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Herpes Simplex Virus Infection in Guinea Pigs: an Animal Model for Studying Latent and Recurrent Herpes Simplex Virus Infection

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Footpad infection of guinea pigs with herpes simplex virus led to an acute local inflammatory reaction, followed by a persistent latent infection of lumbosacral dorsal root ganglia. Spontaneous reactivation of the latent virus occurred, leading to recurrent lesions at the site of the initial infection. Clinical observations and virological studies during the acute latent and recurrent infection are reported.

Primary herpes simplex virus (HSV) infections of man, whether developing with clinical symptoms or asymptomatically, may lead to persistent latent infections. Goodpasture's (4) hypothesis that the virus remains in a noninfectious state within sensory ganglia has been substantiated recently (1-3). In many individuals the virus can be reactivated by certain stimuli, such as exposure to sunlight and physical or emotional stress, to produce a recurrent disease.

The mechanism by which these reactivations occur is far from being understood. Animal models should help to investigate why and how the virus is kept in a latent state during most of the time and can escape the host's control at other times. Experimentally infected rabbits and mice were shown to harbor latent infections after recovery from the acute disease. After ocular infections of rabbits latent virus was demonstrated in the ganglion Gasseri (7). Recurrence of clinical symptoms and of virus production at the site of the initial infection occurred spontaneously or could be induced by epinephrine administration (6). In addition, latent virus was recovered from the spinal ganglia of mice infected in the footpad (8). However, all attempts to induce recurrence of clinical symptoms failed in these animals (7). The present report presents another animal model, i.e., HSV infection in guinea pigs. In these animals primary infection in the footpad results in a local inflammatory reaction only. However, the virus persists in a latent form in the spinal ganglia and gives rise to spontaneous recrudescence at the site of the initial infection.

MATERIALS AND METHODS

Virus. HSV type 2, strain 72, was grown in primary rabbit kidney (PRK) cells in Eagle minimal essential medium without serum. Virus plaque titrations were performed in secondary chick fibroblasts under a fluid overlay containing 0.2% rabbit anti-HSV hyperimmune serum.

Infection of animals. Male white Hartley guinea pigs, weighing 250 to 450 g, were inoculated subcutaneously in one or both hind footpads with 10^4 plaque-forming units of HSV in 0.2 ml.

Direct virus isolation from various organs. Animals were exsanguinated at the appropriate times. Pertinent tissues (liver, spleen, kidney, brain) were removed, weighed, ground in a mortar with sea sand, and made up to a 10% (wt/vol) suspension of tissue in Eagle minimal essential medium. All soft tissue (skin, muscles, tendons) was thoroughly removed from the bones of the feet and then treated as described above. Blood was defibrinated, and the cells were disrupted by three cycles of freezing and thawing. The suspensions obtained were centrifuged at low speed, and the supernatant were used for virus assay. All quantitative virus determinations were performed as plaque assays on secondary chick fibroblasts. Qualitative assays were done by inoculation of PRK cells, which were then observed for cytopathic effect for 2 weeks.

Virus isolation from lymph nodes. Lymph nodes were minced and cells were suspended in a small amount of medium by heavy pipetting. These cell suspensions were seeded on monolayers of PRK cells.

Virus isolation from spinal ganglia and sciatic nerves. The dorsal root ganglia of the 4th lumbal to the 2nd sacral segment were removed from exsanguinated animals, finely minced, washed once in Hanks balanced salt solution, and treated with 0.5% trypsin for 20 min at 37 C. The trypsin was removed by centrifugation, and the ganglia fragments were washed once in medium and then suspended in about 2 ml of Eagle minimal essential medium. The suspension was usually divided into two aliquots, one of which was assayed for latent virus by co-cultivation on PRK cells. The other one was subjected to three cycles of freezing and thawing. After low-speed centrifugation the supernatant was assayed for infectious virus. The sciatic nerves were treated similarly.

Co-cultivation of tissues. Suspended fragments of ganglia or sciatic nerves were seeded on monolayers of

PRK cells. Two to four petri dishes were inoculated with each sample, and 5 ml of Eagle minimal essential medium containing 5% inactivated fetal calf serum was added per culture. The short trypsinization of the tissues improved the adherence of the ganglia on the monolayer and the outgrowth of cells considerably. The medium was changed weekly and the cultures were observed for cytopathic effect for 6 to 8 weeks. Virus isolates were harvested from cultures showing complete cytopathic effect by three cycles of freezing and thawing. The isolates were passed once on PRK cells before serological identification.

Identification of virus isolates. All isolates from ganglia and nerves, as well as isolates from those feet showing recurrence of the infection, were identified by neutralization by a rabbit anti-HSV hyperimmune serum.

RESULTS

Clinical observation. HSV inoculation in the footpad resulted in a local inflammatory reaction, starting about 24 h after infection. Maximum reddening and swelling occurred at about 48 h. Most of the infected animals also developed vesicles between 4 and 7 days after infection. The symptoms declined gradually after 1 week and usually disappeared completely between 2 and 3 weeks. Very few animals (less than 1 in 100) developed paresis in the ipsilateral leg between 1 and 3 weeks, progressing into paraplegia. Observation of the animals over a prolonged period of time revealed that nearly all of the infected guinea pigs developed recurrent lesions at the site of the initial infection. The foot would again show signs of inflammation, usually accompanied by vesicles or hemorrhagic macules. The frequency with which herpetic lesions recurred varied considerably.

Between 80 and 90% of the infected guinea pigs developed at least two exacerbations during the first 4 months after primary infection. The frequency of the recurrent lesions decreased later. However, exacerbations occurred up to at least 1.5 years after initial infection, the longest time animals have been under observation until now. The duration of clinical symptoms varied between 2 days and 4 weeks. Figure 1 shows the time course of recurrent infections in a group of guinea pigs observed between 20 and 200 days after infection. No exogenous stimuli responsible for the recrudescence could be traced so far, since all animals were kept under identical conditions in one room, caged in groups of three or four.

Virus assays. Virus in the inoculated foot were detectable during the acute infection for several days (Fig. 2). Titers varied considerably from animal to animal, but peak titers occurred generally at 24 h after infection. At 4 and 7 days after infection virus was recovered from a proportion of the animals only (8 out of 10 and 5 out of 13, respectively). By day 11 virus was no

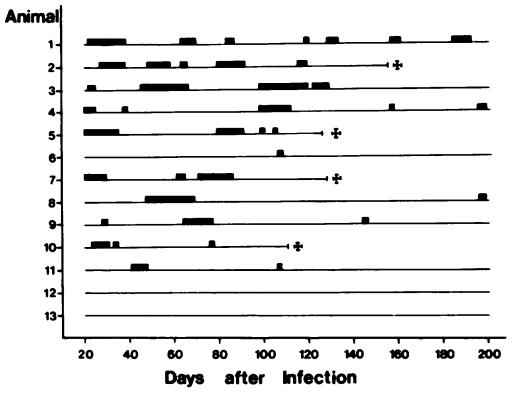

FIG 1. *Time course of clinical symptoms of recurrent infections. Bars represent the duration of recurrent lesions.*

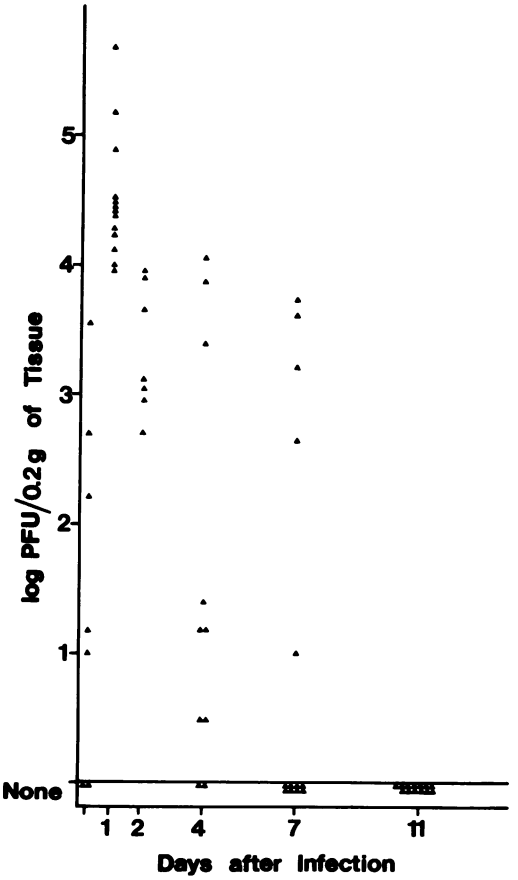

FIG. 2. *Virus titers in infected feet at different times after infection. Each point indicates the titer in the infected foot of one animal.*

longer detectable in the infected foot. Spread of virus to draining lymph nodes was demonstrated during the early phase of infection (Table 1). However, no virus was ever isolated from homogenized blood, liver, kidney, spleen, and brain between 1 and 56 days after infection (data not shown).

To examine whether the virus spreads to sensory ganglia and can establish latent infections there, the sciatic nerve and its dorsal root ganglia were assayed for infectious or latent virus as described in Materials and Methods (Table 2). HSV was isolated from the sciatic nerve during the acute phase of infection. Positive results were obtained by both methods, i.e., by maintenance of nerve sections in culture as well as by inoculation of PRK cells with tissue extracts. Recovery of HSV from spinal ganglia, on the other hand, was possible only after cultivation of ganglia explants. By this method virus could be detected in ganglia as early as 24 h after infection (two out of six animals). From then, the ganglia of a considerable proportion of animals were shown to harbor latent HSV.

Cytopathic effect developed in the co-cultures of ganglia with PRK cells between 10 days and 8 weeks after onset. Replicate cultures of specimens of the same animal often differed widely in that time, after which cytopathic effect appeared. In addition, often only a proportion of the replicas became positive. Table 3 shows some examples of the course of these co-cultivation experiments. These variations of the results obtained in replicate cultures suggest the probably only very few ganglionic cells or focal areas of the cells harbor the latent infection. Viral assays, performed at the time of recurrent infections, showed that HSV could be recovered from the affected foot and the sciatic nerve. Moreover, infectious virus was also isolated from the spinal ganglia on these occasions (Table 4).

TABLE 1. *Recovery of HSV from draining lymph nodes*

Days after infection	Virus recovered from lymph nodes (animals positive/animals tested)		
	Popliteal nodes	Deep inguinal nodes	Superficial inguinal nodes
0.25	2/2	0/2	Not tested
2	4/4	4/4	4/4
7	1/4	0/4	1/4

TABLE 2. *Recovery of HSV from sciatic nerves and lumbosacral ganglia at different times after infection*

Days after infection	Virus recovered (animals positive/animals tested)			
	Dorsal root ganglia		Sciatic nerves	
	Extract	Organ culture	Extract	Organ culture
0.25	0/2	0/2	0/2	0/2
1	0/6	2/6		
2	0/4	3/4	2/4	3/4
7	0/4	7/14	1/4	2/4
25	0/4	3/4		
87	0/5	4/5		
197	Not tested	1/1		

TABLE 3. *Appearance of cytopathic effect in cultures of dorsal root ganglia or sciatic nerve co-cultivated with PRK cells*

Tissue taken on day (post-infection):	Animal	Cultures of:				Negative cultures discarded on day:
		Ganglia		Sciatic nerve		
		Positive	Day	Positive	Day	
2	1887	1/4	12	1/4	33	33
	1889	1/4	26	4/4	26	33
	1881	0/4		2/4	11	25
	1880	1/4	25	0/4		25
87	1734	0/4				35
	1752	2	13			
		1	18			
		1	20			
		4/4				
	1758	2/4	29			37
	1760	2	29			
		1	43			
		3/3				
	1766	1	9			
		1	14			
		1	31			
		3/4				44

TABLE 4. *Recovery of HSV during recurrent infection*

Days after infection	Virus recovered from:				
	Foot homogenate	Sciatic nerve		Dorsal root ganglia	
		Extract	Organ culture	Extract	Organ culture
152	−	−	+	+	+
193	+	+	+	+	−
207	+	−	−	−	+
239	+	−	−	−	−

DISCUSSION

Stevens and Cook (7) and Stevens et al. (8) recently demonstrated in experimentally infected mice and rabbits that HSV travels from the site of primary infection via the peripheral nerve to the sensory ganglia. After an initial period of productive infection in ganglionic cells, the virus establishes a persistent latent infection there. The HSV persists in some subviral state, since no virus can be isolated from homogenized ganglia, and no virus particles or viral antigens can be detected within the cells. However, reactivation of this latent virus can be induced in vitro by explanting latently infected ganglia in tissue culture. The demonstration of latent infections of sensory ganglia has recently been extended to the finding of latent HSV infections in the Gasserian ganglion and in dorsal sacral ganglia in humans (1-3).

In the guinea pig model described in this report subcutaneous HSV infection resulted in the virus spreading along the sciatic nerve to the lumbosacral ganglia within 24 to 48 h. However, unlike in infections of mice and rabbits, virus was never detectable in extracts of homogenized ganglia during the acute phase. The HSV strain used in these experiments has a very low neuropathogenicity for guinea pigs, demonstrated by the rare cases of paresis in the animals. This low incidence of neurological symptoms might be correlated with a primary nonproductive infection of ganglionic cells. However, the experiments presented do not allow a distinction between possible nonproductive infection and low-degree productive infection. The latter form of infection might escape detection by standard virus isolation procedure. Explants of the infected cells in tissue culture probably provide a more sensitive assay for detection of very few virus particles. The same considerations apply to the demonstration of "latent" virus during the clinically silent phases of persistent infection. Further studies by means of electron microscopy and immunofluorescence techniques should help to clarify the mode of the acute and persistent HSV infection of guinea pig ganglia.

In contrast to the findings of virus, detectable only by cultivation of the ganglia during acute and persistent infection, infectious virus was isolated from ganglia during the recurrent disease. These findings, together with the simultaneous isolation of HSV from the sciatic nerve, support Stevens' suggestion (7) that recurrent lesions occur after reactivation of the latent virus in the neurons and the subsequent centrifugal migration of the virus along the nerve to the skin.

Compared to experimental HSV infections of rabbits and mice the guinea pig model may have several advantages for investigations of latent and recurrent HSV infections. (i) Guinea pigs are more convenient than rabbits for studies needing large numbers of animals. On the other hand, they are big enough to allow follow-up studies of immune responses or other factors possibly influencing the course of the infection. (ii) Unlike latently infected mice guinea pigs undergo recurrent disease. (iii) No animals are lost by the primary infection.

In conclusion, this model may offer a possibility for investigation of the pathogenesis of latent and recurrent HSV infections and the mechanisms involved in the regulation of the virus-host relationship, such as humoral or cell-mediated immunity and hormonal or metabolic variations.

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