Latent Infection of Sensory Ganglia with Herpes Simplex Virus: Efficacy of Immunization

Abstract. *Mice were used to test the efficacy of active immunization in preventing latent infection of local sensory ganglia that follows inoculation of superficial epithelial surfaces with herpes simplex virus. Substantial but not complete protection was observed in animals immunized and challenged with herpes simplex virus type 1, but no protection was noted in animals immunized and challenged with herpes simplex virus type 2. Latent ganglionic infection can develop in immunized animals despite the presence of high titers of neutralizing antibody.*

A vaccine that would effectively prevent the recurrent eruptions of skin and mucous membranes caused by herpes simplex virus (HSV) would represent a major advance in controlling this disease. These eruptions are common, but not always benign, and may in fact give rise to considerable morbidity depending on the location and severity of the lesions. Clinically, HSV type 1 (HSV-1) produces the typical fever blister or cold sore in millions of people (*1*). It is a leading cause of corneal disease and in some cases produces blindness. In addition, the virus can produce encephalitis, with severe neurologic sequelae and death. HSV type 2 (HSV-2) is the cause of genital herpes and is perhaps the second most common venereal disease in the United States (*1*). Mortality may be as high as 70 percent in infants contracting the disease from the infected maternal genital tract (*2*). Recently, HSV-2 has been linked on a seroepidemiologic basis to carcinoma of the cervix (*3*). Currently available modes of therapy are inadequate for the treatment of most forms of herpes infection.

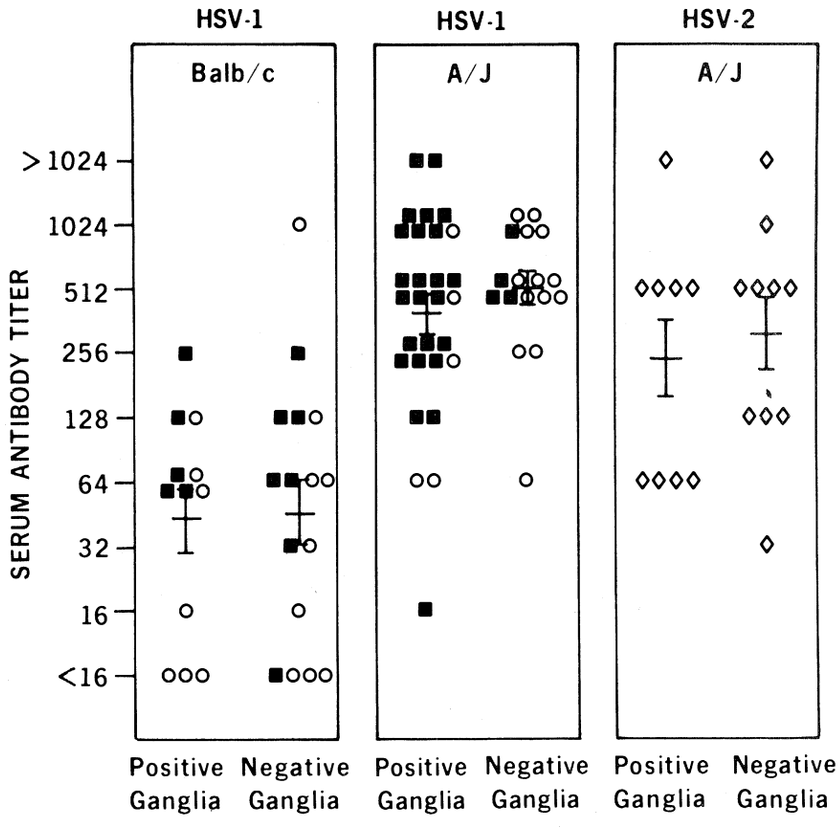

Fig. 1. Comparison of neutralizing antibody titers in immunized mice that did and did not develop a latent ganglionic infection. Animals were immunized intraperitoneally with HSV over a 6-week period prior to epithelial challenge with the virus. Symbols represent antibody titers of individual mice sampled 2 to 6 days before challenge with HSV by the footpad (squares), corneal (circles), and vaginal (diamonds) routes. Twenty-one days after challenge, the animals were killed, and the local sensory ganglia were assayed for virus by the explantation method. The results are grouped according to viral type (HSV-1 and HSV-2) and mouse strain (BALB/c and A/J). Antibody titers represent the reciprocal of the highest serum dilution producing a 50 percent reduction in viral plaques. Cross bars depict the mean ± the standard error of the mean.

Recent studies with the use of tissue explantation methods have established that HSV persists for months or years in the sensory ganglia of experimentally infected animals (*4*, *5*) and in naturally infected human subjects (*6*). These findings support the theory that recurrent epithelial eruptions are due to reactivation of endogenous virus with the sensory ganglia serving as the viral reservoir. It is generally thought that virus synthesized within the ganglion is transported via the neuronal axoplasm to the epithelial surface where subsequent replication causes a local lesion. Once the ganglion becomes infected, the intraneural location of the virus prevents the host's immunological defense mechanisms from eliminating the infection. We now report our experiments directed toward answering the question whether immunization can prevent the ganglion from becoming infected.

Female BALB/c and A/J mice (Jackson Laboratory), 4 to 6 weeks old, were used. Our preparations of HSV-1 and HSV-2, the assay with primary rabbit kidney cells, the techniques for processing ganglia by homogenization, and the methods of explantation have been described (*5*). Inoculation of epithelial surfaces with HSV results in an infection of the sensory ganglia innervating the inoculated area. During the acute phase of this infection (less than 14 days) virus can be recovered from local sensory ganglia either by homogenization or explantation, while during the latent phase (21 days to more than 1 year) virus can be recovered only by explantation (*4*, *5*).

The mice were immunized by intraperitoneal injection of live HSV-1 (CHR-3 strain) or HSV-2 (MS strain). In general, three injections were given at intervals of 10 to 14 days starting with approximately 10^4 plaque-forming units (PFU) and increasing to 10^7 PFU. Serums for determinations of antibody were collected from the retro-orbital plexus approximately 10 days after the final immunizing dose and 2 to 6 days before epithelial challenge with infectious virus; end points are expressed as the reciprocal of the highest serum dilution producing a 50 percent reduction in the number of viral plaques (*7*).

Methods of infecting local sensory ganglia by inoculating the cornea, lip, footpad, and vagina with HSV have been described (*5*). Epithelial surfaces were abraded with an emery board or wooden applicator stick, and a drop of virus or a virus-soaked cotton pledget was placed on the abraded surface. In the case of skin (thigh) infection, epilation of the area was first accomplished with Nair (Carter Products). Pooled stock virus used for HSV-1 in-

oculation contained 1.0×10^8 PFU/ml and that for HSV-2 inoculation contained 1.0×10^7 PFU/ml.

Immunization with HSV-1 decreased the mortality observed with skin and vaginal challenge with HSV-1 from a high of 52 percent to zero (Table 1). It also reduced the number of positive homogenates obtained during the acute phase of the ganglionic infection. With one exception, virus was not recovered from ganglionic homogenates of immunized animals.

In contrast, virus was recovered from explants of immunized animals inoculated with HSV-1, but the number of positive ganglia depended on the route of epithelial challenge. Prior immunization failed to protect animals from developing latent ganglionic infection when challenged by the footpad route, whereas after corneal, lip, skin, and vaginal challenge, a consistent reduction in latent infection was observed (70.4 to 95.7 percent protection). In no case did the intraperitoneal immunization produce a latent infection of the trigeminal or lumbosacral dorsal root ganglia (data not shown).

Table 1 also shows that prior immunization with HSV-2 was quite effective in reducing the high mortality produced when animals were challenged with this virus. Immunization, however, failed to prevent the development of a latent ganglionic infection with HSV-2. When the A/J strain of mice was challenged by the vaginal route, 30 percent of the surviving nonimmunized mice were latently infected, while 51 percent of the immunized mice had positive ganglia. Similarly, 41 percent of the immunized BALB/c mice were latently infected after vaginal challenge, while none of the surviving unimmunized mice had positive ganglia. This seeming increase in latent ganglionic infection in the immunized animals can be attributed to the decrease in mortality in this group, immunization preventing death but failing to inhibit the development of a latent ganglionic infection. This is most evident in the groups inoculated with HSV-2 by the footpad and corneal routes. All the unimmunized mice died while all the immunized survivors developed latent ganglionic infections. Thus it seems that immunization is able to interrupt the course of HSV-2 disease but not before a latent ganglionic infection is established; spread beyond the ganglia appears to be aborted by immunity and fatal central nervous system disease prevented. If the assumption is made that all dead animals developed ganglionic infection in the course of the illness (*8*), a formulation of total ganglionic infection can be calculated to include both dead animals and survivors with positive ganglia. Accordingly, in contrast to the reduction in ganglionic infection in immunized mice challenged with HSV-1 by the vaginal, corneal, lip, and skin routes, little difference was observed with respect to the overall development of ganglionic infection between immunized and unimmunized animals after challenge with HSV-2 by the vaginal and corneal routes (*9*).

To determine whether the reduction in ganglionic infection correlated with antibody titers, serum was collected from individual immunized animals before epithelial challenge; antibody titers of animals demonstrating positive ganglia upon explantation were compared with those of

Table 1. Viral recovery from local sensory ganglia after epithelial challenge with HSV in unimmunized and immunized mice. BALB/c or A/J mice were immunized intraperitoneally with either HSV-1 or HSV-2 while control mice were given saline. The animals were later challenged by inoculation of the footpad, cornea, lip, skin (upper thigh), or vagina with either HSV-1 or HSV-2. Animals were killed and local sensory ganglia (trigeminal ganglia after corneal and lip inoculation; lumbosacral dorsal root ganglia after footpad, skin, and vaginal inoculation) were assayed both during the acute (4 to 7 days after challenge) and chronic (3 weeks or more after challenge) phases of the infection. Ganglia obtained during the acute phase were assayed by homogenization, while ganglia obtained during the chronic phase were assayed by explantation. Results are expressed as the ratio of the number of animals with positive ganglia to the number of animals assayed. Mortality figures are given as the ratio of the number of animals that died to the number challenged. With the exception of mice inoculated by the skin and vaginal routes, the mortality incurred with HSV-1 was invariably less than 10 percent. The percentage of protection provided by immunization was computed by dividing the difference between the percentage of animals with latent ganglionic infection in the unimmunized and immunized groups by the percentage of animals with latent ganglionic infection in the unimmunized group (times 100).

Mouse strain	Intraperitoneal immunization	Route of challenge	Mortality		Homogenization of ganglia (acute phase)		Explantation of ganglia (chronic phase)		Protection (percent)
			Ratio	Percent	Ratio	Percent	Ratio	Percent	
				Challenged with HSV-1					
BALB/c	Saline	Footpad			22/29	75.9	16/21	76.2	
BALB/c	HSV-1	Footpad			0/31	0	34/41	82.9	0
A/J	Saline	Footpad			9/10	90.0	16/17	94.1	
A/J	HSV-1	Footpad			0/10	0	28/34	82.3	12.5
BALB/c	Saline	Cornea			20/20	100	28/29	96.6	
BALB/c	HSV-1	Cornea			1/20	5.0	13/71	18.3	81.1
A/J	Saline	Cornea			7/13	53.8	26/33	78.8	
A/J	HSV-1	Cornea			0/20	0	9/45	20.0	74.6
BALB/c	Saline	Lip			10/16	62.5	15/23	65.2	
BALB/c	HSV-1	Lip			0/25	0	1/36	2.8	95.7
BALB/c	Saline	Skin (thigh)	9/29	31.0	10/10	100	19/20	95.0	
BALB/c	HSV-1	Skin (thigh)	0/30	0	0/10	0	5/20	25.0	73.7
BALB/c	Saline	Vagina	10/54	18.5			22/39	56.4	
BALB/c	HSV-1	Vagina	0/36	0			6/36	16.7	70.4
A/J	Saline	Vagina	12/23	52.2			7/11	63.6	
A/J	HSV-1	Vagina	0/20	0			1/20	5.0	92.1
				Challenged with HSV-2					
A/J	Saline	Vagina	44/64	68.8			6/20	30.0	
A/J	HSV-2	Vagina	6/51	11.8			21/41	51.2	0
BALB/c	Saline	Vagina	39/66	59.1			0/23	0	
BALB/c	HSV-2	Vagina	6/40	15.0			14/34	41.2	0
BALB/c	Saline	Footpad	30/30	100					
BALB/c	HSV-2	Footpad	15/24	62.5			9/9	100	0
BALB/c	Saline	Cornea	24/24	100					
BALB/c	HSV-2	Cornea	5/24	20.8			19/19	100	0

randomly selected serums of cohorts with negative ganglia (Fig. 1). No significant difference in antibody titers ($P > .3$ by t-test) was noted between the positive and negative groups. Ganglionic infection occurred in the presence of relatively high titers of neutralizing antibody (512, or greater). Moreover, no difference in protection was observed between BALB/c and A/J mice (Table 1) even though the antibody titers of the BALB/c mice were consistently lower (Fig. 1).

The effect of hyperimmunization on viral reactivation was studied. Recently we demonstrated that latent virus can be reactivated by neurectomy (*5*). These studies showed that, in animals infected for more than 14 days, virus could only be recovered by explantation and not by homogenization of the dorsal root ganglia. However, when sciatic neurectomy was performed on such latently infected animals, virus could be recovered from homogenates of the dorsal root ganglia. In our experiment, immunized (mean neutralization titer, 1024) and unimmunized mice were challenged with HSV-1 by the footpad route and 21 days later were subjected to sciatic neurectomy; 3 days after this operation the dorsal root ganglia were removed, homogenized, and assayed for virus. Positive homogenates were obtained in 25 percent of the unimmunized and 18 percent of the immunized mice; thus, despite high titers of neutralizing antibody, virus could still be reactivated.

Our studies demonstrate that prior immunization with HSV-1 produced substantial but not complete protection against the development of a latent ganglionic infection after corneal, lip, skin, and vaginal challenge, while animals challenged by the footpad route were not protected. One possible explanation for the difference in incidence of ganglionic infection in immunized animals challenged via different routes may have to do with the degree of injury to the local epithelium at the time of viral contact. Once the virus adsorbs to nerve endings and enters the axoplasm of the neurons it is no longer accessible to antibody or immune cells. In the case of the thick glabrous surface of the footpad no bleeding was incurred by the method used to inoculate the virus. However, the thinner epithelial surfaces of the lips and thighs exhibited mild bleeding or exudation of serum after inoculation. Considerable local tissue injury also was induced after corneal scarification and vaginal-cervical abrasion. Thus, our findings are consistent with the hypothesis that, in the case of severe injury to epithelial surfaces, antiviral antibody or immune cells (or both) are able to accumulate locally, making contact with and neutralizing the virus before the virus adsorbs to nerve endings, while in the case of mild tissue injury, the virus adsorbs to nerve endings before a sufficient amount of antibody or cells has a chance to reach the virus. Also it should be recognized that the degree of protection afforded by immunization may vary with the actual amount of the viral inoculum that reaches the nerve endings and the density of the nerve endings at the injured site.

Although immunization with HSV-1 did not always prevent the development of latency, immunization did prevent the recovery of infectious virus during the acute phase of the infection when ganglia were assayed by homogenization. A possible explanation is that the explantation procedure is a more sensitive method for detecting virus than the homogenization procedure (*5, 10*). Thus, immunization, either by (i) reducing the amount of virus adsorbed to nerve endings (for example, by neutralizing the inoculum or aborting local replication), (ii) inhibiting viral spread within the ganglion, or (iii) suppressing replication of virus through direct action upon the infected cell (*11*), may have decreased the viral content of the ganglion to a level below the threshold for detection by homogenization. This possibility is supported by our studies showing that virus could be recovered from the ganglia of immunized animals during the acute phase of the infection by explantation (data not shown). Alternatively, virus in the homogenate might be inactivated in vitro by small amounts of antibody contaminating the ganglionic tissue, but the reactivation experiment described above in which virus was recovered from homogenates of hyperimmunized animals argues against this being a major factor.

Previously, in most animal models designed for studying the efficacy of immunization against HSV, mortality or skin lesions were used as indices of protection (*12*). Since it is now generally accepted that recurrent herpetic lesions are due to persistence of virus in the sensory ganglia, an important question is whether immunization can prevent the host from developing latent ganglionic infection. Our animal model appears useful for studying this question and has already pointed to several major biological problems that must be considered in protective immunization against HSV. In addition to the potential oncogenicity of partially inactivated virus (*13*), inoculation with live virus can itself establish a latent ganglionic infection. Moreover, our experiments show that infection of ganglia can take place in immunized animals and that the state of the epithelial surface at the time of viral challenge may be an important factor in determining whether immunization is protective. Indeed, even when the neutralizing antibody titer was high, a latent ganglionic infection developed in a significant number of animals immunized and challenged with HSV-1, while no protection was observed in animals immunized and challenged with HSV-2. Whether HSV-2 is more "gangliotropic" than HSV-1, or whether the rate of neutralization of HSV-2 at the site of inoculation is slower than with HSV-1, or whether some other factors are involved remains to be determined. Before, however, it can be concluded that immunization against HSV-2 is invariably less effective in preventing a ganglionic infection than immunization against HSV-1, additional studies must be carried out with other prototype strains of the virus in mice and other animals. These studies are of particular importance because of the potential link between HSV-2 and carcinoma of the cervix (*2, 13*).

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8. In the case of vaginal and footpad inoculation this assumption is supported by the observation that all dying animals first exhibited bilateral hind leg paralysis. This paralysis was due to the development of transverse myelitis at the spinal cord segment corresponding to the involved ganglia.
9. Total ganglionic infection was calculated by taking the sum of the number of mice with positive ganglia plus the number of mice that died and dividing it by the number of animals challenged. In the few cases where not all survivors were assayed, a correction factor was employed. When these calculations were carried out for animals infected with HSV-2 by the vaginal route, it was found that 78 percent of the unimmunized and 63 percent of the immunized A/J mice had positive ganglia, while 59 percent of the unimmunized and 50 percent of the immunized BALB/c mice had positive ganglia. By the same formulation, 100 percent of the immunized and unimmunized mice infected by the corneal and footpad routes with HSV-2 had positive ganglia.
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