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7 February 1974

Latent Ganglionic Infection with Herpes Simplex Virus Types 1 and 2: Viral Reactivation in vivo after Neurectomy

Abstract. *Inoculation of the cornea, lip, or footpad of mice with herpes simplex virus type 1 resulted in a latent infection of the local sensory ganglia. Inoculation of the vagina and cervix with herpes simplex virus type 2, as well as type 1, also induced a latent ganglionic infection. With the use of sciatic nerve section as a stimulus, a reproducible model of viral reactivation in vivo was established.*

While it has long been suspected that herpes simplex virus (HSV) might reside in latent form in the sensory ganglia of the nervous system (*1*), direct evidence substantiating this theory has only recently been obtained. By means of methods of in vitro organ explantation and cocultivation, HSV has been recovered from sensory ganglia of chronically infected animals (*2, 3*) and from asymptomatic human subjects (*4*).

Our study was initiated to determine whether latent infection of local sensory ganglia could be induced after exposure of diverse epithelial surfaces to HSV type 1 (HSV-1). In addition, because HSV type 2 (HSV-2) differs from HSV-1 in a number of biological properties (*5*), the ability of HSV-2 to establish a latent ganglionic infection was also examined. Vaginocervical inoculation was chosen as the route of infection with HSV-2 because it is this serotype which is usually responsible for herpetic infection of the human genitalia (*5*).

Further studies were undertaken to develop a model of in vivo viral reactivation. Previous attempts to simulate the human condition of herpes reactivation with a reproducible animal model have met with only limited success (*6–8*). Since nerve section provokes herpetic lesions in man (*9*), sciatic neurectomy was chosen as the stimulus for effecting viral reactivation in latently infected dorsal root ganglia (DRG) of mice.

Pools of stock virus HSV-1 and HSV-2, which had been grown on primary rabbit kidney cells (*10*), contained approximately 4.0×10^8 and 1.3×10^7 plaque-forming units per milliliter, respectively. Female mice (4 to 6 weeks old) (*11*) were inoculated with undiluted virus at one of four sites, the methods varying according to the site. For corneal inoculation a drop of virus was placed on each eye, and both corneas were scarified with a hypodermic needle. For inoculation of both the right hind footpad and the lips, 10 percent saline was first injected locally, and 4 to 6 hours later the skin was abraded and virus was rubbed into these areas (*12*). Vaginocervical infection was accomplished by abrasion of the mucous surfaces followed by intravaginal insertion of a virus-soaked cotton pledget.

Trigeminal ganglia and lumbosacral DRG were removed under sterile conditions, washed three times, and then homogenized or explanted. The ganglia were disrupted with a Ten Broeck homogenizer; the homogenate was frozen and thawed three times, and samples were plated on duplicate monolayers of primary rabbit kidney cells. Explantation was achieved by placing either intact DRG or fragments of trigeminal ganglia on primary rabbit kidney monolayers. Cultures were observed for the appearance of typical HSV cytopathic effect. Isolated virus was identified by neutralization with specific rabbit antiserum to HSV.

Animals used for in vivo reactivation experiments were inoculated in the right hind footpad with HSV-1 at least 3 weeks before surgery. All surgical procedures were done on the animals' right sides under ether anesthesia. Sciatic neurectomy was performed peripheral to the DRG (that is, outside the spinal column) by cutting the lumbosacral roots of the sciatic nerve close to the exit foramina of the spinal column. Sham operations consisted of skin

Table 1. Latent infection of ganglia with HSV-1 and HSV-2. Virus was inoculated into the cornea, lip, footpad, and vagina. Sensory ganglia were removed bilaterally at intervals after infection and were assayed for virus by homogenization and explantation. Results are given as the ratios of the number of animals positive to the number of animals assayed.

Inoculation site	Virus type	Ganglia assayed	Method of assay	Assay after inoculation of virus on days: 4	7	10–12	14–28
Cornea	HSV-1	Trigeminal*	Homogenate	14/16	11/16	3/16	0/39
			Explant	8/16	8/16	9/16	31/39
Lip	HSV-1	Trigeminal	Homogenate	15/16			0/12
			Explant				14/24
Footpad	HSV-1	Dorsal root	Homogenate	7/10	8/10	0/10	0/34
			Explant				40/41
Vagina	HSV-1	Dorsal root	Homogenate		5/7		0/8
			Explant				7/15
Vagina	HSV-2	Dorsal root	Homogenate		22/32		0/27
			Explant				13/43

* In the corneally infected animals, trigeminal ganglia were removed bilaterally and divided into fragments of approximately 0.5 mm. Pieces were equally apportioned for explantation or homogenization. Figures for this group represent results of these two methods on the same animal. In all other experiments mice were divided into groups to be explanted or homogenized; only one method of assay was employed for each animal.

Table 2. Reactivation of HSV-1 after surgical procedure. The number of animals harboring latent virus was established by explantation of dorsal root ganglia (DRG) from unoperated controls. Other groups were assayed for virus by homogenization of DRG. In experiments 1, 3, and 4, an interval of 21 to 28 days separated viral inoculation (footpad) and surgery, while in experiment 2, mice were infected 16 weeks before surgery. Results are given as the ratios of the number of animals positive to the number of animals assayed. Ganglia from sham-operated and neurectomized animals were assayed 3 days after surgery; ND, Not determined.

Experiment	Explantation of DRG: control*	Homogenization of DRG				
		Control*	Sham A (hip)	Sham B (shoulder)	Sham C (exit foramina)	Sciatic neurectomy
1	11/11	0/11	ND	0/15	ND	3/9
2	ND	ND	0/6	ND	ND	3/12
3	ND	0/13	ND	ND	1/13	3/13
4	15/15	ND	0/9	ND	3/13	11/32
Total	26/26	0/24	0/15	0/15	4/26	20/66

* Unoperated.

incisions and dissection of local muscle and bone in the region of the hip (sham A), shoulder (sham B), and spinal column exit foramina (sham C).

The data in Table 1 show that HSV was recovered from local sensory ganglia after inoculation of each of the four sites (cornea, lip, footpad, and vagina). Regardless of the route of inoculation, virus was detected by both homogenization and explantation within the first 2 weeks, but thereafter only by explantation. At 14 to 28 days after infection, the combined results of all groups showed that none of the 120 animals assayed by homogenization yielded virus, while 105 to 162 animals (65 percent) yielded virus by explantation.

Because HSV was recovered in all groups after day 14 only when ganglia were explanted and cocultivated, viral detection by homogenization of the ganglia after this time was used as an index for measuring viral reactivation in vivo. Preliminary experiments revealed that sciatic nerve section induced viral reactivation. After neurectomy, HSV-1 could be recovered by homogenization of lumbosacral DRG on days 2 through 5, with day 3 being optimal. The results of four subsequent experiments in which animals were killed 3 days after surgery are presented in Table 2. Explantation of ganglia from unoperated control animals showed latent infection in all (100 percent), while homogenization of ganglia from either unoperated controls or animals sham-operated at the hip or shoulder (shams A and B) failed to yield virus. However, sciatic neurectomy resulted in viral reactivation in 20 out of 66 animals. Also, when sham operation was performed in the region of the exit foramina (sham C), reactivation occurred in 4 of 26 animals.

Our investigation of infection of lip, cornea, footpad, and vagina extends previous findings (*2, 3*) and demonstrates that viral latency can be established in local sensory ganglia after inoculation by a variety of routes. Viral latency following lip abrasion offers a model for studying the common cold sore. Moreover, these observations provide the first experimental demonstration that HSV-2 can spread from the vagina and cervix to lumbosacral ganglia and thus establish a latent infection. In this respect genital herpes resembles oral and ocular herpes in that local sensory ganglia may be the site of HSV latency allowing periodic reactivation and recurrence of symptoms. Ganglia may then serve as a reservoir for venereal transmission of virus. Neural spread may also account for occasional parasthesias reported to precede genital lesions and for episodes of meningitis associated with HSV-2 (*13*).

Of the many environmental events which appear to be capable of reactivating HSV in man, retrogasserian rhizotomy for the treatment of trigeminal neuralgia is perhaps the best documented (*9*). Because sciatic neurectomy peripheral to the DRG interrupts the conduit for carrying virus from ganglia to skin, and because of the limited success of previous workers who have relied upon the recurrence of keratitis (*7*), encephalitis (*6*), or other neurologic manifestations (*8*) as indices of reactivation, in our study we used the ganglia themselves as a measure of reactivation. Since virus was never detected by homogenization 14 days or more after inoculation in any of our experiments, the return of the ability of the virus to be recovered from ganglionic homogenates was taken as evidence of viral reactivation. With this method a reproducible system of HSV reactivation from DRG was achieved; this system thus provides a model for studying the effect of other stimuli on viral reactivation.

The molecular basis of HSV latency in chronically infected animals is not known. Stevens and Cook were unable to detect either viral antigen by fluorescent antibody techniques or viral particles by electron microscopy in ganglia of chronically infected animals and have suggested that the viral genome may be integrated into host DNA (*8*). However, the electron microscopic findings of Baringer and Swoveland that ganglion cells containing HSV can be found if exhaustive search methods are used (*4*) suggest the possibility that a constant but indolent productive infection may take place in vivo. In any event, insufficient amounts of virus are produced in the ganglia in vivo to allow detection by homogenization. Moreover, since HSV is a labile virus, homogenization results in considerable loss of infectivity (*14*). In vitro, however, the virus can replicate in the explanted ganglia (*3, 8*) and is readily recovered by cocultivation.

The explanation of viral reactivation after nerve section also remains unknown. At present, it is unsettled whether latent HSV resides in neurons or in surrounding satellite cells (*3, 8*). Both of these cell types undergo profound changes in metabolism after axotomy; neurons undergo chromatolysis or axonal reaction, while satellite cells react by proliferation as well as hypertrophy (*15*). Regardless of cell type, such changes allow the production of fully infectious HSV particles (recoverable by homogenization). Trauma to the sciatic nerve and cutting of small contributing branches were difficult to avoid during sham operation on group C (Table 2) which was carried out in a manner similar to that of neurectomy, entailing disarticulation of the pelvis and dissection adjacent to the spinal column. The induced viral reactivation may, therefore, be explained in a manner similar to that which followed frank nerve section itself.

The ability to reactivate virus by neurectomy raises the question of whether more subtle stimuli acting upon sensory nerves (such as minor trauma to nerve endings, alteration in sensory stimuli giving rise to changes in the pattern of neuronal discharge, or chemical modifications of the extracellular milieu) might also reactivate HSV. The capacity of such changes to

reactivate virus would be of importance not only in understanding the pathogenesis of herpetic lesions, but also in regard to the association of HSV-2 and cervical cancer (*5*, *16*). Our studies demonstrating both the establishment of HSV latency in lumbosacral ganglia after vaginocervical inoculation and the reactivation of the virus after nerve injury raise the possibility that latent HSV "activated" by the presence of a local tumor may travel down sensory nerves and secondarily infect tumor cells. This hypothesis is deserving of further investigation.

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13 February 1974

Taurine Concentrations in Congestive Heart Failure

Abstract. *The concentration of taurine in the left ventricular muscle of hearts of patients who died of chronic congestive heart failure was twice that of patients who died of other causes and had no cardiac pathology. There was no corresponding difference in taurine concentrations in aortic tissue between the two groups. Stress-induced hypertension in rats also led to an increase in taurine concentration in the heart, whereas that in skeletal muscle and brain showed no significant alteration when compared to unstressed animals. Spontaneously hypertensive rats, of the Wistar-derived Okamato strain, showed a similar elevation in cardiac taurine compared to age-matched control Wistar rats.*

Taurine (2-aminoethanesulfonic acid) is abundantly present in mammalian heart, yet its possible function there is undefined despite much speculation. Reed and Welty postulated that it has a role in ion movement in the heart (*1*), and reported that taurine reverses digoxin and epinephrine-induced arrhythmias (*2*). It increases the retention of calcium by the heart (*3*), and potentiates the inotropic effect of strophanthin-K (*4*). Since little is known about taurine in human heart, or how it is affected by drug treatment or disease states, we undertook the study described below.

The content of taurine in the left ventricle of the heart was compared in patients who died from congestive heart failure to patients who died from other causes not involving myocardial pathology (Table 1, groups 1 and 2). Patients with congestive heart failure had twice the taurine content of other patients. Samples of heart tissue received varied widely in terms of degree of hydration, fibrous nature, and percentage of fat content, making it desirable to report in several ways the specific concentrations of taurine.

Patients were diagnosed as having had congestive heart failure if they had clinical signs and symptoms of chronic congestive heart failure, secondary to rheumatic or atherosclerotic heart disease, of not less than a week and more typically of months or years duration. These diagnoses were made independently by clinicians and were confirmed by pathologists at autopsy. For most samples, analyses were performed without any knowledge on our part of the patient's medical history. Subjects less than 10 years old were excluded from the study because of the high taurine levels in the newborn (*5*) and in young children. Also excluded from groups 1 and 2 were subjects who had had any myocardial abnormality not clearly definable as chronic congestive heart failure. Infarcted or scarred muscle was not included in samples taken for analysis. All the subjects having congestive heart failure were males, but even if females were excluded from the control group, taurine concentrations in the congestive heart failure group were still significantly higher (group 3 compared to group 1).

The age at death for congestive heart failure subjects (group 1) ranged from 53 to 88 years, whereas for the controls (group 2) it ranged from 12 to 77 years. The mean age of death for group 1 was 10 years higher than that for group 2. However, a regression analysis showed no correlation between taurine concentration and age (correlation coefficient .08). This indicates that the difference in taurine concentration between group 1 and group 2 is not due to the age difference. There was no difference in

Table 1. Taurine content of left ventricular muscle of human heart. In a number of cases the sample was too fibrous and caused too much light scattering to permit a protein determination. "Wet weight" refers to sample weight as received from the morgue. Acid precipitate refers to material precipitated by trichloroacetic acid, after homogenization of the sample. Protein was determined by the biuret procedure (*9*). Numbers in parentheses are numbers of samples. Data are reported as means ± S.E.M. The significance of differences was measured by Student's *t*-test, groups 2 and 3 being compared to group 1.

Group	Sex	Age (years)	Taurine (μmole/g)		
			Wet weight	Acid precipitate	Protein
		Congestive heart failure			
1	M	65.8 ± 4.5	13.2 ± 2.1 (13)	36.7 ± 3.3 (13)	47.3 ± 8.4 (9)
		No congestive heart failure			
2	M and F	55.8 ± 3.7	5.6 ± 0.5* (23)	18.2 ± 1.6* (23)	23.6 ± 3.5† (18)
3	M	65.3 ± 3.3	5.9 ± 1.0 (8)	17.4 ± 2.9* (8)	17.4 ± 3.5‡ (7)

* $P < .001$; † $P < .005$; ‡ $P < .025$.

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