INFECTION AND IMMUNITY, Feb. 1979, p. 373-383
0019-9567/79/02-0373/11$02.00/0

Vol. 23, No. 2

Route of Infection, Systemic Host Resistance, and Integrity of Ganglionic Axons Influence Acute and Latent Herpes Simplex Virus Infection of the Superior Cervical Ganglion

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Received for publication 20 November 1978

The character of acute and latent herpes simplex virus (HSV) infection of the superior cervical ganglion (SCG) in mice depended on the route by which the virus reached the ganglion, the level of systemic host resistance, and the integrity of postganglionic nerves. Prevention of ganglionic infection by postganglionic neurectomy carried out before intraocular (i.o.) virus challenge established the importance of the neural route in the development of SCG infection. However, hematogenous virus dissemination also led to SCG infection although with reduced frequency compared to that with i.o. inoculation. Enhanced host systemic antiviral resistance had two divergent effects on ganglionic infection depending on the dose and timing of virus inoculation. Thus, both acute and latent ganglionic infections were concomitantly reduced when resistant C57Bl/6 mice were challenged with low doses of virus or when less resistant BALB/c mice were actively immunized 1 week before virus challenge. On the other hand, when resistant mice were challenged with high doses of virus or when either active or passive (antibody) immunization was delayed long enough to assure viral access to the ganglion, intraganglionic viral replication during the acute phase of infection was reduced, but the prevalence of subsequent latent infection was either unaffected or actually enhanced. Postganglionic neurectomy, performed after virus had reached the ganglion, altered the course of SCG infection in a direction opposite that of immunization, augmenting the acute phase of viral replication while reducing latency. In athymic nude mice and mice immunosuppressed with cyclophosphamide, intraganglionic viral replication was prolonged. These results emphasize that host factors both extrinsic and intrinsic to the SCG modify the course of ganglionic infection.

Herpes simplex viruses (HSV) types 1 and 2, are exquisitely adapted for survival in the human community. Once these viruses infect the human host, they are capable of sustaining a prolonged, perhaps lifelong, latent infection. Latent virus may then periodically reactivate and be shed at epithelial surfaces or into external secretions where it is available to infect susceptible contacts. These three stages of HSV infection, i.e. primary infection, latency, and recurrence, are evidence of a variability in the virus-host relationship. Initial invasion is accompanied by a productive infection resulting in a sufficient quantity and distribution of viral progeny to allow the virus to reach the site(s) of eventual latency. Productive infection then gives way to the latent stage, during which virus either no longer replicates or replicates in insignificant amounts. Finally, recurrent infection indicates a switch back to an active productive phase with release of HSV in amounts sufficient to infect contacts. Host responses are important in determining the course of each phase of infection and presumably fall within a limited range for both host and virus to survive. If the host is too restrictive, initial infection will not occur or recurrence will be aborted. On the other hand, if the host fails to restrict virus replication, it will be overwhelmed, and the period during which infection is passed on will be markedly abbreviated. A number of recent studies have provided substantial support for the hypothesis that sensory ganglia of the nervous system play a central role in latent and reactivated HSV infections (2, 13). Sensory ganglia have been shown to harbor latent HSV types 1 and 2 in both experimentally infected animals (14, 17) and in humans (1, 3).

To explore those factors which modify or regulate the course of HSV infection, we have employed an experimental model of infection of the

superior cervical ganglion (SCG). When the anterior chamber of the mouse eye is inoculated with HSV, infection of the ipsilateral SCG ensues. This ganglion, located in the neck adjacent to the bifurcation of the common carotid artery, is part of the sympathetic division of the autonomic nervous system and supplies postganglionic nerve fibers to target organs in the head, including the iris within the anterior chamber of the eye. Infection of this sympathetic ganglion resembles in all respects thus far tested experimental infection of sensory ganglia (10, 12). After eye inoculation, an acute infection of the SCG is produced, during which active viral replication takes place and HSV can be detected in homogenates of the ganglion. This acute phase subsides within 7 to 10 days, and, thereafter, the conventional homogenization assay no longer allows recovery of virus. However, the continued presence of latent HSV can be demonstrated when explantation and co-cultivation methods are used for virus detection. Latent virus can be reactivated in vivo when postganglionic nerve fibers emanating from the SCG are surgically severed (12). Recently, HSV has been recovered from the SCG of humans (18) supporting the suggestion that the autonomic nervous system may participate in the pathogenesis of human herpetic infection (11).

We now describe a series of experiments which demonstrate that the character of ganglionic infection depends upon the route by which virus reaches the ganglion, systemic host resistance to HSV, and the integrity of postganglionic nerves.

MATERIALS AND METHODS

Tissue culture and media. Rabbit kidney (RK) cells were used either as primary cell monolayers or after one or two passages, whereas human embryonic lung (HEL) cells were obtained commercially (Microbiological Associates, Rockville, Md.) and used between passages 17 and 26. Cultures were maintained as previously described (12).

Virus propogation and inoculation. The F strain of HSV type 1 was prepared on RK monolayers (6) with the stock virus pool for inoculation containing 2×10^7 plaque-forming units (PFU) of HSV/ml. Intraocular (i.o.) inoculation was carried out as previously described (12); unless otherwise specified, mice received 4 μl of undiluted virus stock pool i.o. on the right side.

Animals. With the exception of two experiments, 4- to 6-week-old BALB/c female mice, purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass., were used for these studies. C57B1/6 mice and the BALB/c mice used in the experiment comparing these two mouse strains were simultaneously purchased from Jackson Laboratories, Bar Harbor, Maine; both strains were received at 6 weeks of age and used when 10 weeks old. Nude mice (nu/nu) and heterozygous littermates (nu/+), obtained from the Sloan-Kettering Institute Animal Care Facility, were used at 3 to 5 weeks of age and possessed an ICR genetic background.

Preparation of antisera and determination of serum antibody titers. Anti-HSV antiserum was prepared in both BALB/c mice and rabbits. Mouse antiserum was induced by two intraperitoneal (i.p.) injections of 2×10^5 PFU of live HSV suspended in 0.2 ml of Dulbecco phosphate-buffered saline (PBS) spaced 2 weeks apart. Mice were bled 10 to 14 days after the second injection. Rabbit antiserum was obtained by using a similar schedule with intradermal injections of undiluted stock virus pool for induction. Serum neutralizing antibody titers, determined using a plaque-reduction assay (16), were expressed as the reciprocal of the highest serum dilution producing a 50% reduction in viral plaques.

Assay of ganglia and eyes for virus. SCG were analyzed for HSV by either homogenization or explantation–co-cultivation techniques as previously described (12). Viral titers of ganglion homogenates were determined using RK cell monolayers and results were expressed as $\log_{10}$ mean viral titers (sum of $\log_{10}$ viral titers of individual ganglia per number of ganglia assayed); in cases where ganglia were negative for virus, the convention that 1 PFU of HSV ($\log_{10}$ value of 0) was detected in these ganglia was employed in calculating mean values. Significance of differences in viral titers was assessed by the Student t test. For the explantation–co-cultivation assay, ganglia were placed on HEL monolayers and monitored for 3 weeks for the appearance of HSV-induced cytopathology. Results of this assay were presented as a ratio (the number of ganglion explants positive for virus per number of ganglia assayed), and the significance of results were assessed by the chi-square contingency method or, when the number of ganglia was less than 20, by the Fisher exact test. Viral titration of eye homogenates was carried out in a manner similar to that used for ganglia. In brief, eyes were washed three times in PBS containing antibiotics, homogenized with a Ten Broeck tissue grinder, frozen and thawed three times, and, after centrifugation at 5,000 rpm for 10 min, HSV titers of the supernatants were determined by using RK monolayers.

Surgery. All neurectomies and sham operations were performed aseptically under pentobarbital anesthesia with the aid of a dissecting microscope.

RESULTS

Effect of postganglionic nerve section on the acquisition of SCG infection. To determine whether HSV reaches the SCG over neural pathways after inoculation of the anterior chamber of the eye, mice were subjected to postganglionic nerve section 1 day before i.o. HSV injection. Under ether anesthesia, the three major postganglionic nerve fibers emanating from the right SCG were severed; the efficacy of operation was judged by the appearance of eyelid ptosis on the operated side. On the day after surgery, the neurectomized mice, along with unoperated control mice, were infected by i.o. HSV inoculation of the right eye. Mice were then sacrificed

for ganglion assay both at the height of the acute infection (day 5) and during the latent phase of infection (day 21). The ipsilateral (right) SCG of mice sacrificed during the acute phase of infection were assayed for HSV by homogenization, whereas ganglia of mice sacrificed during the latent phase were assayed by explantation-cocultivation. The data in Table 1 show that postganglionic neurectomy almost completely prevented ganglionic infection. While in the control group during the acute phase of the infection ganglion homogenates of all 13 animals contained HSV with a mean viral titer approaching 2.9×10^3 PFU per ganglion, in none of the neurectomized mice was HSV detected in ganglion homogenates. Similarly, during the latent phase in only 1 of 11 SCG explants in the neurectomized group was HSV detected, compared to 10 of 15 ganglia in the control group.

The most likely explanation for these results is that the postganglionic nerves serve as the major route of virus passage from the eye to the SCG. An alternative explanation is that virus circulating in the bloodstream preferentially infects the non-operated ganglion, i.e., that postganglionic neurectomy inhibits virus uptake and subsequent replication or latency. To test this possibility and also to assess the efficiency with which hematogenously disseminated virus infects the SCG, mice were subjected to unilateral (right) postganglionic neurectomy and inoculated intravenously (i.v.) (tail vein) with 1.0×10^6 PFU of virus diluted in 0.2 ml of PBS; both the right (ipsilateral to neurectomy) and the left (nonoperated) SCG were then assayed for HSV. Table 2 summarizes the results of three experiments in which mice were neurectomized at various intervals from 21 days to 1 day before i.v. virus inoculation; the animals were sacrificed either 5 days (preliminary results indicated that this was the optimal time to detect virus in the SCG after i.v. injection either by homogenization or explantation) or 21 days after virus injection. As the data in the table show, there was no difference in the acquisition of infection between the neurectomized and non-neurectomized ganglia. During the acute phase, SCG homogenates were negative in all but approximately 4% of the ganglia, and in those ganglia in which HSV was detected, the viral titers were low, ranging from 1 to 33 PFU per ganglion. Although the more sensitive explant method detected HSV in between 25 and 29% of explants during the acute phase, the prevalence of detectable latent infection fell to less than 7% of ganglia in both groups by 21 days, indicating that the efficiency of establishing latent infection by the i.v. route was far lower than that following ipsilateral i.o. virus injection.

TABLE 1. *Effect of postganglionic neurectomy before inoculation of the ipsilateral eye on the acquisition of acute and latent HSV infection of the SCG*

Group	Acute phase (homogenates)		Ratio of latent phase explants[c]
	Ratio[a]	Mean titer (PFU, $\log_{10}$)[b]	
Unoperated controls	13/13	3.5	10/15
Postganglionic neurectomy	0/12	0	1/11

[a] $P < 0.001$.
[b] $P < 0.001$.
[c] $P < 0.01$.

TABLE 2. *Acute and latent SCG infection after i.v. HSV inoculation*[a]

Ganglion assayed	Acute phase		Latent phase (explants)[c]
	Homogenates[b]	Explants	
Left (unoperated)	2/49	13/51	2/31
Right (postganglionic neurectomy)	2/49	15/52	2/31

[a] Results all express the ratio of the number of ganglia positive for HSV per number of ganglia assayed. The table gives the combined results of three experiments (see text).
[b] Quantitative assay of viral titers in positive ganglion homogenates revealed 1 PFU and 30 PFU in the left-sided ganglia and 2 PFU and 33 PFU in the right-sided ganglia. These ganglia were from four different mice.
[c] Positive ganglion explants were obtained from three mice; in one of these both ganglia were positive, whereas in single animals only the right or only the left ganglia was positive for HSV.

Latent SCG infection in C57Bl/6 mice compared with that in BALB/c mice. The morbidity and mortality of mice subjected to systemic HSV infection varies among inbred mouse strains (9). To determine whether genetic differences in systemic resistance to virus influence the development of latent ganglionic infection, SCG infection of C57Bl/6 mice, a resistant strain, was compared to infection in BALB/c mice, the strain used in previous studies that is moderately susceptible to systemic HSV infection (9). Because resistance to infection changes rapidly with age in young mice, 10-week-old animals were used for this study. Female mice of the two strains were received, housed, infected, and sacrificed, and ganglia were assayed at the same time to minimize variables that might arise if the groups had been tested sepa-

rately. To compare the development of latent ganglionic infection, mice of both strains were inoculated i.o. with from 8 to 8 × 10^5 PFU of HSV suspended in 4 μl of PBS, and 3 weeks later ganglia ipsilateral to the inoculated eye were assayed for latent HSV by explantation-cocultivation. The data in Fig. 1 demonstrate that although both mouse strains were capable of supporting a latent SCG infection, the dose-response curves of the two strains were quite different. In the case of the C57Bl/6 mice, the animals were resistant to the development of latency at lower doses of virus inoculum, but there was a steady increase in the percentage of mice harboring latent infection as the dose increased, and maximum prevalence of latency occurred in the group receiving the highest virus dose. In contrast, latent infection of BALB/c mice developed at a lower dose (e.g., the 40% infection rate in the BALB/c mice occurred at a virus dose of less than one one hundredth of that inducing a similar rate in the C57Bl/6 mice). In addition, the highest frequency of latent infection was detected in BALB/c mice injected with a relatively low concentration of virus (8 × 10^2 PFU), whereas higher concentrations of virus inoculum led to progressively lower rates of latency.

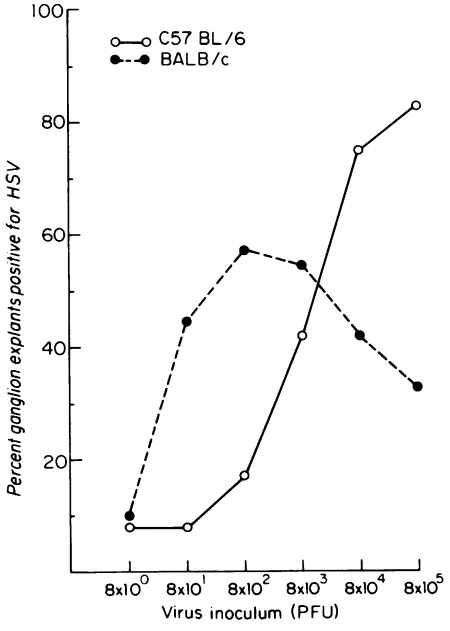

FIG. 1. *Comparison of latent SCG infection in C57Bl/6 and BALB/c mice. Mice of both strains were inoculated i.o. with the dilutions of HSV shown suspended in 4 μl of PBS and were sacrificed 3 weeks later. Each point represents the results of explantation of ipsilateral ganglia from nine or more mice.*

To determine if the extent of HSV replication either in the SCG or at the site of virus inoculation during the acute phase of infection correlated with the subsequent development of latent ganglionic infection, viral titers of homogenates of the SCG as well as of the injected eyes of BALB/c and C57Bl/6 mice inoculated with the highest dose of virus (8 × 10^5 PFU) were compared during the acute phase of infection. As shown in Table 3, the level of virus replication in the two mouse strains during the acute phase of infection differed, and, in fact, correlated inversely with the subsequent development of latency. Viral titers of ganglia and eyes from BALB/c mice were more than 10 times higher than titers of the same organs of C57Bl/6 mice, indicating that interference with viral replication did not account for the lower prevalence of latency at high doses in the BALB/c mice.

Effect of active immunization before i.o. virus challenge on the development of latent SCG infection. To explore further the effect of systemic viral resistance on both the development of latent infection and on the course of the acute lytic phase of the infection, a series of manipulations of anti-HSV immunity in BALB/c mice was carried out. In the first of these studies, the effect of active immunization with live HSV, given i.p. at various intervals before virus challenge, was assessed. Initial studies showed that when mice were actively immunized by i.p. injection of 4 × 10^5 PFU of HSV 7 days before virus challenge, latent SCG infection was completely prevented with none of 13 explants positive for HSV; this agrees with the results of previous observations (10) and also indicates that i.p. virus inoculation is unlikely to itself lead to latent SCG infection. Immunization 1 or 3 days before i.o. challenge, on the other hand, failed to reduce the prevalence of latency compared to that in unimmunized controls with 55 to 60% of explants positive for virus in each group. Further studies were then carried out to assess whether similar active immunization

TABLE 3. *Comparison of viral titers of inoculated eyes and ipsilateral SCG of BALB/c and C57Bl/6 mice during the acute phase of infection*[a]

Mouse strain	Mean tissue titers (acute phase) (PFU, $\log_{10}$)	
	Eyes[b]	SCG[c]
BALB/c	5.4	3.0
C57Bl/6	4.2	1.1

[a] Mice were sacrificed 5 days after i.o. inoculation. Results express the mean values of eye and SCG homogenates from five animals of each mouse strain.
[b] $P < 0.02$.
[c] $P < 0.02$.

shortly before i.o. HSV challenge would influence the course of the acute phase of ganglionic infection. The data in Table 4 indicate that while active immunization before i.o. virus challenge again did not reduce the subsequent prevalence of latency, immunization effectively reduced the extent of virus replication during the acute phase of infection by over 100-fold compared to the unimmunized controls.

Effect of passive immunization with anti-HSV antibody on the development of latent SCG infection. Similar studies assessing the influence of passively administered antiviral antibody on acute and latent infection were next undertaken. BALB/c mice were immunized by i.p. injection of 0.2 ml of immune BALB/c mouse serum possessing an anti-HSV neutralizing antibody titer of >1,024. Mice receiving serum 1 day before, 1 day after, and 3 days after right-sided i.o. HSV challenge were compared to unimmunized control animals. As shown in Fig. 2, during the acute phase of infection, mean viral titers in ipsilateral ganglion homogenates of control animals (group D) were high, approaching 1.0×10^4 PFU per ganglion. In each of the antibody-treated groups viral titers of ganglion homogenates were reduced. Greatest reduction occurred in mice receiving immune serum 1 day after virus challenge (group B) with lesser reduction in groups A and C. In contrast to the results of ganglion assay during the acute phase of infection, when latent infection in these groups was assessed by explantation, the highest prevalence of latency was found in group B, with progressively lower rates of latent infection in groups A, C, and D. Thus, an inverse relation was observed between viral titers of ganglion homogenates acutely and the subsequent prevalence of latency.

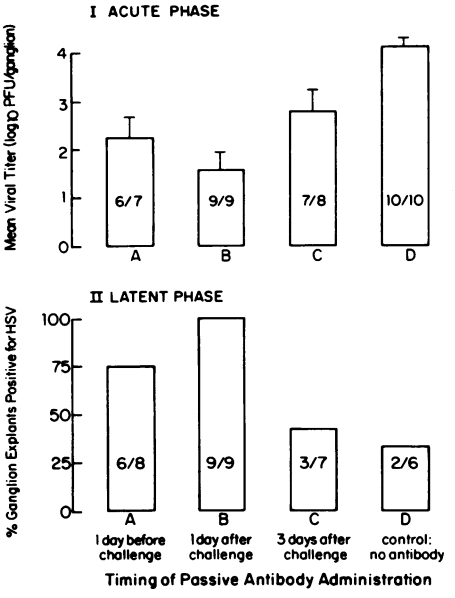

FIG. 2. *Effect on the development of acute and latent SCG infection of passive immunization with mouse anti-HSV antibody administered at various time intervals. Mice were injected i.p. with anti-HSV antibody 1 day before (group A), 1 day after (group B), and 3 days after (group C) i.o. virus challenge; group D did not receive antibody. (I) Acute phase: Mice were sacrificed on day 5, and viral titers in ganglion homogenates were determined. The columns depict the mean viral titers and standard error of the mean, whereas the numbers within the columns give the number of ganglion homogenates positive for HSV per number of ganglia assayed. Significance: A vs. D, $P < 0.01$; B vs. C, $P < 0.05$; B vs. D, $P < 0.001$; C vs. D, $P < 0.02$; other comparisons not significant. (II) Latent phase: Mice were sacrificed 21 days after viral challenge, and ganglia were assayed by explantation-co-cultivation. Columns depict the percentage of ganglion explants positive for HSV, whereas the numbers within the columns give the actual number of ganglion explants positive for HSV per number of ganglia assayed. Significance: A vs. C, $P < 0.05$; A vs. D, $P < 0.01$; B vs. C, $P < 0.01$; B vs D, $P < 0.01$; other comparisons not significant.*

TABLE 4. *Effect of active immunization by i.p. injection of HSV 1 day before i.o. virus challenge on acute and latent SCG infection*[a]

Group	Acute phase (homogenates)		Ratio of latent phase explants[d]
	Ratio[b]	Mean titer (PFU, $\log_{10}$)[c]	
Unimmunized	16/17	3.6	17/20
Immunized	3/18	1.3	20/20

[a] Mice were sacrificed on day 5 after i.o. HSV inoculation during the acute phase of infection and on day 28 after i.o. inoculation during the latent phase of infection. Immunized mice received 4×10^5 PFU of HSV i.p. 1 day before i.o. virus challenge while unimmunized controls were injected i.p. with PBS on the same schedule.

[b] $P < 0.001$.

[c] $P < 0.001$.

[d] Not significant.

The suppression of viral replication and enhancement of latent infection by passively administered antibody were reproducible when larger numbers of animals were tested and when higher titer antiserum produced in rabbits was used. The data in Table 5 show that rabbit anti-HSV serum administration (0.2 ml of serum i.p. with a neutralizing titer of >4,000 resulting in a serum titer of 128 in recipient mice) 1 day after i.o. virus challenge markedly decreased both the percentage of positive homogenates and the

mean viral titers in ganglia during the acute phase while again enhancing the development of latent infection.

Effect of cyclophosphamide on the course of acute SCG infection. The effect of immunosuppression with cyclophosphamide on the course of ganglionic infection was then assessed. BALB/c mice were divided into four groups: untreated controls; mice treated with cyclophosphamide (250 mg/kg i.p.) 1 day after i.o. virus challenge; mice receiving rabbit anti-HSV serum (0.2 ml i.p. of serum with a neutralizing titer of >4,000) 1 day after i.o. virus challenge; and mice similarly treated with both cyclophosphamide and immune serum. Figure 3 depicts the results of SCG homogenates of these four groups of animals when sacrificed on days 2 through 11 after virus inoculation. In the untreated animals, maximum viral titers were noted on day 4, decreasing thereafter to undetectable levels by day 7. In the cyclophosphamide-treated mice, viral titers continued to rise after day 4, peaked at day 5 and then declined to a mean of approximately 2.0×10^2 PFU per ganglion on day 7; all mice in this group died before day 11. Immune serum treatment, as in previous studies, markedly reduced viral titers in SCG homogenates of immunocompetent mice on each day tested. In the mice receiving both cyclophosphamide and immune serum, viral titers of ganglion homogenates were somewhat reduced early in the course of the infection, but further virus elimination was delayed, with HSV being detected in all of the five ganglia tested at day 7, and at day 11 one of four ganglia contained a small amount of infectious HSV. Antibody levels were measured at day 5 using pooled serum specimens from each group. In the two groups of mice passively transferred with rabbit anti-HSV serum, antibody titers were 128; in the untreated mice a serum titer of 4 was measured, whereas in animals treated only with cyclophosphamide, serum antibody was undetectable.

TABLE 5. *Effect of passive immunization with rabbit anti-HSV antiserum on acute and latent SCG infection*[a]

Group	Acute phase (homogenates)		Ratio of latent phase explants[d]
	Ratio[b]	Mean titer (PFU, $\log_{10}$)[c]	
Unimmunized	22/23	3.5	24/32
Immunized	3/23	1.2	31/32

[a] Mice were immunized with rabbit anti-HSV serum injected i.p. 1 day after i.o. HSV challenge (see text).
[b] $P < 0.001$.
[c] $P < 0.001$.
[d] $P < 0.02$.

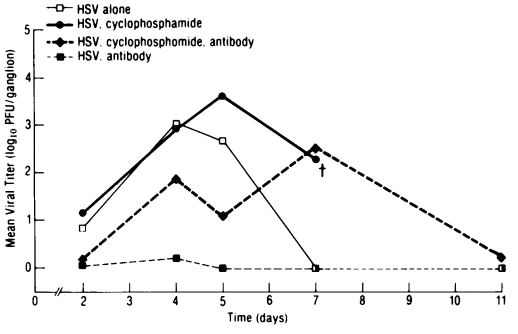

FIG. 3. *Effect of cyclophosphamide treatment on the course of acute SCG infection. All mice were challenged i.o. with HSV on day 0, and viral titers of ganglion homogenates from mice sacrificed between 2 and 11 days after HSV challenge were measured. Both cyclophosphamide (250 mg/kg) and rabbit anti-HSV antiserum were administered 1 day after i.o. virus challenge. The four groups included mice receiving no additional treatment, mice treated with cyclophosphamide alone or in combination with antibody, and those passively immunized with antibody only. All remaining mice in the cyclophosphamide alone group died between the days 7 and 11. Each point represents the results of determinations on five or more ganglia.*

Acute SCG infection in nude mice. Mice homozygous for the nude trait (nu/nu) were injected i.o. with HSV bilaterally, and both the right and left SCG were assayed for HSV by homogenization at 5, 8, and 10 days after inoculation. Results in nude mice passively immunized with 0.2 ml of immune rabbit serum (anti-

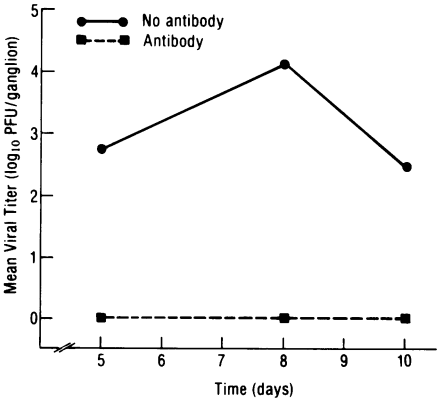

FIG. 4. *Acute SCG infection in nude mice. Nude mice were injected with either immune rabbit serum (anti-HSV neutralizing antibody titer >4,000) or with normal rabbit serum 1 day after bilateral i.o. virus challenge (see text). Animals were sacrificed for assay of SCG homogenates 5, 8, and 10 days after i.o. challenge. Each point represents the results of assay of four or more ganglia.*

HSV antibody titer of >4,000) given i.p. 1 day after i.o. HSV challenge were compared to unimmunized nude mice similarly injected i.p. with 0.2 ml of normal rabbit serum devoid of neutralizing antibody. Figure 4 shows that in the unimmunized nude mice highest viral titers were observed on day 8 after infection and that infectious virus remained detectable in SCG homogenates at day 10. Antibody treatment of the nu/nu mice reduced SCG infection to undetectable levels on the days assayed. Infection in unimmunized and passively immunized heterozygous (nu/+) littermates was also assayed and resembled that observed in BALB/c mice (data not shown).

Effect of postganglionic neurectomy after i.o. HSV injection on the course of acute SCG infection in passively immunized mice. We have previously shown that postganglionic neurectomy reproducibly reactivates HSV in the SCG of latently infected mice (12). We next sought to determine whether this same operative procedure would alter the acute phase of ganglionic infection if neurectomy were delayed until after virus reached the ganglion. BALB/c mice were injected with 0.2 ml of rabbit anti-HSV serum (titer >4,000) i.p. 1 day after i.o. virus challenge and subjected to postganglionic neurectomy at various time intervals before and after HSV inoculation. All animals were sacrificed for ganglion assay by homogenization 5 days after i.o. virus challenge. As shown in Table 6, when neurectomy was carried out between 24 h before and 6 h after virus challenge, HSV was undetectable in SCG homogenates. However, in animals neurectomized between 20 and 72 h after i.o. virus challenge, ganglionic HSV replication was detected, with highest viral titers being noted in mice subjected to neurectomy at 48 h. The results of ganglion assay in these neurectomized, passively immunized mice contrasted sharply with those in sham-operated (20 h), immunized animals in which mean viral titers of SCG homogenates remained below 1 PFU per ganglion.

A further experiment was undertaken to evaluate the time course of neurectomy-induced enhancement of virus replication during the acute infection. Mice were divided into animals subjected to ipsilateral postganglionic neurectomy 48 h after i.o. HSV inoculation and those simultaneously subjected to sham operation in which the SCG was surgically exposed but left untouched. The two groups were in turn subdivided into those passively immunized with rabbit anti-HSV serum (titer >4,000) 24 h after virus challenge and those receiving normal rabbit serum at the same time. As indicated by the data in Fig. 5, neurectomy profoundly influenced

TABLE 6. *Effect of postganglionic neurectomy on the acute phase of SCG infection in passively immunized mice*

Operative procedure	Time of operation[a] (h)	Acute phase[b] (homogenates)	
		Ratio	Mean titer (PFU, $\log_{10}$)
Postganglionic neurectomy	−24	0/4	0
Postganglionic neurectomy	−1.5	0/4	0
Postganglionic neurectomy	+6	0/5	0
Postganglionic neurectomy	+20	2/4	3.8
Postganglionic neurectomy	+29	2/5	3.8
Postganglionic neurectomy	+48	4/5	4.6
Postganglionic neurectomy	+72	4/4	2.9
Sham	+20	1/5	0[c]

[a] Time of operative procedure in relation to time of i.o. challenge. Negative sign indicates operation before virus injection, whereas positive sign indicates operation following virus injection.

[b] Mice were sacrificed 5 days after i.o. HSV challenge.

[c] The single positive ganglion contained 1 PFU of virus.

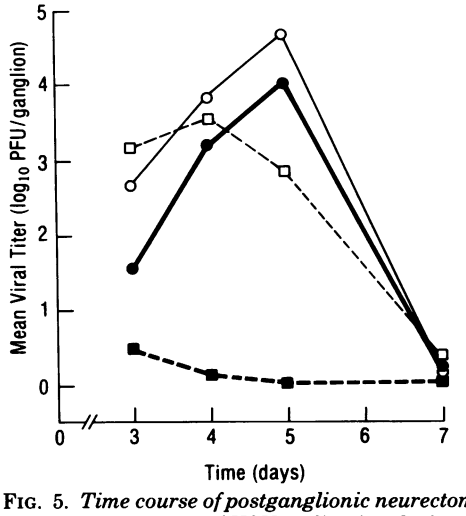

FIG. 5. *Time course of postganglionic neurectomy-induced augmentation of HSV replication during the acute phase of SCG infection. All mice received i.o. HSV challenge on day 0. The immunized groups were injected i.p. with rabbit anti-HSV antiserum and the unimmunized groups were injected with normal rabbit serum 1 day after i.o. virus challenge. Postganglionic neurectomies and sham operations were done 2 days after i.o. injection. Mice were sacrificed on days 3 through 7, and viral titers of ganglion homogenates were determined. Each point represents the results of assay of between four and six ganglia. Symbols: □, unimmunized, sham operation; ■, immunized, sham operation; ○, unimmunized, neurectomy; ●, immunized, neurectomy.*

the course of viral replication. In the unimmunized mice subjected to sham operation, viral titers peaked at day 4 and declined to nearly undetectable levels at day 7. In contrast, in the neurectomized, unimmunized group viral titers continued to rise after day 4 reaching a mean titer above 4.5×10^4 PFU per ganglion on day 5; this compares with a mean titer of 5.9×10^2 on the same day in the sham-operated, unimmunized group. Even more striking, however, was the course of viral replication in the immunized, neurectomized group. In these animals, HSV was detected at day 3 and rose to a peak level of nearly 1.0×10^4 PFU per ganglion on day 5, again demonstrating that neurectomy induced high viral titers in the SCG despite passive transfer of immune serum which, in the sham-operated group, resulted in minimal viral titers in the SCG at days 3 and 4 and undetectable levels thereafter. Mice from the two immunized groups were also sacrificed at 21 days to assess the effect of neurectomy on the development of latent infection. In the neurectomized group 5 of 15 (33%) ganglion explants were positive for HSV, whereas 11 of 14 (79%) ganglia from sham-operated control mice were positive for virus ($P < 0.05$), indicating that neurectomy not only augmented viral replication acutely but also led to a reduction in the subsequent prevalence of latency.

DISCUSSION

The present studies show that the course of experimental SCG infection with HSV is influenced by a number of factors, including the route by which the virus reaches the ganglion, the level of systemic host resistance, and the integrity of postganglionic axons. These factors importantly determine not only whether or not the ganglion becomes infected but also the character of both the acute and latent phases of ganglionic infection.

Viral access to the SCG may occur via either neural or hematogenous pathways. Disruption of the nerves connecting the eye and the SCG by postganglionic neurectomy before i.o. virus challenge prevented both acute and latent ganglionic infection, thereby demonstrating the importance of the neural route in establishing autonomic infection. Whether neural passage entails primarily intra-axoplasmic (retrograde) transport (8) or perineural virus passage (7) cannot be directly inferred from these studies, although both the detection of virus in ganglia as early as 24 h after i.o. injection (5) and the failure of neurectomy performed later than 24 h after inoculation to prevent ganglionic infection suggest involvement of the more rapid intra-axoplasmic route. Similarly, the failure of passively administered antiviral antibody, which might be expected to interfere more effectively with perineural than with axoplasmic passage of virus (5), to prevent latent SCG infection also favors the importance of axoplasmic viral transport. However, in unimmunized animals HSV may well reach the ganglion by both intra-axonal and perineural passage. Moreover, the present studies also demonstrate that virus can reach the ganglion hematogenously, although with poor efficiency. Thus, i.v. injection of HSV led to low levels of virus replication in the ganglion and to a lower prevalence of subsequent latency compared to anterior chamber inoculation.

Both immunologically specific and nonspecific defenses influenced whether or not HSV gained access to the SCG. The protective effect of specific immunity was demonstrated by the prevention of ganglionic infection in BALB/c mice actively immunized i.p. with HSV 7 days before i.o. virus challenge. Although anti-HSV antibody may have contributed to this protection, the failure of antibody alone, when administered 1 day before i.o. virus challenge, to exert a similar protective effect suggests that other, presumably virus-specific, cell-mediated, immune mechanisms were also involved in preventing virus from reaching the ganglion in actively immunized mice. On the other hand, the resistance to the development of ganglionic infection seen in the C57Bl/6 mice compared with that of BALB/c mice when low doses of HSV were used for inoculation suggests that nonspecific host defenses can also participate in preventing ganglionic infection. Virus gains access to the SCG within 24 to 48 h of i.o. injection, and, therefore, early events at the site of inoculation and perhaps along the nerve are paramount in determining whether the ganglion becomes infected. Because these early events take place rapidly, before specific immune defenses can be mobilized by the virgin host, it is likely that other genetically determined but immunologically non-specific antiviral defenses account for the differences in ganglionic infection seen in the two mouse strains at low virus doses.

The ganglionic infection which ensues once HSV reaches the SCG has been analyzed in two phases, an acute and a latent phase. The acute phase was quantitatively assessed by the viral titers of ganglion homogenates which reflect the extent of intraganglionic viral spread and replication. The latent phase was assessed by the explantation-co-cultivation assay which detects the continued presence of the HSV viral genome. The present studies demonstrate that the outcome of the acute and latent phases of infection may be dissociated. Thus, appropriately timed immunization or genetically determined sys-

temic resistance decreased the magnitude of viral replication acutely while failing to reduce, or actually enhancing, the subsequent prevalence of latent infection. Conversely, postganglionic neurectomy 48 h after i.o. virus challenge augmented the acute phase of ganglionic infection while reducing subsequent latency.

How does systemic host resistance favor the development of latency over acute productive infection? Stevens and Cook have hypothesized that one particular aspect of host defenses, antiviral immunoglobulin G antibody, exerts a direct effect on the infected ganglion cell and modulates the permissiveness of the cell for virus replication (15). Aspects of the present studies can be interpreted as consistent with this hypothesis. Passively administered antiviral antibody, particularly when given 1 day after virus challenge, not only reduced productive infection but potentiated latency. Likewise, active immunization induced by i.p. virus injection between days 1 and 3 before i.o. virus challenge reduced acute virus replication without reducing latency; this effect could also be interpreted as secondary to circulating antibody which becomes detectable between days 4 and 6 after immunization. In both instances it can be speculated that antiviral antibody directly signaled ganglionic cells to "commit themselves" to the latent state rather than allowing productive infection.

The results of other experiments, however, suggest that antiviral immunoglobulin G alone is inadequate to explain the influence that systemic host resistance exerts in inhibiting acute productive infection and enhancing latency. In the studies of timed passive immunization, it might have been expected that transfer of antiserum 1 day before i.o. virus challenge (Fig. 2, group A) would have produced the greatest reduction in the acute infection if antibody alone was responsible for inhibiting virus replication. In fact, maximal reduction occurred in mice given antibody 1 day after i.o. virus challenge (Fig. 2, group B). One possible explanation for this result is that the 24-h interval between i.o. virus challenge and later antibody administration in group B allowed clearer exposure of the host's own immune system to the virus, thereby stimulating a stronger endogenous cell-mediated immune response. Furthermore, in mice immunized with anti-HSV antibody and also treated with cyclophosphamide, productive infection continued beyond the usual peak at 4 to 6 days, indicating that antibody, by itself, was incapable of inducing the ganglion cells in these animals to enter the latent state, but rather required the participation of a cyclophosphamide-sensitive cell population. The experiments employing athymic nude mice indicate that T-lymphocytes contribute to eliminating intraganglionic HSV replication, but the observation that anti-HSV antibody largely abrogates the acute infection in the SCG of nude mice suggests that one reason for the prolonged infection in the thymus-deficient animals may have been an absence of antibody production rather than a lack of T-lymphocyte effector cells; HSV acts as a thymus-dependent antigen in these animals, and thus they require T-lymphocyte helper function to produce neutralizing antibody (4). The results of studies of C57B1/6 compared to BALB/c mice inoculated with higher titers of virus also suggest that mechanisms other than antibody contribute to concomitant reduced acute infection and increased latency (9).

Although the present investigations do not in each instance precisely define the immune defenses involved, they do suggest that a constellation of host defenses can cooperate in reducing the level of intraganglionic HSV replication acutely and that the effect of antibody is perhaps complemented by T-lymphocytes and other cell populations. But how do these antiviral mechanisms, which eliminate infectious virus acutely, actually promote the development of latency? In part, they may act by preserving the anatomic integrity of the ganglion. In unimmunized BALB/c mice, infected ganglia have at times been noted to be reduced in size when assayed for latency, and light microscopic examination has revealed a loss of ganglionic cells in these animals. Immunization, by either active or passive administration of antibody, lessened the degree of atrophy and neuronal loss (unpublished data). Ganglionic atrophy was similarly less evident in C57B1/6 than in BALB/c mice. Reduced latency may then result from a quantitative reduction in surviving neurons. On the other hand, it can be argued that ganglionic atrophy provides only a partial answer and does not fully explain why the prevalence of latency is higher in the surviving cells of the immunized mice compared with that of the nonimmunized animals. Despite destruction of some cells, the surviving cells of both groups are still faced with a "decision" as to whether latent or productive lytic infection will ensue following viral exposure. An alternative hypothesis to explain the potentiation of latency by increased host resistance is that the input multiplicity plays a critical role in determining whether a ganglion cell is to be productively or latently infected, and that when a cell is infected with a low input, latent infection occurs, although higher doses of infecting virus lead to productive infection. Such a mechanism would account for the diverse circumstances in which heightened resistance leads to latency. The several means discussed earlier

by which host defenses dampen productive infection might also thereby insure latency by reducing, but not preventing, initial viral access to the ganglion, by eliminating productively infected cells and by quantitatively reducing intraganglionic virus spread with the overall effect of reducing the input multiplicity to individual ganglion cells. It may additionally be speculated as a corollary to this hypothesis that the route by which virus reaches the ganglion also influences the outcome of infection. The ability of host defenses to more effectively prevent perineural propagation of HSV, which entails multiple cycles of virus replication and spread to contiguous cells, compared to axoplasmic viral transport provokes the question of whether the balance between productive and latent infection depends on which of these neural routes serves to introduce virus to the ganglion. If virus reaches the neuronal soma as part of a wave of infection involving Schwann cells, other non-neuronal supporting cells, and adjacent neurons, productive infection may be more likely to occur than if virus reaches the soma via its own axonal process.

Whatever the mechanisms by which heightened resistance reduces acute infection and promotes latency, the experiments employing postganglionic neurectomy establish that the state of the ganglion cells also profoundly influences the balance between productive and latent infection although in a direction opposite that conferred by immunization. When postganglionic neurectomy was delayed 48 h after i.o. virus challenge so that viral access to the ganglion was assured, HSV replication during the acute phase was augmented. This was evident in nonimmunized mice, but was even more striking in mice passively immunized with antiviral antibody in which viral titers of nearly 1.0×10^4 PFU per ganglion were detected, compared to minimal viral titers in sham-operated immunized controls. Indeed, the effect of neurectomy was similar in magnitude, although not in duration, to that seen when mice were immunosuppressed with cyclophosphamide. Furthermore, this augmented viral replication in the acute phase was followed by a reduced prevalence of subsequent latency. The mechanism involved in this neurectomy-induced alteration of acute ganglionic infection is uncertain, although very likely it is the same as that responsible for reactivation of latent infection by postganglionic neurectomy (12). We have previously speculated that neurectomy might alter permissiveness of ganglion cells for HSV replication either as a part of the axon reaction (chromatolysis) which is accompanied by augmented and redirected RNA and protein synthesis, or as a result of altered trophic influence conferred by innervated target tissue (12). Whatever the mechanisms involved, it is evident that although increased systemic host resistance favors an outcome of latent rather than productive SCG infection, postganglionic neurectomy favors the opposite. Also, neurectomy can clearly overcome the effect of antiviral antibody, allowing vigorous productive infection to occur despite high levels of serum neutralizing antibody.

Host factors both extrinsic (e.g., systemic antiviral resistance) and intrinsic (e.g., the integrity of postganglionic axons) to the ganglion determine the course of acute and latent ganglionic infection. For the most part the mechanisms underlying these host influences remain uncertain. For this reason, the foregoing discussion has been rather speculative. Support for or rejection of the offered hypotheses requires additional studies of in vivo ganglionic infection, and, perhaps more importantly, awaits the development of relevent in vitro models of ganglionic latency.

ACKNOWLEDGMENTS

This work was supported by Public Health Service research grant NS-12396 from the National Institute of Neurological and Communicative Disorders and Stroke and Basil O'Connor Starter research grant 5-123 from the National Foundation-March of Dimes.

We gratefully acknowledge the additional technical assistance of Joanne Levin.

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