cooled on ice, and diluted with hybridization mix (2× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 1 mM Tris, pH 7.4, 1 mM EDTA, 1× Denhardt solution [0.02% polyvinylpyrrolidone, bovine serum albumin, and Ficoll], 10% dextran sulfate, 45% formamide, and 100 mg of polyadenylic acid, 1 mg of mouse brain RNA, and 1 mg of mouse brain DNA per ml). The probe concentration was adjusted to contain 1 ng of DNA per 5 μl (ca. 10^5 cpm per tissue section). The hybridization mix including probe was heated for 30 s at 100°C and cooled on ice; 10 mM dithiothreitol was added, and the complete mixture was prehybridized at 45°C for at least 1 h.

Portions of the prehybridized probe solution (5 μl) were placed on tissue sections and covered with baked, siliconized cover slips and paraffin oil. After hybridization at 50°C for 48 to 65 h, the paraffin oil was removed by two 5-min incubations in chloroform. Cover slips were removed by gently shaking the slides in wash solution, and unhybridized probe was removed by extensive washing for 2 to 3 days at room temperature, as described (44). Following dehydration in ethanol ammonium acetate (0.3 M), slides were dipped in NTB2 nuclear track emulsion (Kodak) that was diluted 1:1 with 0.6 M ammonium acetate. After 2 to 4 days of exposure at 4°C, slides were developed with D19 (Kodak) and stained with hematoxylin and eosin. On average, 10 sections were examined per probe per mouse.

RESULTS

Reactivation of latent HSV-1 from mouse tissues. Previous studies have shown that HSV-1 DNA is present in mouse PNS and CNS tissues (37, 38). In this study, latent HSV-1 could be reactivated from 100% of the ganglia (PNS) either by explant cocultivation with susceptible cells (CV-1) (40 of 40 tested by 7 days postexplant) or by incubating explanted ganglia in culture for 4 days and titrating ganglion homogenates (18 of 18 tested). None of the ganglia or brain stems tested contained infectious virus at explant (data not shown). However, these techniques were completely ineffective in stimulating reactivation in brain stems explanted from la-

TABLE 2. In situ hybridization of HSV-1 RNA in latently infected tissue with probes encompassing the whole genome

Probe[a]	No. positive/no. examined[b]	
	Ganglia	Brain stem
*Bam*HI-E	4/4*	4/4
*Xba*I-C	0/8	0/8
*Eco*RI-D	ND[c]	0/4
*Eco*RI-G	0/2	0/5
*Xba*I-F	0/5*	0/4
*Xba*I-E	0/5*	0/8
*Eco*RI-A	0/3	0/2
*Xba*I-D	3/3	5/5
*Eco*RI-I	0/4	0/4
*Bam*HI-B	4/4*	2/2
1.1-kb *Sal*I fragment	0/4*	0/4
6.8-kb *Sal*I fragment	7/7*	4/4
2.6-kb *Sal*I fragment	4/4*	3/3
*Bam*HI-Sp[d]	4/4*	2/2
1.6-kb *Sac*I fragment	5/5*	4/4
4.2-kb *Sac*I fragment	0/5*	0/8
*Bam*HI-Y	0/9*	0/3
*Bam*HI-N	0/10*	0/4
*Bam*HI-J	0/4	0/4
*Bam*HI-X	0/10*	0/5
*Bam*HI-Z	0/4	0/4

[a] Listed in order from left to right of the prototype HSV-1 genome (Fig. 1). Indented probes are subfragments of the fragments under which they are listed. All probes hybridized to HSV-1 RNA in acutely infected PNS and CNS tissues, but did not hybridize to RNA in mock-infected or uninfected tissues.
[b] *, Data from Deatly et al. (10).
[c] ND, Not done.
[d] *Bam*HI-SP also encodes two small *Sac*I fragments (300 base pairs each). We tested those small fragments with tissues from acutely, mock, and latently infected tissues. No hybridization was detected in any tissue.

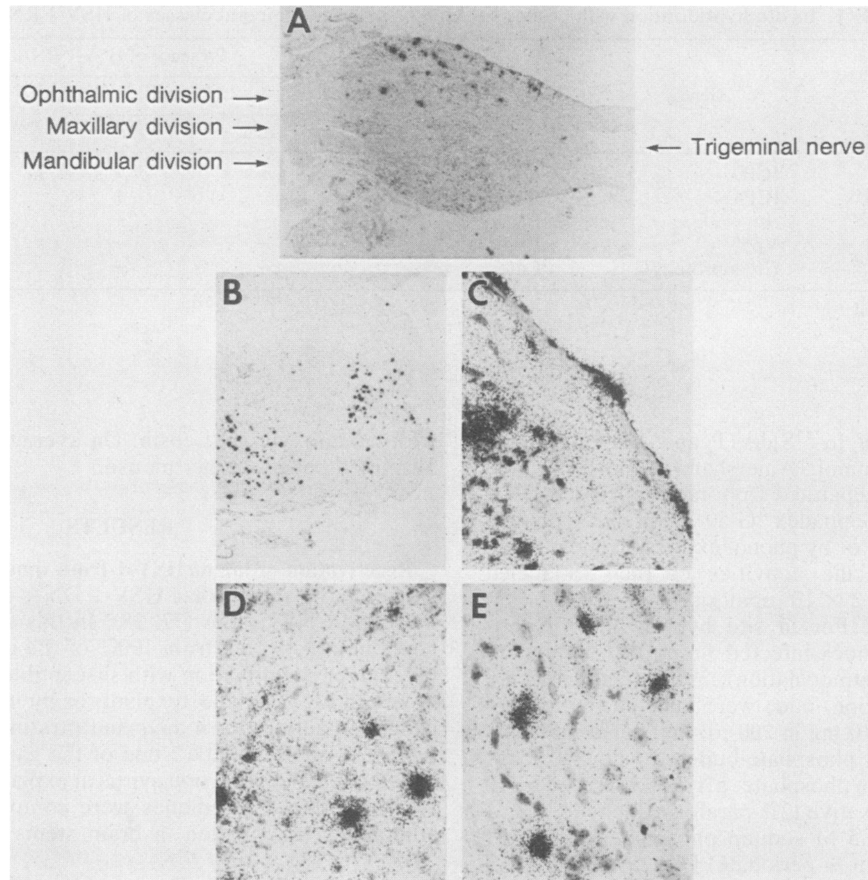

FIG. 2. HSV-1 RNA localization during acute infection of the trigeminal ganglia and the CNS as detected by in situ hybridization. (A) Detection of HSV-1 RNA is restricted to the ophthalmic division of the trigeminal ganglia. (B) HSV-1 RNA in the region of the entry route of the trigeminal nerve into the brain stem at the level of the pons. Hybridization is detected in the trigeminal nerve (5), sensory nucleus, and the trigeminal spinal tract (for orientation, see Fig. 3). (C) Detection of HSV-1 RNA in meningeal cells, adjacent to positive cells in the trigeminal spinal tract. (D) HSV-1 RNA is seen in neurons of the pontine division of the trigeminal spinal tract. (E) HSV-1 RNA is detected in the posterior horn of the upper cervical spinal cord in the caudal portion of the trigeminal spinal nucleus. Trigeminal ganglion sections are longitudinal, and brainstem sections are transverse. Probe: (A and B) *Bam*HI-N; (C, D, and E) *Bam*HI-B. Exposure times were 2 to 4 days. Magnification: A, 10×; B, 70×; C, 650×; D and E, 550×.

tently infected mice (0 of 6 [incubated for 15 days] and 0 of 9 [titrated at 4 days postexplant] for the two methods, respectively).

Analysis of HSV-1 transcription in latently infected mouse PNS and CNS tissues. To compare latent herpesvirus infections in the mouse PNS and CNS in terms of viral gene expression, we performed in situ hybridization with brain stem and trigeminal ganglia with probes specific for HSV-1 genes representative of the temporal gene classes (immediate-early, early, early-late, and late genes). A difference in the extent of latent viral transcription could indicate a difference in the maintenance stages of latent infections in the PNS and CNS. The data obtained from these experiments are summarized in Table 1. The 2.6-kb *Sal*I fragment of *Bam*HI-B (F) and *Bam*HI-Y (F) (Fig. 1) were used to detect RNA from the regions encoding the immediate-early genes ICP0 and ICP4, respectively (6, 28). The LE578 (Patton) probe which corresponds to *Bam*HI-Q (F) was used to detect RNA from the region encoding the early gene *tk*. *Bam*HI-a' (KOS) was used to detect RNA from the major capsid protein gene (Vp5) (an early-late gene) and an *Eco*RI-*Bam*HI-I-I (KOS) probe was used to specifically detect glycoprotein C RNA (a late gene). These probes all hybridized to HSV-1 RNA in acutely infected mouse trigeminal ganglia and brain stem tissues. None of these probes showed positive hybridization with uninfected or mock-infected mouse trigeminal ganglia and brain stem tissues. A virion DNA probe hybridized with nucleic acid in acutely infected brain stem and trigeminal ganglia but did not hybridize with acutely infected brain stem or trigeminal ganglia tissues treated with RNase prior to hybridization, demonstrating hybridization to RNA. Hybridization specificity was further demonstrated by lack of hybridization with a probe for a different viral genome (adenovirus), pBR322, and a cellular gene (Δ-2globin) not expressed in nervous system tissues (data not shown). Only the 2.6-kb *Sal*I fragment of *Bam*HI-B (F), which encodes part of the immediate-early ICP0 gene, hybridized to RNA in latently infected brain stem tissues.

To perform a complete analysis, probes spanning the entire HSV-1 genome were used. The only region of the HSV-1 genome which hybridized to HSV-1 RNA in latently infected brain stem tissues (CNS) was the repeat region which brackets the long unique sequence (Table 2). This region is represented by the *Bam*HI restriction fragments B, SP, and E. As illustrated in Fig. 1, RNAs of four gene products have been mapped to this region, the immediate-

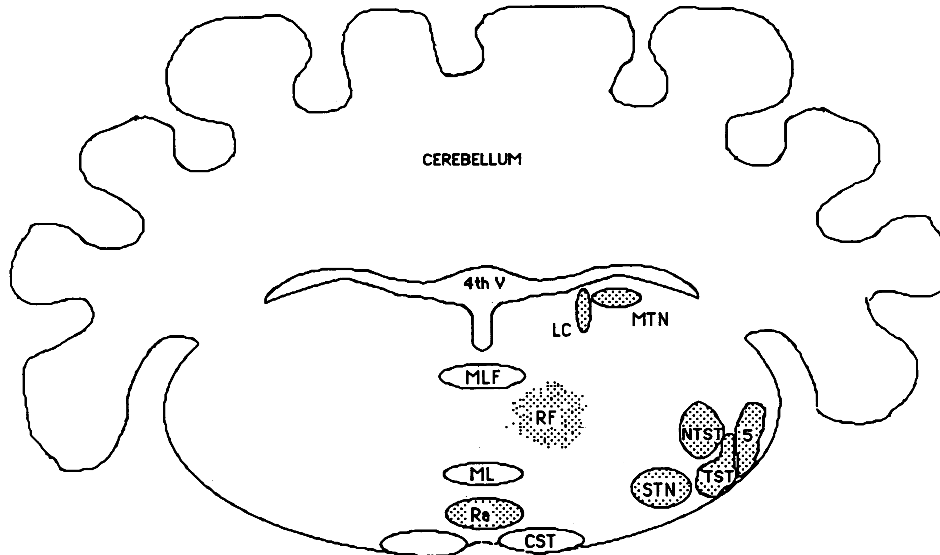

FIG. 3. Schematic representation of a transverse section of the brain stem at the level of the pons, containing the sites of HSV infection: trigeminal nerve (5), sensory trigeminal nucleus (STN), trigeminal spinal tract (TST), nucleus of the trigeminal spinal tract (NTST), mesencephalic trigeminal nucleus (MTN), raphe nucleus (Ra), and reticular formation (RF). Included for reference are the medial lemniscus (ML), corticospinal tracts (CST), medial longitudinal fusciculus (MLF), locus coeruleus (LC), and the fourth ventricle (4th V). Areas positive for latent HSV-1 RNA are stippled.

early gene products ICP27, ICP0, and ICP4, as well as the late-gene product γ34.5. Recently, the 6.8-kb *Sal*I subfragment of *Bam*HI-B has been shown to encode 0.9-kb and 1.1-kb transcripts in infected cells (29, 39) and three latency-specific transcripts by Northern (RNA) blotting (39, 43). To separate the four mapped genes, the region was subdivided to determine which genes might encode latent HSV-1 RNA. *Bam*HI fragment B was cut into three *Sal*I fragments of 1.1 kb, 6.8 kb, and 2.6 kb, and *Bam*HI fragment SP was subdivided into two *Sac*I fragments of 1.6 kb and 4.2 kb. Since *Bam*HI-E sequences are duplicated in *Bam*HI-B, similar results would be expected. Of these subfragment probes, only the *Sal*I 2.6-kb and 6.8-kb subfragments of *Bam*HI-B hybridized strongly, and the 1.6-kb *Sac*I subfragment of *Bam*HI-SP hybridized less strongly to RNA in latently infected tissues. As illustrated in Tables 1 and 2, there appeared to be an identical pattern of restricted transcription of latent HSV-1 genomes in the CNS, as has been previously reported for trigeminal ganglia (10). Hybridization to the 1.6-kb *Sac*I subfragment was noticeably weaker than to the other two fragments but clearly above that of fragments such as the *Xba*I fragments shown in Fig. 1.

Anatomical distribution of HSV-1 RNA in the PNS and CNS during acute infection. Pathogenesis of herpesvirus infections in the mouse PNS and CNS was compared in an attempt to determine whether the difference in reactivation of the two tissues was due to a difference in infection in the two tissues. During acute infections, HSV-1 RNA was detected in the ophthalmic branch of the trigeminal ganglia (Fig. 2A) in both neuronal and nonneuronal cells. In the CNS, HSV-1 RNA was detected in cells in the trigeminal system extending from the mesencephalon to the spinal cord, as described previously (44). Viral RNA was detected in cells in the region of the route of entry of the trigeminal nerve into the pons region of the brain stem (Fig. 2B), in the main sensory nucleus of the trigeminal nerve, in the descending trigeminal spinal tract (Fig. 2D) and nucleus, and in the posterior horn of the upper cervical spinal cord (Fig. 2E) (44). Detection of HSV-1 RNA in meningeal cells is shown in Fig. 2C. The location of these anatomical regions is shown in a transverse brain stem section in Fig. 3.

Anatomical distribution of HSV-1 RNA in the PNS and CNS during latent infection. The distribution of the viral infection in latently infected PNS and CNS tissues was similar to that of acutely infected tissues. HSV-1 RNA was found predominantly in cells of the ophthalmic branch of the trigeminal ganglia (Fig. 4A to C) and in cells of the trigeminal system of the CNS, including the mesencephalic nucleus of the trigeminal nerve (5) (Fig. 4D), sensory nucleus (Fig. 4E), trigeminal spinal tract (Fig. 4F), and nucleus. In addition, HSV-1 RNA was detected in nontrigeminal locations, including the raphe nucleus, the pontine reticular formation (Fig. 4G to I), the cerebellum (locations which have not been reported before as sites of HSV-1 latency), the hippocampus, and the entorhinal cortex. During latency in the CNS, cellular infiltration was also evident in the regions of the trigeminal nuclei and tracts of the pons region of the brain stem as described previously (44), indicative of a chronic focal encephalitis. Figure 3 is a schematic representation of the regions of the mouse brain stem which are infected during HSV-1 latency.

Latent HSV-1 RNA was detected primarily in neurons. However, a small percentage of latently infected cells were identified morphologically as either glial cells (in the CNS) or satellite cells (in the PNS) (Fig. 4C). Such cells were also found in areas such as the trigeminal spinal tract which are devoid of neurons (Fig. 4F). Compartmentalization of the HSV-1 RNA within the cells was also compared. Latent RNA appeared to be associated primarily with the nucleus, with no detectable difference between PNS and CNS tissue.

DISCUSSION

The objective of this work was to compare latent herpesvirus infections in the mouse CNS and PNS. HSV-1 was reactivated from 100% of the trigeminal ganglia tested, but

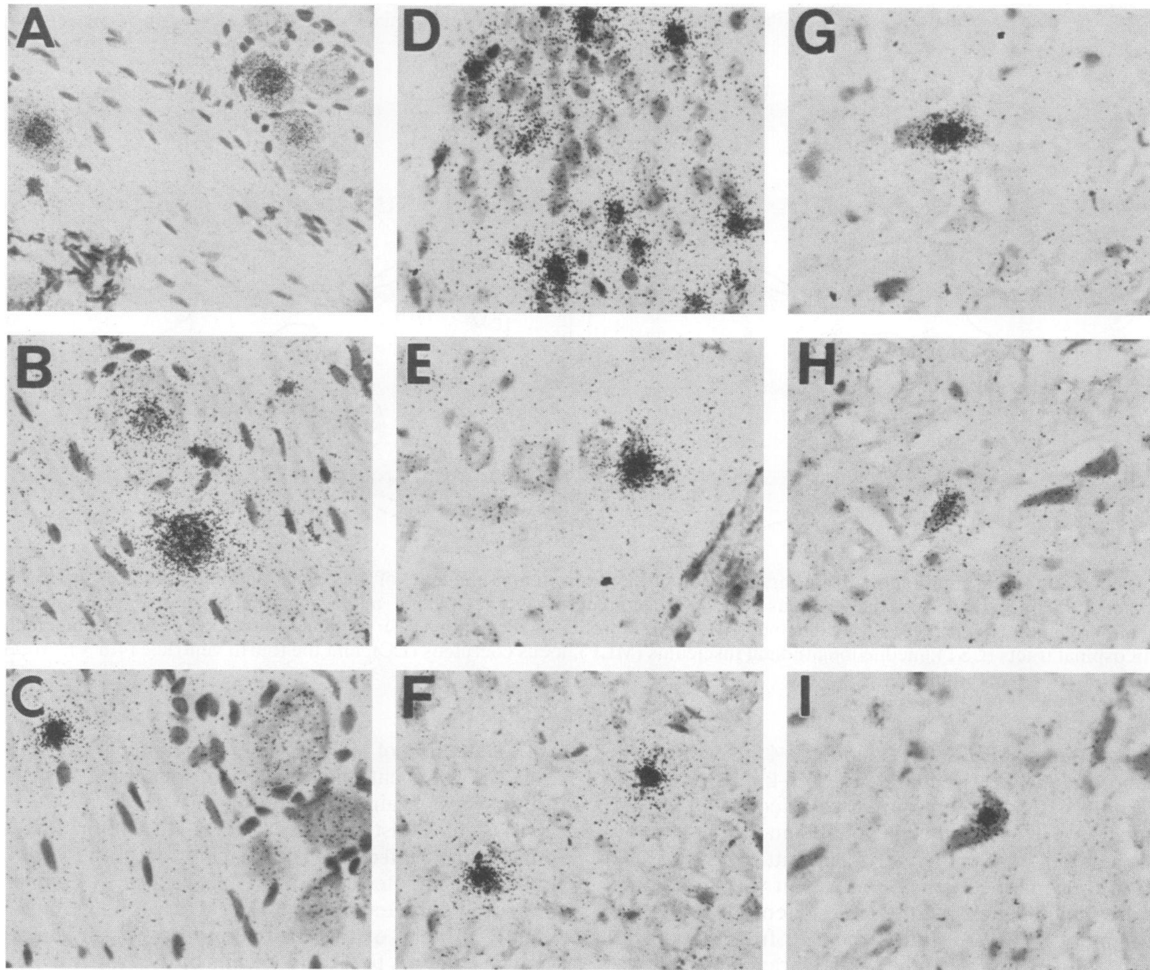

FIG. 4. Detection of HSV-1 RNA during latency in the trigeminal ganglia (A to C), the trigeminal system of the brain stem (D to F), and nontrigeminal system regions of the brain stem (G to I) by in situ hybridization. Positive hybridization is detected in cells of the trigeminal mesencephalic nucleus and the locus ceruleus (D), in neurons of the main trigeminal sensory nucleus (E), in glial cells of the trigeminal spinal tract (F), and in neurons of the pontine reticular formation (G to I). Latent HSV-1 RNA is detected in neuronal (A, B, D, E, G to I) as well as in nonneuronal cells both in the PNS (C) and in the CNS (F). *Bam*HI-B probes were hybridized with tissue from a mouse 9 months postinfection (A to C) and 1 month postinfection (D to I). Panels A to C were exposed for 3 days, D for 4 days, and E to I for 2 days. Magnification: A, 420×; D, 200×; others, 670×.

not from any brain stem samples. Other investigators have also been unable to reactivate latent HSV-1 from the CNS of latently infected mice (22, 47) or were only able to reactivate HSV-1 from the CNS at a low frequency (4, 7). We attempted to determine whether the difference in reactivation in the two tissues was at the level of latent virus transcription.

Latent viral transcription in CNS and PNS is restricted to the same region of the genome. Using cloned HSV-1 DNA fragments encompassing the whole genome, we have shown in this report that latent HSV-1 RNA, in the mouse CNS, is encoded by the repeat regions which bracket the long unique sequence. Further analysis with cloned DNA subfragments from these repeat regions demonstrated limited transcription during a latent infection to the region known at present to encode the immediate-early ICP0 gene, two recently mapped open reading frames (20 kb and 22 kb [29, 39]), and the recently detected latency-associated transcripts (39, 43). Recent reports show that latent RNA is transcribed from the strand opposite to that producing ICP0 RNA (39, 43) and is polyadenylated to a limited extent (35, 39).

This region of the genome, which hybridizes to the latent RNA, is 11 kb and extends from a *Sal*I restriction site close to the U_L/IR_L boundary in the *Bam*HI B fragment to a *Sac*I restriction site in *Bam*HI-SP (Fig. 1). Since the latency-associated RNAs detected by Northern blotting map to a 3.0-kb region within this 11-kb region, we suggest that there may be other latent HSV-1 RNAs in addition to those already mapped (35, 39). It is interesting that the hybridization to the 1.6-kb *Sac*I subfragment of the *Bam*HI SP fragment was less than that to the 2.6-kb and 6.8-kb *Sal*I subfragments of *Bam*HI-B. It is also worth mentioning that the *Sal*I-1 subfragment of *Bam*HI-B and *Sac*I-4.2 subfragment from *Bam*HI-SP fragment, although scored as negative, did show occasional positive cells. It is possible that these regions are transcribing low levels of HSV RNA or that we are observing some cross-hybridizing sequences, either virus-virus or cell-virus in nature. This region of weak hybridization will be the subject of further study.

The conclusion from the in situ hybridization data obtained with brain stem tissues (CNS) is that the maintenance stage of latent infections in the CNS and PNS appears to be

the same in terms of the regions of the genome which encode latent RNA. Altogether, these data suggest that transcription during the maintenance stage of a latent herpesvirus infection is latency specific and might be regulated by a different mechanism than during a productive infection. It is possible that a function(s) encoded by the virus in this specific region of the genome is involved in the tightly controlled regulation of restricted transcription during herpesvirus latency. These results, however, do not eliminate the possibility that different transcripts or different HSV-1 genes from this region may be synthesized in the different tissue types that affect reactivation.

To determine whether differences in reactivation of latent HSV-1 infections of the CNS and PNS tissues are due to differences in the host nervous system tissues, we studied the pathology of latent herpesvirus infection of the mouse PNS and CNS. Latent HSV-1 RNA was found primarily in the trigeminal system, as reported previously (44). HSV-1 RNA was also detected in other systems of the CNS not previously reported, including the raphe nucleus (Ra), the reticular formation (RF) in the pons region of the brainstem, and the cerebellum (Fig. 2). It is possible that the establishment of latent infections in these areas of the mouse CNS may be due to spread of the virus through the olfactory system, as already suggested (44). For the most part, the cells which harbor the latent herpesvirus in the PNS and CNS tissues appear to be neurons, as previously reported (8, 17, 32, 44, 46). However, a small percentage of cells in which latent HSV-1 RNA is detected appear to be glial (CNS) or satellite cells (PNS) (Fig. 4), which may affect reactivation.

It is possible that the number or density of latently infected cells differs in the CNS relative to the PNS and that a lower number or density in the CNS reduces the possibility that a detectable reactivation event will occur. An estimation of the number of cells transcribing latent HSV-1 RNA per transverse CNS tissue section (2 to 10) appears to be on average less than the number of cells detected from a longitudinal PNS tissue section (4 to 40) (unpublished data). We and others have detected latent RNA in ganglia by Northern blot analysis (39, 43). However, it has been more difficult to detect latent RNA in the CNS (unpublished data). Nevertheless, the amount of HSV-1 DNA in the CNS and PNS appears to be similar on a per gram of tissue basis (4, 11, 37, 38). The structure of latent HSV-1 DNA in the PNS and CNS is indistinguishable but different from that of virion DNA, as revealed by Southern blotting (11, 37, 38). However, there may be subtle differences in the structure or secondary modifications of the latent viral DNA in the different tissues. Presently we cannot detect viral DNA in latently infected tissue by in situ hybridization. Thus, it is not possible to determine whether all of the latently infected cells are expressing viral RNA.

The difference in cell density or other neuroanatomical features of the PNS and CNS tissues may be the major determinant of potential reactivation of a latent herpesvirus infection. Different cellular factors in the CNS and PNS could play a major role in reactivation differences. It is also possible that the two tissues differ in their ability to survive in explant culture. Neurons of the CNS do not have the same ability as neurons of the PNS to repair nerve processes (3, 20). Therefore, the required metabolic change in the host cell for reactivation to occur may be related to the ability of the host cell to repair itself (23).

This in vitro phenomenon (the inability to reactivate virus from the mouse CNS) may or may not be related to the in vivo human virus-host interaction. It is interesting to speculate that the detectable reactivation of a human herpesvirus infection could be from a PNS infected cell and that the rare reactivation from a CNS infected cell could lead to the rare and severe cases of herpes encephalitis and meningitis. In any case, an understanding of the factors involved in reactivation of a latent herpesvirus infection will aid in understanding the maintenance of a latent herpesvirus infection.

ACKNOWLEDGMENTS

We thank H. Koprowski for his continued interest in this work, Anita Jackson for superb help in embedding and preparing the tissue sections, and Richard W. Peluso and Israel Steiner for critical reading of the manuscript.

This study was supported by Public Health Service grants NS-11036 and A1-23968 from the National Institutes of Health.

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