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Herpes Virus Latency in Spinal Ganglia of Mice Without Illness[4] (38251)

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Sensory ganglia in both man and mouse are important sites of latency for herpes simplex virus (HSV) (1–3). Heretofore, disease from the initial infection has been considered a precondition for development of latent viral infection. In man, the initial disease is gingivostomatitis, pharyngitis, keratitis, or other mucocutaneous sites of infection. In mice, foot pad inoculation of herpes virus results in a clinical progression of monoplegia paraplegia, ascending myelitis, and diffuse encephalitis, which frequently ends in death. Among survivors, virus becomes latent in spinal ganglia and is not demonstrable by electron microscopy, staining of specific viral antigen or recovery by inoculation of susceptible tissue cultures. Infectious virus can be unmasked in explants of sensory ganglia. Using this model we recovered latent HSV from infected mice that developed no neurologic signs or clinical disease.

Materials and Methods. Outbred albino ICR mice, 3.5–8 weeks of age, were inoculated with HSV isolated from a patient with pharyngitis and having the cultural characteristics of HSV type 1. Stock virus containing $10^{3.5}$ $TCID_{50}$/0.1 ml was prepared by passing the isolate three times through HEp-2 cells (a human nasal carcinoma). Animals were inoculated with 10^2, 10^3, or 1.6×10^3 $TCID_{50}$ in the right rear foot pad and were observed daily for constitutional symptoms, monoplegia or paraplegia, ascending myelitis, or encephalitis. To uncover minimal disease, the mice were evaluated by normal walking, walking and running against resistance, foot grasp reflex, and ability to climb a ladder-like apparatus. Animals were judged monoplegic upon detection of consistent lateralized motor difficulty in any of these tests, paraplegic upon detection of bilateral paralysis, and myelitic upon detection of urinary incontinence with paralysis. All animals with any detected deficits were excluded from subsequent studies. From the remaining asymptomatic animals, those inoculated at different ages were selected and sacrificed at varying periods postinoculation. The lumbosacral dorsal root ganglia from each side were dissected free and placed as explants in 25mm petri dishes with an overlay of 20% fetal calf serum in Medium 199 containing 100 μg of penicillin and 100 μg of streptomycin. Supernates were harvested at 2–5 day intervals and assayed for herpes virus by inoculation onto WI-38 and HeLa cell cultures. Isolates from each animal were identified as HSV by specific neutralization with hyperimmune guinea pig serum.

Results. Table I summarizes the data on unmasking and recovery of latent virus. A diseased animal with an initial paraplegia

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TABLE I. Viral Recovery from Supernates of Sensory Ganglia Explants.

Animal Status	Time postinoculation		Day of explant 4	6	7	9	11	15	18	21	25	29	32	36
Controls	9 mos.	L		—	—	—	—	—	—	—	—	—	—	
para/monoplegia		R		—		+	+	+	+	+	+	—	—	
Uninfected Control	9 mos.	R	—	—	—	—	—	—	—	—	—	—	—	
Test														
Asymptomatic	14d	L	—		—	—	—	—	—	—	—	[a]		
		R	—		+	+	+	+	+	+	+	[a]		
Test														
Asymptomatic	22d	L	—		—	—	—	—	—	—	[a]			
		R	—		+	+	+	+	+	+	[a]			
Test														
Asymptomatic	22d	L	—		—	—	—	—	—	—	—			
		R	—		—	—	+	+	+	+	+	[a]		
Test														
Asymptomatic	28d	L	—		—	—	—	—	—	—	—	—	—	—
		R	—		—	+	+	+	+	—	—	—	—	
Test														
Asymptomatic	28d	L	—		—	—	—	—	—	—	—	—		
		R	—		—	—	—	—	—	—	—	—		
Test														
Asymptomatic	35d	L	—		—	—	—	—	—	—	—	—	—	[a]
		R	—		+	+	+	+	+	+	+	+	—	[a]
Test														
Asymptomatic	35d	L	—		—	—	—	—	—	—	—	—	—	—
		R	—		—	+	+	+	+	+	+	+	—	—
Test														
Asymptomatic	99d	L	—		—	—	—	—	—	—	—	—	—	—
		R	—		—	+	+	+	+	+	+	+	+	[a]
Test														
Asymptomatic	99d	L	—		—	—	—	—	—	—	—	—	[a]	
		R	—		+	+	+	+	+	+	+	+	[a]	

[a] Cultures lost at this time because of contamination.

and a residual monoplegia served as a positive control. Virus was recovered only from ganglia on the right (infected) side. Cultures from a matched control animal, receiving inoculum without virus, yielded no virus. Nine asymptomatic animals were sacrificed in 3 separate experiments 14–99 days after inoculation. Eight contained latent virus recoverable only by explant technique and only from the right dorsal root ganglia. Ganglia from the noninoculated left side were carried in parallel as internal controls and were negative. No virus was recovered in tissue cultures inoculated with a homogenate of a pool of lumbosacral ganglia from four animals at the time of sacrifice. Explants of other lumbosacral ganglia from three of these same animals yielded virus. The explants required seven to eleven days cultivation before virus production was detected. Infectious virus was then present in the surrounding media from 9 to 23 days. In cultures that were not terminated by bacterial contamination viral titres in the supernatant gradually dropped to unrecoverable levels and remained negative until the experiment was completed.

In Table II, data presented show that the induction of latency in the dorsal root

TABLE II.

	Infecting dose		
	10^3TCID$_{50}$	10^2TCID$_{50}$	10^3TCID$_{50}$
Age of mice	4 week	4 week	8 week
Number inoculated	10	10	10
Neurologic disease	5	3	0
No neurologic disease	5	7	10
Virus positive animals Number tested	4/4	2/3	2/2

ganglia is an efficient process. Among 10 animals receiving 10^2 or 10^3 TCID$_{50}$ at 4 weeks of age or 10^3 TCID$_{50}$ at 8 weeks of age, 22 remained asymptomatic but nearly all ganglia tested harbored latent virus.

To examine whether the induction of latent virus in young animals was simply a manifestation of age-related susceptibility (8), we tested older mice. Figure 1 shows that the incidence of overt disease dropped from 100% at 3.5 weeks of age to 0% at 6 weeks. Incorporation of HSV as latent virus was shown to occur in 8 week old animals which were markedly resistent to disease (Table II, column 3). Overall, ganglia from 8 of 9 animals with no signs of initial or reactivated infection, regardless of age at the time of inoculation or dose, were positive for virus. Thus, the development of latency does not depend on these factors.

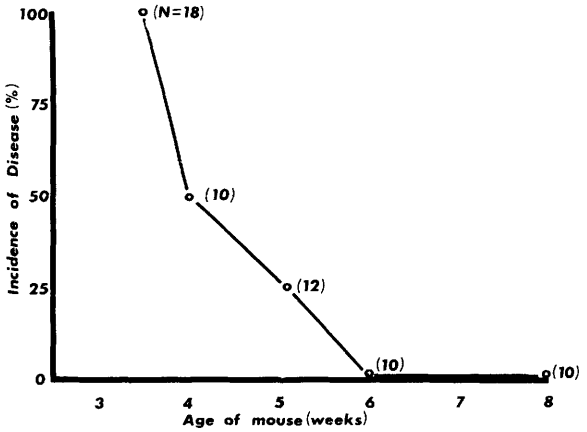

FIG. 1. Relation of neurological disease and age at time of infection. Dose of virus was 1000 TCID$_{50}$ for all but 5 week old group which received 1600 TCID$_{50}$.

Discussion. The absence of primary infectious virus, but its production in explants, indicates the latent state of virus in the ganglia. The induction of latency without antecedent disease is an important new finding which extends understanding of the role of the nervous system as a reservoir for latent Herpes simplex and perhaps other viruses. That the induction of latency in asymptomatic animals is equivalent to that reported in mice recovered from disease during the primary infection is remarkable. Also noteworthy is that a relatively small inoculum of virus, perhaps with local replication, induced latent infection of ganglia in nearly all cases. The lateralization to the infected side confirms that viral access to the ganglia is by ascent of the peripheral nerve (4–7). The regionalization without mononeuritis supports the concept of the neuronal axon, rather than extraneuronal pathways, as the facilitating channel for transport virus. The efficiency and route of infection suggested by these studies are compatible with reports that a high proportion of humans have latent HSV in the trigeminal ganglia (3). Consideration must be given to the possibility that the human reservoir of HSV is initiated and maintained in part by asymptomatic infection.

Summary. Mice that remained free of illness after infection with herpes virus had latent virus in spinal ganglia on the side ipsilateral, but not contralateral to the limb inoculated for 14–99 days. The data demonstrate that induction of latency in neuronal cells is an efficient process which can occur

independently of appreciable clinical manifestations.

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