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Pathogenesis of Herpes Simplex Virus Infections in Guinea Pigs

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The pathogenesis of herpes simplex virus types 1 and 2 has been studied in guinea pigs after inoculation by various routes (subcutaneous and intradermal infection in footpads and vaginal infection). Clinical observations as well as virus isolation studies are reported. Herpes simplex virus type 2 infection by all three routes of inoculation led to acute primary and recurrent lesions. Virus persisted in the nervous system, particularly in sensory ganglia, and locally at the site of inoculation. Herpes simplex virus type 1 infection induced no or very mild primary symptoms. Recurrent lesions were only observed after intradermal inoculation. Invasion of the nervous system and consequent establishment of latent ganglionic infection was less efficient than after herpes simplex virus type 2 infection. Peripheral persistence was, however, equally common.

The pathogenesis of herpes simplex virus (HSV) has been studied in a variety of animal models, most extensively in mice and rabbits (1a, 3, 8, 10, 17, 18, 21, 22). We have previously reported on subcutaneous (s.c.) and vaginal infections of guinea pigs with HSV type 2 (HSV-2) as an animal model for studying acute, latent, and recurrent herpes (12, 13). The HSV-2 infection of guinea pigs differs from experimental infections of other species in two main aspects: (i) guinea pigs develop frequent spontaneously recurring herpetic lesions; and (ii) virus persists in this animal species not only in the nervous system but also in peripheral tissue, i.e., skin or mucosa at the site of initial inoculation (14, 15).

We now present considerably extended data from clinical observations and virological studies of this model. In addition, the difference between pathogenesis of HSV-1 and HSV-2 in these animals has been studied.

Concerning the nomenclature for long-term infections used in this paper, the term "latent" infection defines a presumably nonproductive state of the virus, whereas "chronic" infection means a productive infection. "Persistent" infection is used for all long-term infections without consideration of the state in which the virus is maintained.

MATERIALS AND METHODS

Viruses. HSV-2 strain 72 was used in its 8th to 10th tissue culture passage after isolation from a patient's lesion. HSV-2 strain MS and HSV-1 strains HF and McIntyre were obtained from the American Type Culture Collection. HSV-2 strain Curtis was received from H. F. Maassab, Ann Arbor, Mich. All other virus strains were isolated from clinical material in Erlangen, Germany (H. zur Hausen) and in Copenhagen (B. Faber Vestergaard) and were used in their third or fourth tissue culture passage. These strains had been typed as HSV-2 or HSV-1 either by deoxyribonucleic acid hybridization (11) or by crossed immunoelectrophoresis (20) and in addition by their ability to form plaques in chicken fibroblasts (7). The viruses were propagated in primary rabbit kidney cells (PRK) or in MRC-5 cells. Viruses were plaque-titrated on Vero cells under a fluid overlay containing 0.2% of an antiserum against HSV-2 prepared in rabbits.

Animal experiments. Outbred white Hartley guinea pigs were bought from dealers in Germany. Inbred strain 2 guinea pigs came from our own breeding colony. All animals were infected at between 2 to 3 months of age. Three different routes of inoculation were used. (i) For s.c. footpad inoculation, 10^4 plaque-forming units (PFU) in 0.2 ml was injected into the left hind footpad. (ii) For intradermal (i.d.) footpad inoculation a drop of virus suspension was applied on the left hind footpad, and the virus was injected into the skin with a spring-loaded vaccination instrument (Sterneedle, Ormont Drug Co.) as described by Hubler et al. (4). (iii) For vaginal inoculation, a piece of gelatin foam (Spongostan, Ferrosan, Denmark) was soaked in 0.2 ml of virus suspension, containing 10^4 PFU, and immediately placed into the vagina. All animals were monitored for clinical symptoms daily for 2 weeks and 3 times per week subsequently.

Virus isolations. Animals were exsanguinated, and pertinent tissues were removed and finely minced. For isolation of virus from homogenated organs, a small volume of Eagle minimal essential medium was added to the tissue fragments, and samples were frozen and thawed three times and then either homogenized by ultrasonication or ground up in a mortar with sea sand. After removal of the debris by low-speed centrifugation, the supernatants were inoculated onto PRK. For virus isolation from tissue explants, the tissue fragments were treated with 0.25% trypsin for

20 min at 37°C. Trypsin was removed by centrifugation of fragments, and the fragments were washed once in minimal essential medium and then explanted on PRK. All cultures were maintained in minimal essential medium (in which glucose was replaced by galactose to stabilize the pH) supplemented with 5% fetal calf serum and incubated at 36°C. The medium was changed 7 days after establishing the cultures. If cytopathic effect did not occur earlier, cocultures of nerve tissue and all cultures of tissue extracts were maintained for 2 weeks, whereas cocultures of nonneural tissues were maintained for 7 days only. Negative cultures were then passaged once on PRK after three cycles of freezing and thawing. The different incubation time of primary cocultures resulted from the experience that in cocultures of nonneural tissues infectious virus usually appears within the first 3 to 5 days and never later than 7 days. In nerve tissue, particularly in ganglia, on the other hand, latent virus is only rarely activated to a productive stage before day 7 (15). All isolates were identified as HSV by neutralization with a hyperimmune serum prepared in rabbits.

RESULTS

s.c. infection. (i) Clinical observations. s.c. footpad inoculation with the standard dose of 10^4 PFU of HSV-2 strain 72 resulted in an inflammatory reaction of the infected footpad in about 60% of the animals, accompanied by vesicle formation. These symptoms usually disappear between days 14 and 20, although they may occasionally persist longer. Recurrent vesiculation may develop at any time thereafter. Thus, the period from days 0 through 20 is arbitrarily taken as the acute phase of infection; the chronic phase starts on day 21. The details of the acute and recurrent clinical symptoms have been described earlier (12).

Table 1 summarizes clinical observations in a total of 124 white female guinea pigs infected within the period of 1 year. All animals were observed for 100 days after infection. Only development of vesicles was assessed as herpetic lesions, since inflammation alone may be caused by other means, particularly by trauma. By the criterion of vesiculation, 58% of animals developed primary lesions and 62% developed recurrent lesions. The development of recurrent lesions was apparently not related to prior development of primary lesions, since the rate of animals showing recurrent herpes was not significantly different in the group with or without primary lesions as assessed by the χ^2 test. The majority of animals with recurrent herpes had their first eruption before day 50 postinfection (p.i.), and most of them also developed more than one exacerbation within the observation period.

Subsequently, a number of HSV-2 as well as HSV-1 strains were assayed for their ability to induce primary and recurrent herpes in guinea pigs. Laboratory-adapted strains as well as recent clinical isolates were used. The data in Table 2 show that all strains of HSV-2 tested induced primary and recurrent lesions in a fashion comparable to that of strain 72. None of the HSV-1 strains, on the other hand, induced recurrent herpes. Only strain Brand, after a high-

TABLE 1. *Clinical symptoms observed in a total of 124 guinea pigs infected s.c. with 10^4 PFU of HSV-2 strain 72*

Animals	No. with symptoms/no. tested (%)
With primary lesions	72/124 (58)
Transient paresis	1/124 (0.8)[a]
Recurrent lesions	76/123 (62)
With primary lesions	
Developing recurrent lesions	49/71 (69)
Without recurrent lesions	22/71 (31)
Without primary lesions	
Developing recurrent lesions	27/52 (52)
Without recurrent lesions	25/52 (48)
With recurrent lesions	
Developing first exacerbation between days 21 and 50	64/76 (84)
Developing two or more exacerbations until day 100	55/76 (72)

[a] One animal was killed on day 20, because it gnawed off its foot.

TABLE 2. *Clinical observations after s.c. infection with various strains of HSV*

Strain	Dose (PFU)	Ratio of animals with	
		Primary lesions	Recurrent lesions
HSV-2			
Old laboratory strains			
Curtis	10^5	9/12	4/12
	10^3	0/12	0/12
MS	10^4	3/6	3/6
Clinical isolates			
Klee	10^5	7/12	9/12
	10^4	2/8	0/8
K 41	10^4	7/8	6/8
K 67	10^4	5/8	3/8
K 979	10^4	5/8	7/8
K 58	10^4	7/8	5/8
K 937	10^4	7/8	4/8
HSV-1			
Old laboratory strains			
HF	10^7	0/16	0/16
McIntyre	10^5	0/16	0/16
Clinical isolate			
Brand	10^7	12/12	0/12
	10^5	3/12	0/12

dose inoculation, produced vesicles during the acute phase of infection.

(ii) Influence of the genetic background of animals for the development of recurrent herpes. Previously we had reported that up to 80 to 90% of Hartley guinea pigs developed recurrent herpes (12). Later observations of accumulating numbers of animals showed that the proportions varied considerably between different shipments of animals and even more so between animals obtained from different sources. To investigate a possible influence of the animals' genetic background for the development of recurrent herpes, we mated animals which had shown frequent recurrent eruptions on the one hand and animals which had never developed recurrent herpes on the other and assayed their offspring. The progeny animals were routinely infected at the age of 2 months, when the maternal antibodies had disappeared. A significantly higher percentage of the offspring from positive parents developed recurrent lesions (59 of 90 [66%]) compared with the offspring from parents without recurrent herpes (10 of 45 [22%]). In addition, we compared the rate of recurrency-positive animals in inbred strain 2 guinea pigs and in outbred Hartley guinea pigs. Both groups were infected in the same time period and observed for 80 days after infection. Only 27 of 72 (37.5%) of strain 2 animals developed recurrent herpes, compared to 97 of 184 (53%) of Hartley guinea pigs. In addition, strain 2 guinea pigs only rarely had more than one exacerbation.

(iii) Spread of HSV-2 during the acute phase of infection. Table 3 summarizes the results of various experiments of virus isolation from explanted tissues. All animals had been infected with strain 72. Previously we have shown that virus isolation from homogenated footpad skin was successful in all animals up to day 2 p.i. and then gradually declined to 0% by day 11 (12). We now show that by the method of tissue explantation, virus remained recoverable from the inoculated sole. The infection spread within the skin into more proximal parts of the limb and very rapidly into draining lymph nodes. As shown before (12), virus also migrated into the sciatic nerve and up into the dorsal root ganglia (DRG). Analyses of ganglia involved showed that the infection was restricted to the ipsilateral sacral ganglia (S_{1-3}; data not shown). The virus was found in the ganglia in some animals as early as 24 h p.i. and in about 50% of the guinea pigs from day 7 onward.

(iv) Virus isolation during the chronic phase of HSV-2 infection. Animals infected with HSV-2 strain 72 were assayed for persistent virus infection between 20 and over 300 days p.i. All animals were killed at times when they did not show clinical signs of recurrent infection, and various organs were tested by the cocultivation method (Table 4). Persistent virus was found in the DRG of 55% and in the lumbosacral part of the spinal cord of 32% of animals assayed. In marked contrast to other animal models, however, virus was also detected in sciatic nerves (43%) and almost regularly in the skin of the footpad (95%). The rate of virus recovery in all of these organs seemed independent of the time after infection when the animals were killed. In

TABLE 3. *Spread of HSV-2 during the acute phase of s.c. infection (isolation from tissue explants)*

Tissue	No. positive/no. tested at day p.i.:					
	0.25	1	2	4	7	16
Skin						
Sole of foot			4/4	16/17	10/11	4/4
Upper part of foot				2/4	0/4	1/4
Shin				1/7	1/7	1/4
Thigh					1/4	1/3
Lymph nodes						
Popliteal	2/2		8/8	3/17	1/17	1/4
Inguinal deep	0/2		4/4		0/8	
Inguinal superficial			4/4		0/8	
Sciatic nerve	0/2		4/8	1/7	7/15	2/4
Sacral DRG	0/2	2/6	3/8	3/16	14/29	3/4

TABLE 4. *Recovery of HSV-2 from tissue explants during the clinically inapparent phase of infection (s.c. footpad inoculation)*

Day after infection	No. positive/no. tested					
	Footpad skin	Shin skin	Popliteal lymph node	Sciatic nerve	DRG	Spinal cord
20–30	9/9			4/9	9/19	1/5
31–100	22/22			4/5	34/50	
101–200	62/66	0/11	0/8	6/20	23/46	4/18
201–300	9/9			5/6	5/11	2/2
>300	1/2			1/6	4/9	1/3
Total	103/108	0/11	0/8	20/46	75/135	9/28

spite of the occasional spread of the virus within the skin during the acute phase of infection, persistent skin infection was apparently restricted to the initially inoculated area, i.e., the sole. Also, no virus remained detectable in draining lymph nodes.

Virus isolation studies revealed no difference in the behavior of HSV in Hartley and strain 2 guinea pigs during the acute or chronic phase of the infection (data not shown).

In contrast to virus recovery from explanted tissue, virus was isolated from only 8% of homogenated footpad skin and never from homogenated nerves or ganglia during the quiescent phase of infection. Infectious virus could, however, be demonstrated in ganglia, nerves, and skin at times when fresh recurrent lesions had developed (Table 5).

(v) Virus isolation from HSV-1-infected animals. In contrast to HSV-2, HSV-1 strain Brand apparently did not spread to other areas, but remained restricted to the skin at the site of inoculation (Table 6). In addition, tests with three different strains of HSV-1 showed that persistent virus could be recovered from footpad explants nearly as regularly as the HSV-2, but was never found in the ganglia or central nervous system.

i.d. infection. Clinical observations of animals infected i.d. into the footpad with graded doses of HSV-2 or HSV-1 showed that primary lesions developed regularly with both types of virus and, in addition, that these lesions were more severe than after s.c. infection. In contrast to s.c. infections, i.d. infection with HSV-1 induced recurrent lesions in some animals (Table 7).

The spread of HSV-2 after i.d. infection was shown to be largely similar to the spread after s.c. inoculation. Latent ganglionic infection was, however, more frequent than after s.c. infection. In addition, one of eight animals infected with the high dose of HSV-1 could be shown to harbor latent virus in DRG (Table 8).

Vaginal infection. (i) Clinical observations. The symptoms induced by HSV-2 strain 72 have been described previously (13). Briefly, a rather severe primary disease is observed in most of the animals with inflammation and extensive vesiculation on the vulva, developing into deep ulcers. Urinary retention, diarrhea, and paresis of the hind legs may be observed in addition to the local symptoms. Spreading of inflammatory reactions and sometimes vesicles in the skin of the entire lower part of the body can be observed after removal of hair. Usually, this spread reaches the hind footpads within 10 to 14 days p.i. The infection is always lethal in strain 2 guinea pigs within 10 to 20 days p.i. Some of the Hartley guinea pigs, on the other hand, may develop less severe symptoms (up to 20%) and then survive with complete healing of symptoms during the third week after infection. These animals usually develop frequent recurrent genital herpes which induces, however, only mild symptoms. One or two vesicles on a red halo can be observed on the vulva and usually disappear within 2 to 3 days. Recurrent lesions may also be observed on the hind footpads, sometimes simultaneously with recurrent genital lesions, but also independently.

In contrast to HSV-2 infection, vaginal HSV-1 infections with two different virus strains induced very mild symptoms. Strain McIntyre led only to an inflammation of the vagina, observed between days 3 and 6; animals infected with strain Brand developed one or two vesicles on

TABLE 5. *Virus isolation from homogenated tissues during the quiescent and recurrent state of infection (assayed between day 21 and 200 p.i.)*

Tissue	No. positive/no. tested during phase	
	Quiescent	Recurrent
Footpad skin	3/39	8/10
Sciatic nerve	0/11	2/5
DRG	0/16	3/7

TABLE 6. *Isolation of virus from tissue explants after s.c. footpad infection with HSV-1*

Virus strain	Day p.i.	No. positive/no. tested					
		Footpad skin	Shin skin	Popliteal lymph node	Sciatic nerve	DRG	Spinal cord
Brand	4	3/3	0/3	0/3	0/3	0/3	
	7	3/3	0/3	0/3	0/3	0/3	
	>100	3/3				0/3	0/3
HF	50–100	1/2			1/8	0/8	0/6
McIntyre	50–100	1/5			0/5	0/5	
	>100	6/6			0/6	0/6	0/6

TABLE 7. *Clinical observation after i.d. inoculation with HSV-1 or HSV-2*

Virus	Dose (PFU/ml)	No. with lesions/no. tested	
		Primary lesions	Recurrent lesions
HSV-2 72	2×10^6	8/8	5/8
	2×10^5	8/8	5/8
	2×10^4	8/8	3/8
HSV-1 Brand	6×10^7	8/8	3/8
	6×10^6	8/8	3/8
	6×10^5	8/8	3/8

TABLE 8. *Virus isolation from tissue explants after i.d. infection*

Virus	Tissue	No. positive/no. tested at day p.i.:				
		4–5	7	20–50	51–100	362
HSV-2	Skin					
	Sole of foot	7/8		10/11		
	Upper part of foot	1/4				
	Shin	1/4				
	Thigh	0/4				
	Lymph nodes					
	Popliteal	1/3				
	Inguinal deep	3/3				
	Inguinal superficial	2/3				
	Sciatic nerve	2/4				
	DRG (S_{1-3})	3/8	2/3	10/11	4/5	4/5
HSV-1	Skin of sole				8/8	
	DRG (S_{1-3})				1/8	

the vulva, and the vesicles disappeared after a few days. None of the HSV-1 strains led to recurrent herpes.

(ii) Virus isolation. The spread of HSV-2 strain 72 during the acute phase of vaginal infection has been studied previously in detail by assaying tissue explants as well as homogenates (13). Table 8 shows that invasion of the nervous system after vaginal infection is more efficient than after s.c. footpad inoculation. Virus was recoverable in 100% of the animals from DRG and spinal cord tissue during the acute phase. The proportion of animals harboring latent nervous sytem infection declined gradually with time. Only three of seven animals assayed later than 1 year p.i. were positive for latent virus in their ganglia. Virus remained recoverable from the vagina for up to 200 days p.i. and then disappeared. As with footpad infection, the persistence of virus in the peripheral tissue was restricted to the inoculation site, i.e., the vagina. Virus did not remain in secondary sites of peripheral infection (i.e., vulva, cervix, or footpad skin). HSV-1 also migrated into ganglia after vaginal infection and was shown to persist in the vagina and in one of four animals in the ganglia at 2 months p.i.

DISCUSSION

The pathogenesis of HSV-1 and HSV-2 infection has been studied in guinea pigs after s.c., i.d., and vaginal inoculation. Marked differences have been found in the behavior of the two subtypes of the virus.

HSV-2 was shown to induce acute primary lesions, persistent infection, and recurrent herpes after inoculation by all three routes. The acute phase resulted in herpetic lesions at the site of inoculation; the lesions showed a tendency to spread into neighboring skin areas. This spreading was most pronounced after vaginal inoculation. The vaginal infection also usually resulted in severe symptoms of nervous system involvement: paraplegia and dysfunction of bladder and rectum. Recurrent herpes after footpad inoculation was restricted to the initially inoculated foot. Lesions in the contralateral foot were extremely rare (data not shown). After vaginal infection, however, recurrent herpes occurred not only on the vulva, but also, although less frequently, in the hind footpads.

No differences were observed in the pathogenicity of a number of type 2 strains tested in the s.c. infection. However, differences were found in the development of symptoms in different strains of guinea pigs. Thus, primary vaginal infection was more severe in strain 2 inbred guinea pigs than in Hartley outbred animals. On the other hand, recurrent footpad herpes was significantly less frequent in strain 2 (37.5%) than in Hartley animals (53%). In addition, the offspring of recrudescence-positive Hartley animals developed recurrent herpes more frequently than did the offspring of recrudescence-negative animals. Genetically determined differences in susceptibility to acute HSV infection of mice have been observed by several groups (1, 5, 6, 9). We present here for the first time evidence from an animal model that susceptibility to recurrent herpes might also be genetically controlled. Whatever this control mechanism is, it obviously acts after the establishment of latent infection, since we found no difference between Hartley and strain 2 guinea pigs in the frequency of persistent ganglionic or skin infection. However, establishment of the persistent infection may also be genetically determined. Thus, Donnenberg et al. (2) found that in HSV-2-infected inbred strain 13/N guinea pigs only 45% harbored virus in their skin and 33% harbored virus in their DRG, as compared to 95 and 56%, re-

spectively, of our animals.

In contrast to HSV-2, HSV-1 infections resulted in only mild acute symptoms which remained localized at the site of inoculation and never spread to other areas of the skin. Recurrent lesions were only observed after i.d. inoculation of HSV-1. The pathogenicity of HSV-1 was also strain dependent: a recent clinical isolate was more pathogenic than the old laboratory-adapted strains HF and McIntyre.

The migration of virus into the nervous system and establishment of latent infections in DRGs was markedly different after HSV-1 and HSV-2 infection, but was also dependent on the route of inoculation; i.d. and vaginal inoculation were more efficient in leading to invasion of ganglia with both subtypes than was s.c. inoculation. After s.c. infection, HSV-1 never migrated into ganglia; HSV-2 was found to invade ganglia in about 50% of the animals. Once it got into ganglia, however, it apparently always established a long-lasting latent infection, since no reduction in the proportion of animals harboring latent ganglionic infections was found with increasing time after inoculation (Table 4).

i.d. and vaginal infection with HSV 2, on the other hand, resulted in invasion of the nervous system in almost 100% of the animals. Again, after i.d. infection, the virus, once inside the ganglia, was never eliminated. On the other hand, after vaginal infection the proportion of animals harboring latent ganglionic infection gradually decreased for up to more than 1 year after infection (Table 9). The peripherally persisting virus was concomitantly eliminated, in contrast to the skin infections.

So far, we have only limited data on HSV-1 infection of ganglia after vaginal and i.d. inoculation. However, these data show that HSV-1, after these routes of inoculation in contrast to s.c. inoculation, could invade ganglia during the acute phase of infection but was probably eradicated in most of the infected ganglia later on. The finding that lasting latent infections are established only in a small proportion of those animals whose ganglia were initially invaded by HSV is consistent with the findings of Tenser and Hsiung (19) on acute and latent HSV-1 infections of trigeminal ganglia after corneal infection of guinea pigs. The basis of the different behavior of the two HSV subtypes in ganglia after the different routes of inoculation is not clear.

A unique feature of HSV infections of guinea pigs which differentiates this model from those in other species is that both HSV-2 and HSV-1 can persist locally at the site of initial infection. This local persistence is independent of latent ganglionic infection, as demonstrated by three different observations. (i) HSV-1 after s.c. infections persists exclusively in the skin. (ii) Dissection of the sciatic and saphenic nerve before footpad inoculation with HSV-2 prevents migration of virus into ganglia. The virus does, however, still persist in all animals in the footpad skin (15). (iii) After infection of guinea pigs with bromodeoxycytidine-resistant mutants of HSV-2 it was shown that the virus populations persisting in the ganglia and peripheral tissues, i.e., footpad skin or vagina, differed in their TK phenotype (M. Scriba, B. S. Fong, and J. Botto, manuscript in preparation).

As with latent virus in ganglia, the virus in peripheral tissues was only detectable by the cocultivation method. In addition, no viral antigen was found in the skin by immunofluorescence during clinically quiescent phases of infection (unpublished data). We have, however, presented evidence previously that the state of virus persisting in the skin or the vagina is different from the state of the virus in the ganglia (15). In contrast to the latent infection of ganglia, peripheral tissues probably harbor a chronic productive infection. This productive infection is

TABLE 9. *Virus isolation from tissue explants after vaginal infection*

Virus (PFU)	Tissue	No. positive/no. tested at day p.i.:				
		7	14	25–100	101–210	369–640
HSV-2 72 (10^4)	Vagina	5/5	1/3	7/11	5/17	0/8
	Cervix uteri		2/3	1/5	0/8	0/8
	Corpus uteri			0/3	0/7	0/8
	Vulva			0/3		0/4
	Footpad skin		1/3	0/3	0/4	0/5
	Genital nerves		2/3	3/4	3/4	2/5
	Sciatic nerves		3/3	3/4	1/4	0/5
	DRG	10/10	3/3	13/15	12/18	3/7
	Spinal cord		3/3	4/4	1/4	1/4
HSV-1 brand (10^5)	Vagina	3/3		2/3		
	DRG	3/3		1/4		

maintained on an extremely low level, probably by the host's immune response, so it is undetectable by all direct assays.

The question remains whether recurrent herpes in guinea pigs is induced by virus activated locally or in ganglia, as is the case with recurrent herpes in other species. This question cannot be answered unambiguously at this time. A number of observations indicate, however, that the ganglionic infection might be the major source of recurrent lesions.

(i) Vaginal HSV-2 infections led to recurrent herpes not only in the genital area but also in the hind footpads. No virus persistence in footpad skin after vaginal infection was ever detected. Thus, recurrent lesions in footpad skin after vaginal inoculation must have resulted from virus reactivation in ganglia.

(ii) HSV-2 can be prevented from migrating from the site of skin inoculation to the corresponding ganglia by two means: by immunization of the animals with HSV antigen or by denervation of the inoculated leg before footpad infection. In both cases, virus persisted in the inoculated skin, but invasion of ganglia was inhibited. Concomitantly, no or only rare recurrent lesions developed (15, 16).

(iii) Footpad infection with HSV-1 led to ganglionic infection only after i.d. inoculation, never after s.c. inoculation. Recurrent herpes was only observed after i.d. infection, although both routes of inoculation resulted in virus persistence in the skin. Taken together, these findings suggest that at least the majority of recurrent eruptions are related to virus reactivation in ganglia. However the possibility cannot be excluded that activation of peripheral virus might occasionally occur and lead to development of overt clinical symptoms.

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