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Pathogenesis of Latent Herpes Simplex Virus Infection of the Trigeminal Ganglion in Guinea Pigs: Effects of Age, Passive Immunization, and Hydrocortisone

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Latent herpes simplex virus (HSV) infection of the trigeminal ganglion, after corneal inoculation of virus, was investigated in guinea pigs. The effects of several factors on the establishment of ganglionic latency were investigated. Latently infected guinea pigs were clinically normal, and virus was isolated from the trigeminal ganglia by co-cultivation. It was found that newborn guinea pigs were significantly more susceptible than adult animals to the development of latent HSV infection of the trigeminal ganglion. The susceptibility of newborn guinea pigs was very much decreased, however, if they received passive immunization with immune serum or if they were born of actively immunized mothers. On the other hand, the susceptibility of adult animals, usually somewhat resistant to the development of latent HSV ganglionic infection, was markedly increased by the parenteral administration of hydrocortisone.

Latent herpes simplex virus (HSV) infection of the trigeminal ganglion has been reported in humans (1, 4, 10, 12) and in experimentally infected animals (2, 14, 18). Experimentally infected rabbits and mice have been found to readily develop acute central nervous system (CNS) disease and latent ganglionic infection after corneal inoculation of the virus (2, 7, 18). In studies of the pathogenicity of HSV type 1 in guinea pigs, however, we found that adult guinea pigs were relatively resistant to the development of acute CNS disease after corneal inoculation of virus (16). In considering the pathogenesis of HSV latency, therefore, it was of interest to investigate whether or not latent infection of the trigeminal ganglion in guinea pigs could be readily established.

In the present study, the effect of factors which may permit further understanding of the establishment of ganglionic latency was investigated. Initially, the development of latent HSV infection of the trigeminal ganglion in adult as compared with newborn guinea pigs was determined. The protective effect of humoral immunity on establishment of ganglionic infection was then studied. Finally, the effect of parenteral hydrocortisone administration on increasing susceptibility to development of latent trigeminal ganglion infection was evaluated.

MATERIALS AND METHODS

Virus stock and tissue culture. The prototype McIntyre strain of HSV type 1, obtained from the American Type Culture Collection, was used. The virus was passaged in primary rabbit kidney (RK) cell monolayer cultures four or five times before use. Infectivity titers of virus stocks varied between $10^{6.5}$ and $10^{7.0}$ 50% tissue culture infectious doses per 0.1 ml. Methods of preparation of monolayer cultures were similar to those previously described (15).

Corneal inoculation of HSV. Young adult Hartley guinea pigs (1 to 2 months old) and newborns (2 to 4 days old) were obtained from the Yale University Division of Animal Care. Animals were anesthetized with ether. The cornea of each eye was scarified with a sterile 25-gauge needle, and HSV (0.2 ml) was dropped onto each eye.

Inoculation of hydrocortisone. Adult guinea pigs, previously infected with HSV by corneal inoculation, were inoculated by the intraperitoneal (i.p.) route with hydrocortisone phosphate twice daily on the third and fourth days after virus inoculation. Each animal received either 20 or 200 mg of hydrocortisone/kg in each injection.

Tissue sampling and virus isolation. Guinea pigs were sacrificed by exsanguination while they were acutely infected (3 to 12 days after corneal inoculation of HSV) or while latently infected (21 to 70 days after corneal inoculation). The time of latent virus infection was similar to that used by other investigators (2, 14, 18). The trigeminal ganglia, trigemi-

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nal roots, brainstem, cerebellum, diencephalon, olfactory lobe, optic chiasm, and cerebrum were removed and assayed for the presence of HSV by the following co-cultivation method. Tissues were separately minced in Hanks balanced salt solution (HBSS) with a scalpel. The minced fragments, approximately 1 mm in diameter, were inoculated with individual Pasteur pipettes into monolayer tube cultures of RK cells. Three to eight pieces of tissue were co-cultivated in each of four tube cultures.

In some instances, trigeminal ganglia from individual guinea pigs were homogenized in HBSS (5%, wt/vol) in a Ten Broeck homogenizer. After centrifugation (10 min at 1,200 rpm), 0.1 to 0.2 ml of the cell-free supernatant fluid was inoculated into each of four RK culture tubes.

All cultures were incubated at 37°C and were observed for the development of cytopathic effect (CPE) typical of herpesvirus. Cultures which were negative for CPE after 28 days were considered to be negative for HSV and were discarded. Positive virus isolates obtained from latently infected animals were immunologically identified by neutralization with HSV type 1 specific antiserum prepared in guinea pigs.

Passive immunization procedures. Antiserum was prepared in adult guinea pigs immunized against HSV by inoculation of stock virus three times at biweekly intervals. For each immunization, 0.8 ml of virus suspension was inoculated i.p. and 0.2 ml was inoculated subcutaneously. Neutralizing antibody titers of immune sera were 1:128 to 1:256.

Passive immunization of newborn animals was achieved in one of two ways. Most animals were inoculated i.p. with 2.0 ml of heat-inactivated HSV immune serum obtained from adult guinea pigs. Corneal challenge with HSV was performed 18 h after this passive immunization. A smaller group of newborns was considered to be passively immunized by being born of actively immunized mothers. Blood for serum neutralizing antibody titer determination was drawn by cardiac puncture from passively immunized guinea pigs in both groups immediately before corneal challenge with HSV.

Neutralizing antibody determinations. Sera were obtained from all guinea pigs at the time of sacrifice, and neutralizing antibody titers were determined. The procedure employed was similar to that used previously (15). Virus-serum mixtures were inoculated into RK culture tubes. After 2 to 4 days of incubation, the highest serum dilution which prevented CPE when it was 2+ or greater in control cultures was considered to be the serum antibody titer.

RESULTS

Clinical course: corneal clouding. All guinea pigs developed corneal clouding after corneal inoculation of virus.. This reached a maximum after 3 days and cleared in most animals 7 to 10 days after inoculation. Clearing was slightly slower in newborn than in adult animals, and about 35% of the newborns developed blepharostenosis before corneal clearing. Occasional adult and newborn animals (1 to 2%) showed corneal scarring 2 or more weeks after inoculation.

Encephalitis. Approximately 1 to 2% of adult animals developed signs of CNS disease and died with HSV encephalitis 8 to 10 days after corneal inoculation. However, 30% of guinea pigs inoculated as newborns died with HSV encephalitis within 10 days of inoculation. None of the surviving guinea pigs, many of which were latently infected, showed signs of CNS disease.

Effect of parenteral hydrocortisone. Clearing of corneal clouding in adult animals did not differ significantly between those treated or not treated with parenteral hydrocortisone. However, two died with HSV encephalitis, one in each of the two dosage groups.

Acute and latent infection of trigeminal ganglion and brain in adult guinea pigs. Virus was recovered from the trigeminal ganglia of all of seven adult guinea pigs sacrificed 3 days after corneal inoculation, but from only one of six animals after 11 to 12 days. The decrease in number of animals showing virus infection after day 3 was progressive on days 5 and 7 to 8 (Fig. 1a). The pattern of virus recovery from trigeminal root paralleled that from trigeminal ganglion but at a lower level. HSV recovery from brainstem, the part of the brain proximal to the trigeminal ganglion, was maximal, at 33%, on day 5 (Fig. 1b). Other parts of the brain assayed had maximal virus isolations on days 7 to 8 and ranged from 15% (cerebellum) to 28%

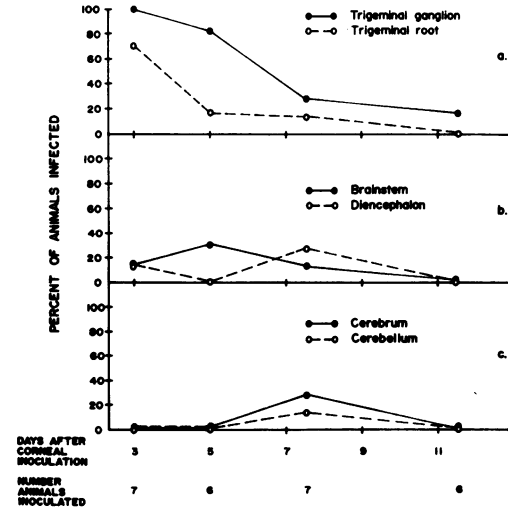

FIG. 1. *Recovery of HSV from neural tissues of acutely infected adult guinea pigs, 3 to 12 days after corneal inoculation of virus.*

(cerebrum, diencephalon) (Fig. 1b and c). HSV was not isolated from the olfactory lobe of any of 13 guinea pigs tested, including 4 on day 3 and 3 on day 5. Virus was isolated from optic chiasm tissue of one animal on day 7 but not from any of six tested on days 3 to 5.

During the period of latent infection, which was considered to extend from 21 to 70 days after corneal inoculation, HSV was isolated from the trigeminal ganglion of only a few adult guinea pigs. In 3 of 12 animals, HSV was isolated from the trigeminal ganglion 21 to 28 days after corneal inoculation (Fig. 2). Virus was isolated from both ganglia in one animal and from one ganglion in each of the other two. HSV was isolated from one ganglion in one of five adult guinea pigs tested 50 to 70 days after infection. In none of 17 animals sacrificed after 21 days was virus isolated from the brainstem or other parts of the brain.

Acute and latent infection of trigeminal ganglion and brain in newborn guinea pigs. After corneal inoculation of HSV in 2- to 4-day-old animals, 30% developed HSV encephalitis, and virus was isolated from different parts of the brain and from the trigeminal ganglia. HSV was most readily isolated from cerebral tissues.

In those guinea pigs that did not develop clinical evidence of CNS disease, HSV infection of the trigeminal ganglia was frequently found despite their normal appearance when they were sacrificed 21 to 70 days postinoculation ($P < 0.01$ when compared with adult animals). Latent infection of ganglia was detected in 88% (eight of nine) of those sacrificed 21 to 28 days after infection and in 78% (seven of nine) 50 to 70 days after corneal inoculation (Fig. 3). Bilateral trigeminal ganglion infection was found in eight animals and unilateral, in seven. HSV was isolated from the brainstem of 22% of animals 21 to 28 days and 50 to 70 days after infection but was not isolated from other parts of the brain.

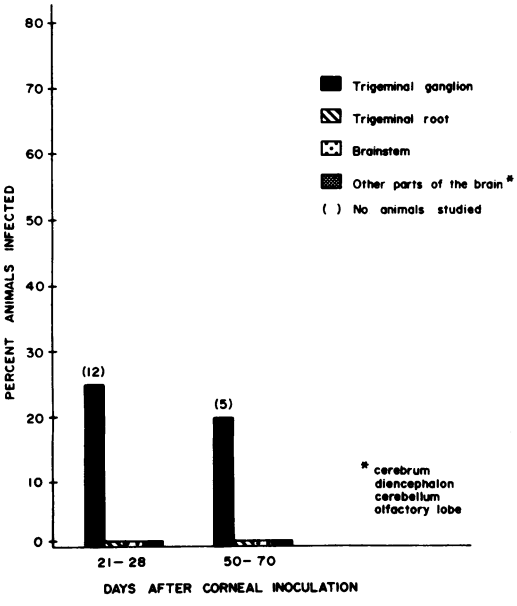

FIG. 2. *Latent HSV infection of the trigeminal ganglion of adult guinea pigs, 21 to 70 days after corneal inoculation of virus.*

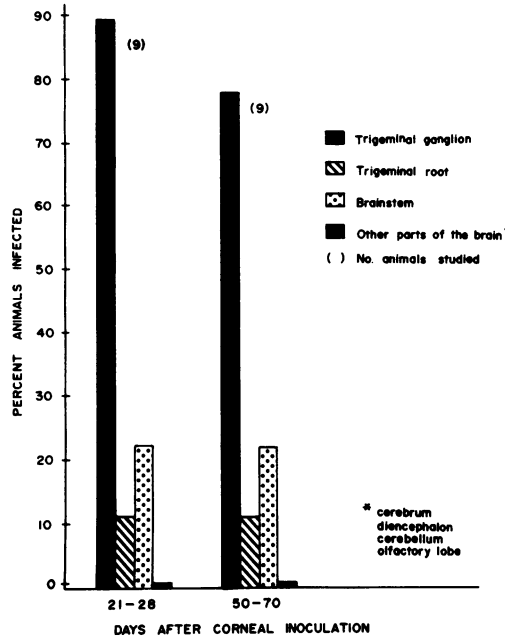

FIG. 3. *Latent HSV infection of the trigeminal ganglion and brain of newborn guinea pigs, 21 to 70 days after corneal inoculation of virus.*

Characteristics of HSV isolation from latently infected trigeminal ganglion. As described above, HSV was isolated from many newborn guinea pigs during the period of latent infection when minced fragments of ganglia were co-cultivated with RK cells. HSV could also be isolated from latently infected ganglia by trypsinization and co-cultivation of the dispersed ganglion cells (data not shown). However, virus could not be isolated from any of seven cell-free suspensions of ganglion homogenates which were cultured with RK cells. On the other hand, HSV was isolated from intact ganglion cells as well as from cell-free homogenates during the period of acute trigeminal ganglion infection 3 to 8 days after corneal inoculation.

The time after co-cultivation prior to the development of CPE differed considerably be-

tween trigeminal ganglion tissues taken from acutely or from latently infected guinea pigs. The average time interval for tissues from acutely infected animals was 5.1 days (standard error of the mean, ±0.4; range, 3 to 14 days), whereas from latently infected animals it was 14.7 days (standard error of the mean ± 0.5; range, 9 to 25 days).

Effect of immunization on acute and latent infection of the trigeminal ganglion. Serum neutralizing antibody titers were determined for all guinea pigs at the time of sacrifice. Antibody titers of 1:4 were detected in some animals on days 7 to 8 after corneal inoculation of HSV. Maximal titers of 1:32 were found 21 to 35 days after inoculation, although in some guinea pigs antibody titers as low as 1:4 were also found at this time. In animals sacrificed during the period of latent infection, 21 to 70 days postinoculation, there was no positive or negative correlation between antibody titer and the presence or absence of latent infection.

To further study the effect of the immune system on latent HSV infection of the trigeminal ganglion, newborn guinea pigs were passively immunized against HSV prior to corneal inoculation of HSV. Among the guinea pigs that received HSV antiserum 18 h prior to inoculation, only 18% (2 of 11) showed HSV infection of the trigeminal ganglion 28 to 50 days after corneal inoculation with HSV (Table 1). The two animals that did have ganglionic infection with HSV had had antibody titers of 1:64 at the time of corneal inoculation of virus; others not latently infected had antibody titers of 1:32 to 1:128 at the time of corneal challenge. Of six animals born of actively immunized mothers, none had evidence of ganglionic infection when sacrificed 28 to 50 days after corneal inoculation with HSV. Animals in the latter group had neutralizing antibody titers of 1:128 to 1:256 at the time of corneal challenge. No clinical evidence of encephalitis was noted in guinea pigs in either of the passive immunization groups. Of 16 nonimmunized control animals, 14 (88%) developed HSV encephalitis or latent ganglionic infection. In other guinea pigs passively immunized with 2.0 ml of normal guinea pig serum 18 h before corneal challenge with HSV, none of 10 was protected against HSV encephalitis or latent HSV infection.

Effect of parenteral hydrocortisone on the establishment of latent HSV infection of the trigeminal ganglion in adult guinea pigs. In adult guinea pigs treated with 20 mg/kg doses of hydrocortisone, virus was isolated from the ganglia of two of seven when tested 21 to 23 days after corneal inoculation (from both ganglia of one and from one ganglion of the other). However, ganglionic infection was found in all of seven after 21 to 25 days when treated with doses of 200 mg/kg (Table 2). In one animal in the latter group, virus was also isolated from trigeminal root. In three of the guinea pigs in the 200 mg/kg group, virus was isolated from both ganglia whereas in the others it was isolated from one ganglion. Compared with untreated guinea pigs, there was a minimal decrease in antibody titer in animals treated with 20 mg of hydrocortisone/kg, but a somewhat

TABLE 1. *Effect of passive immunization on the establishment of latent HSV infection of the trigeminal ganglion in newborn guinea pigs*

Group[a]	No. of animals			Percent latently infected	Neutralizing antibody titer, range and (median)	
	Total studied	Dead with encephalitis[b]	Latently infected[c]		At time of corneal inoculation of HSV[d]	At sacrifice 28–50 days postinoculation of HSV
Controls						
No treatment	16	4	10	83[e]	1:2 (<1:2)	1:4–1:32 (1:8)
Normal guinea pig serum	10	4	6	100[e]	1:2 (<1:2)	1:4–1:32 (1:16)
Passively immunized						
Guinea pig antiserum	11	0	2	18	1:32–1:128 (1:64)	1:4–1:32 (1.8)
Born of immunized mother	6	0	0	0	1:128–1:256 (1:128)	1:8–1:32 (1:16)

[a] $P < 0.01$, by chi-square test, with Yates' correction, between controls and passively immunized animals.

[b] Animals that died with encephalitis within the acute period of infection, 3 to 12 days after corneal inoculation of HSV.

[c] Animals sacrificed 28 to 50 days after corneal inoculation.

[d] Blood was drawn by cardiac puncture immediately before corneal inoculation of HSV. See text for immunization procedures utilized prior to corneal inoculation of virus.

[e] Percentage of guinea pigs that had survived the period of acute infection and were then found to be latently infected.

TABLE 2. *Effect of parenteral hydrocortisone on the establishment of latent HSV infection of the trigeminal ganglion in adult guinea pigs*

Group[a]	No. of animals studied	No. latently infected[b]	Neutralizing antibody titer, range and (median)[b]
Nontreated (controls)	12	3 (25%)	1:4-1:32 (1:8)
Hydrocortisone treated			
20 mg/kg	7	2 (28%)	<1:2-1:16 (1:4)
200 mg/kg	7	7 (100%)	<1:2-1:4 (1:2)

[a] $P < 0.01$, by chi-square test, between latent infection in nontreated (controls) and hydrocortisone-treated animals (200 mg/kg). See text for hydrocortisone treatment schedule.

[b] Determined at time of sacrifice, 21 to 28 days after corneal inoculation of HSV.

greater decrease in those treated with 200 mg/kg. The geometric mean antibody titers in animals in the 200 mg/kg group and in the untreated control animals were approximately 1:2 and 1:8, respectively. (For the purpose of figuring the mean antibody titer in guinea pigs in the 200 mg/kg group, titers of <1:2 were considered to be 1:1.)

DISCUSSION

The present investigation showed that, 3 days after corneal inoculation of HSV in adult guinea pigs, virus was frequently recovered from the trigeminal ganglion, but that latent infection of the ganglion 21 or more days after corneal inoculation of virus was not as frequently found as in newborn guinea pigs. The recovery of HSV from several parts of the brain of adult guinea pigs was most frequent on days 7 to 8. Despite the presence of HSV in the CNS of these animals, signs of neurological disease were rarely seen. This was similar to the lack of neurological findings in guinea pigs with latent HSV type 2 infection of dorsal root ganglia described by Scriba (13).

Newborn guinea pigs, which were very susceptible to the development of latent HSV infection after corneal inoculation of virus, were protected by prior systemic administration of specific immune serum. Protection was also found in newborn guinea pigs which had significant neutralizing antibody titers after being born of actively immunized mothers. Protection against acute HSV infection has also been reported in passively immunized mice and guinea pigs (3, 9, 17). In the passively immunized animals in our study, protection may have been mediated by inhibition of virus entry into corneal free nerve endings and, therefore, elimination of virus passage to trigeminal ganglion neurons. In an experiment by Cook and Stevens, intravenous inoculation of mice with anti-HSV serum 5 or 10 h after footpad inoculation of HSV did not prevent virus from reaching the dorsal root ganglia (5). In conjunction with our findings, the latter report supports the hypothesis that antibody may protect against infection by inhibiting only an early step in pathogenesis, such as virus entry into free nerve endings.

In an experiment in mice, Price et al. reported partial protection against the development of latent HSV infection in actively immunized animals (11), but did not find a clear correlation between serum antibody titer and protection against latent infection. Similarly, in our experiment the two passively immunized animals found to be latently infected had not had the lowest serum antibody levels at the time of corneal challenge with HSV. Nevertheless, our data did indicate that passively administered antibody conferred protection against the establishment of latent HSV infection, similar to passive immunization in utero.

Although it appears that parenteral administration of preformed antibody to newborn guinea pigs can protect against the development of acute and latent HSV infection, the role that antibody plays in the natural age-related susceptibility of normal guinea pigs to acute and latent HSV infection is less clear. In the present experiment, virus was found in the trigeminal ganglia of all normal adult guinea pigs 3 days after corneal inoculation but in few 7 to 8 days postinoculation. Serum neutralizing antibody levels, however, were only minimal by days 7 to 8. Antibody production, therefore, may not be sufficient explanation for the clearing of virus from the trigeminal ganglia in adult animals.

A pharmacological means of increasing susceptibility to the establishment of latent HSV infection of sensory ganglia, such as our use of hydrocortisone, has not been previously reported. Experiments using such methods may provide a useful means for studying the pathogenesis of latent herpetic infections. Although large amounts of hydrocortisone were necessary to induce latency, the animals were treated for only 2 days. The high doses of drug

used were apparently sufficient to overcome the relative resistance of guinea pigs to the immunological inhibitory effect of adrenal corticosteroids (6, 8, 15), as indicated by slight although consistent decreases in serum antibody titers. The effect of smaller amounts of corticosteroids administered for longer periods of time on the development of viral latency is not known. The effect of the large amounts of hydrocortisone on cell-mediated immune mechanisms was not evaluated, but this part of the immune system may also have been inhibited. Despite the decrease in humoral antibody titers and probable inhibition of cell-mediated immunity, however, the means by which hydrocortisone increased susceptibility to HSV latency may not necessarily have had an immunological basis. Direct effects on neuronal function or on virus replication are other possible mechanisms.

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