JOURNAL OF VIROLOGY, Sept. 1988, p. 3493–3496
0022-538X/88/093493-04$02.00/0

Vol. 62, No. 9

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Latent Herpes Simplex Virus Type 1 Transcription in Human Trigeminal Ganglia

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Received 1 April 1988/Accepted 2 June 1988

We studied latent herpes simplex virus type 1 gene expression in human trigeminal ganglia. Two transcripts were mapped to a 3.0-kilobase region within the long repeat region and appeared to be located in neuronal nuclei. These viral RNAs were not abundant during lytic replication and may represent an alternative pattern of herpes simplex virus type 1 gene expression involved in the pathogenesis of latent infection.

After primary infection, latent herpes simplex virus type 1 (HSV-1) persists in sensory ganglia of the peripheral nervous system (1, 3, 11, 15) and can reactivate to produce recurrent mucocutaneous lesions in up to 61% of the adult human population (4, 13, 28). Experimental animal models have been used to explore the molecular basis of the latent viral state within the nervous system. In latently infected mice, HSV-1 DNA is predominantly in a nonintegrated form (16) that differs in structure from linear unit-length virion DNA (9, 18). In situ hybridization data suggest that HSV-1 transcription in neurons of latently infected ganglia is limited to the repeat regions (5–7, 19, 25), which contain *Bam*HI restriction fragments B, SP, and E (see Fig. 1). The study of these transcripts might shed light upon the mechanism for establishment and maintenance of latent HSV-1 infection in the nervous system. Recent reports obtained from latently infected mouse and rabbit models have identified three RNAs (2.0, 1.5, and 1.45 kilobases [kb]) within the long repeat regions of the HSV-1 genome (19, 22, 25). These RNAs are in proximity to the immediate early gene ICP0, which is transcribed in the opposite direction (19, 22, 25). These latency-associated HSV-1 transcripts are expressed at much lower levels during the acute stage of infection (23) and lytic replication than are other HSV-1 RNAs, and they are regulated differently from other HSV-1 genes (24). The animal models may or may not accurately reflect herpesvirus infection in human nervous tissue for the following reasons. (i) High numbers of PFU are required to induce latent infection in some models, (ii) some of the portals of entry are not natural ones, and (iii) the clinical disease and involvement of the central nervous system of the animal models is often more severe than in humans (11, 20). To address this issue, we analyzed human trigeminal ganglia for HSV-1 transcripts by Northern (RNA) blot analysis and in situ hybridization.

Trigeminal ganglia were excised from 12 human cadavers at 12 to 25 h after death (Table 1). Care was taken to avoid samples from human subjects who had clinical evidence of active herpetic disease at death or histological evidence of active infection. Total RNA was extracted from trigeminal ganglia, Northern blotted (14, 22), and hybridized with *Bam*HI fragment B (17) or its *Pst*I-*Mlu*I subfragment (Fig. 1C). Of 13 ganglia (from eight individuals), 8 (from five individuals) were positive for a 2.0-kb band of variable intensity (Fig. 2, lanes 5 to 10). A 1.5-kb band of lesser intensity was detected in six of the eight positive ganglia. These RNAs correspond to the transcripts detected in the trigeminal ganglia of latently infected mice (Fig. 2, panel A, lane 3), except that in mice the 1.5-kb band has been resolved into a doublet (22). Slight variations in band size were observed among the human samples. A 2.0-kb band of much lesser intensity was also present in HSV-1-infected cells (22, 24) and the ganglia of acutely infected mice (22, 23; Fig. 2, lanes 1 and 4). No signal was detected with RNA from latently infected mice or human trigeminal ganglia when *Bam*HI-SP (17) or a mixture containing equimolar portions of the *Bam*HI-*Pst*I and *Mlu*I-*Bam*HI subfragments of *Bam*HI-B were used as a probe (Fig. 1C). This indicated that these transcripts only partially overlap with ICP0 mRNA. A

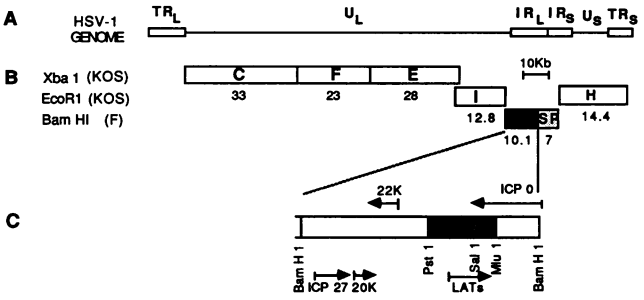

FIG. 1. Map of the HSV-1 genome and location of latency-associated transcripts. (A) HSV-1 genome. The unique long and short regions are represented as lines (U_L and U_S), and the internal and terminal long and short repeats are represented as open boxes (IR_L, TR_L, IR_S, and TR_S). (B) Location of the HSV-1 restriction fragments (and their sizes in kilobases) used for in situ hybridization. Shading denotes fragments positive by in situ hybridization (see Fig. 3): heavy shading, strong hybridization signal (*Bam*HI-B); light shading, less intense signal (*Bam*HI-SP). (C) Locations of ICP0, ICP27, 20K, and 22K protein RNAs and the latency-associated transcripts (LATs), marked by arrows that indicate direction. The shaded regions (between enzyme restriction sites *Pst*I and *Mlu*I) hybridized in Northern blots of RNA from human trigeminal ganglia (see Fig. 2).

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TABLE 1. Clinical data and results of latent HSV-1 RNA analysis of human trigeminal ganglia[a]

Patient no. (age [yr]; sex; cause of death)	Ganglion	In situ hybridization to the following probe:				Hybridization to the following probe in Northern blots:					
		*Bam*HI-B	*Bam*HI-SP	Mix a	Mix b	*Bam*HI-B-*Pst*I-*Mlu*I	Mix c	HSV-1	*Bam*HI-SP	SS18	SS19
1 (78; M; heart failure)	R	+	+	−	+						
	L	+	+	−	+						
2 (33; M; ICH)	R	+	+	−	+						
	L	−	−	−	−						
3 (56; F; PTE)	R					−		−			
	L	−	−	−	−						
4 (46; M; ICH)	R	−	−	−	−						
	L					−		−			
5 (76; F; carcinoid tumor)	R	−	−	−	−						
	L	−	−	−	−						
6 (41; F; Ca of breast)	R	+	−	−	+						
	L	+	+	−	+						
7 (67; F; PTE)	R	+	+	−	+						
	L					+	−	+	−	+	−
8 (60; F; Ca of breast)	R					+	−	+	−	+	−
	L					+	−	+	−	+	−
9 (59; M; heart failure)	R					+	−	+	−	+	−
	L					−		−			
10 (63; M; MI)	R					+	−	+	−	+	−
	L					+	−	+	−	+	−
11 (79; F; heart failure)	R					+	−	+	−	+	−
	L					−		−			
12 (45; M; ABE)	R					+	−	+	−	+	−
	L					+	−	+	−	+	−

[a] Each ganglion was either fixed for in situ hybridization or processed for Northern blot analysis. Mix a, Equimolar mixture of *Xba*I restriction fragments C, F, and E and *Eco*RI fragments H and I; Mix b, *Mlu*I-*Bam*HI restriction fragments of *Bam*HI-B; SS18, *Sal*I-*Bam*HI single-strand probe synthesized in M13 mp18; SS19, *Sal*I-*Bam*HI single-strand probe synthesized in M13 mp19. M, Male; F, female; R, right ganglion; L, left ganglion; ICH, intracerebral hematoma; PTE, pulmonary thromboembolism; MI, myocardial infarction; Ca, carcinoma; ABE, acute bacterial endocarditis.

purified HSV-1 virion DNA probe hybridized to the bands which were positive with the *Bam*HI-B or *Pst*I-*Mlu*I probe but did not hybridize to any additional RNAs (data not shown), indicating limited viral gene expression. Since both HSV-2 and varicella-zoster virus can establish latent infections in human sensory ganglia, the human RNA samples were probed with virion DNAs from these viruses (8, 27). Under HSV-1 hybridization conditions, neither viral DNA probe hybridized to the 2.0- or 1.5-kb RNA.

To determine the direction of the transcripts, a *Sal*I-*Bam*HI subfragment of *Bam*HI-B DNA (Fig. 1C) was cloned in both directions into M13 (22). In one orientation, the single-strand-labeled DNA (12) hybridized to ICP0 mRNA but not to the latency-associated transcripts in mouse or human trigeminal ganglia (mp19, Fig. 2B), while the complementary single-strand-labeled probe hybridized to the 2.0-kb latent RNA but not to ICP0 RNA (mp18, Fig. 2C). In latently infected mice, the latency-associated transcripts, 2.0, 1.5, and 1.45 kb, are all transcribed in the same direction (i.e., rightward) (22). This may also be true of latent HSV-1 infection in humans, although the 1.5-kb transcript was not clearly detected with the single-strand-labeled probe.

To determine the cell types harboring HSV-1 transcripts and to localize them in cells, in situ hybridization was used (6, 7, 10, 26). In latently infected mice, the HSV-1 regions positive by in situ hybridization during latency are *Bam*HI fragments B, SP, and E (Fig. 1) (6, 7, 19, 25). *Bam*HI-B was chosen as a probe because it encodes the latency-associated transcripts detected by Northern blot analysis, and it hybridized most strongly in latently infected mice (6, 7). Eleven ganglia obtained from seven adults were tested by in situ hybridization (Table 1). In six ganglia from four patients, hybridization to HSV-1 RNA was detectable. A strong signal was confined to neurons and was located on and around cell nuclei (Fig. 3A and B). No signal was detected in the human newborn control ganglia that served as negative control tissue. To examine other regions of the viral genome, (i) *Bam*HI-SP (Fig. 1) was used as a probe, and it hybridized weakly to human trigeminal ganglia (Fig. 3C; Table 1), suggesting that other RNAs in addition to those detected by Northern blots are present during latency and (ii) a mixture, spanning 74% of the genome, which contained equimolar amounts of the following HSV-1 DNA fragments was prepared: *Xba*I (KOS) fragments C, F, and E (2) and *Eco*RI (KOS) fragments I and H (21). Whereas strong hybridization was detected in ganglia from acutely infected mice, no signal

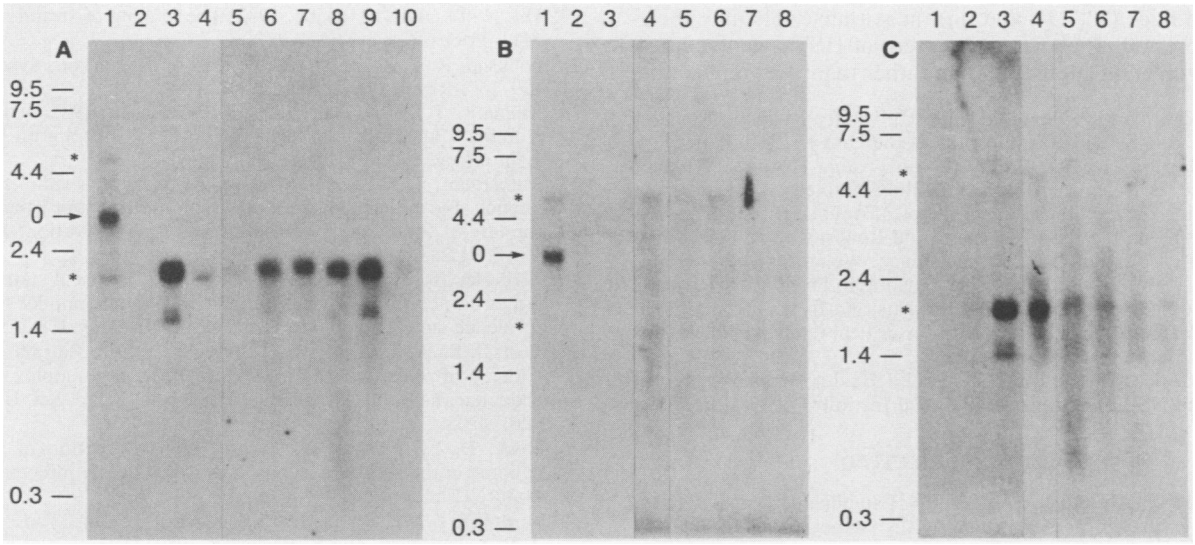

FIG. 2. Detection of HSV-1 RNA in human trigeminal ganglia by Northern blot analysis. (A) *Pst*I-*Mlu*I probe. Lanes: 1, 2 μg of RNA from HSV-1-infected CV-1 cells (5 PFU per cell) at 5 to 6 h postinfection; 2 to 4, 5 μg of RNA from uninfected mouse brain, trigeminal ganglia of latently infected mice, and mice at 5 days postinfection, respectively; 5 to 10, 5 μg of RNA from individual human trigeminal ganglia. (B and C) Single-strand-labeled probes synthesized from the 2.6-kb *Bam*HI-*Sal*I fragment of *Bam*HI-B in M13 mp19 (panel B) and mp18 (panel C) vectors (16). Lanes: 1 to 3, as in panel A; 5 to 8, 5 μg of RNA from human trigeminal ganglia. The positions of RNA markers are labeled in kilobases, and 28S and 18S rRNAs are marked by asterisks on the left. The location of ICP0 RNA is marked with an arrow labeled 0.

was present in latently infected mice or human ganglia unless an equimolar amount of *Bam*HI-B was included in the mixture (data not shown).

We have demonstrated that the HSV-1 RNAs transcribed during latency in human tissue are similar in size, location, and direction to the overlapping and colinear RNAs in latently infected mice (19, 22, 23, 25). The human in situ hybridization data are in accordance with the findings in the various animal models (6, 7, 19) and suggest the possibility that additional HSV-1 RNAs are present during latent infection that are below the limits of detection of Northern blots. In conclusion, these data demonstrate that (i) in the peripheral nervous systems of humans, HSV-1 gene expression is restricted during latency, (ii) the transcripts detected on Northern blots are from HSV-1 genes expressed at higher levels during latency than in infected cells or in acutely

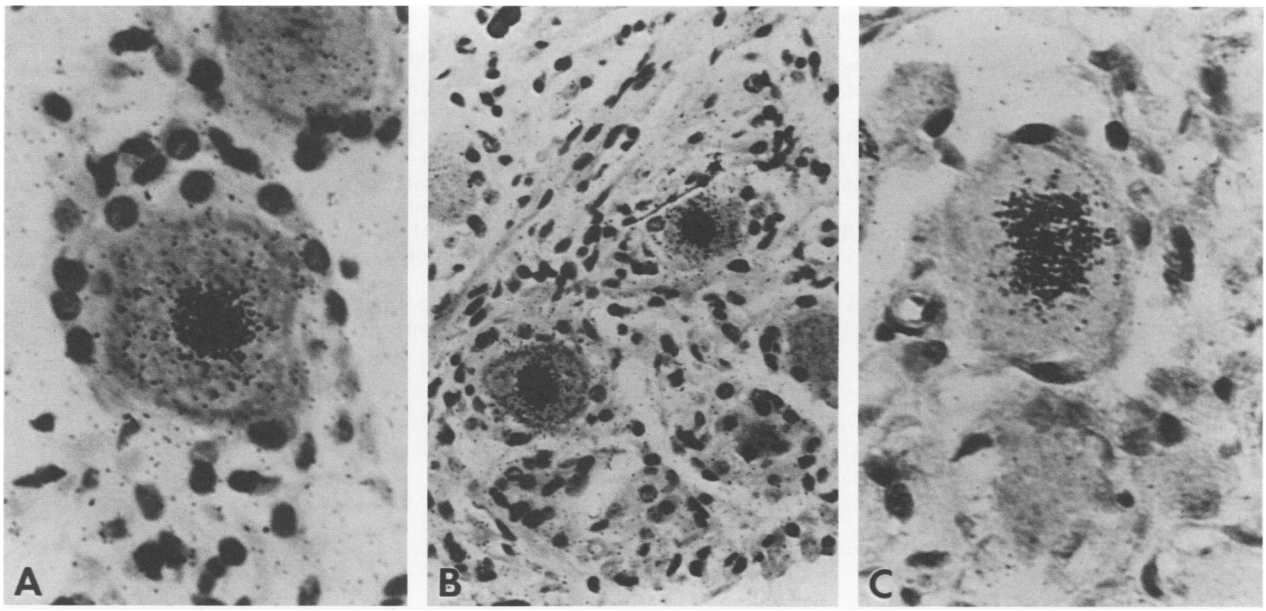

FIG. 3. In situ hybridization of viral RNA in human trigeminal ganglia. Hybridization with ^{35}S-labeled nick-translated probes. Each panel is from a different ganglion. Positive hybridization signals are present over neuronal nuclei. Panels: A and B, *Bam*HI-B; A, magnification, ×400; 24-h exposure; B, magnification, ×200; 48-h exposure; C, *Bam*HI-SP; magnification, ×400; 72-h exposure.

infected mice (22, 23), and (iii) these transcripts may therefore represent an alternative pattern of HSV-1 gene expression involved in latent infection rather than viral replication.

We thank E. M. Hoenig, Temple University Hospital, Philadelphia, Pa., N. K. Gonatas, Hospital of the University of Pennsylvania, Philadelphia, M. J. Warhol, Pennsylvania Hospital, Philadelphia, and J. Rogers, Institute for Biogerontology Research, Sun City, Ariz., for providing human samples, Anita Jackson for embedding and preparing tissue sections, and Robert Carroll and Wayne Eisenberg for expert technical assistance. We are indebted to Y. J. Gordon, University of Pittsburgh, Pittsburgh, Pa., for discussion of human in situ hybridization experiments and to R. Ricciardi, The Wistar Institute, Philadelphia, Pa., for critical reading of the manuscript and useful suggestions.

This work was supported by Public Health Service grants AI 23968 and NS 11036 from the National Institutes of Health.

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