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Reactivation of Latent Herpes Simplex Virus Infection of the Autonomic Nervous System by Postganglionic Neurectomy

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Latent herpes simplex virus infection of the superior cervical autonomic ganglion was reactivated in vivo by postganglionic neurectomy. Two methods were used to demonstrate viral reactivation: (i) recovery of infectious herpes simplex virus in ganglion homogenates and (ii) acceleration of virus expression in ganglion explants in culture. Both the percentage of mice exhibiting reactivated ganglion infection and the viral titers detected in ganglia increased when neurectomized mice were treated with cyclophosphamide. Antithymocyte serum treatment prolonged the time course over which neurectomy-induced virus could be detected, but neither antithymocyte serum nor cyclophosphamide reactivated herpes simplex virus in the absence of neurectomy. These results demonstrate that postganglionic neurectomy provides a specific stimulus for herpes simplex virus reactivation and that cell-mediated immune defenses are involved in the highly efficient elimination of reactivated virus from the ganglion in vivo.

Recent studies (2, 22) have provided substantial support for the theory that the sensory ganglia of the nervous system play a central role in latent and reactivated herpes simplex virus (HSV) infections. However, despite evidence that sensory ganglia can harbor latent HSV type 1 (HSV-1) and type 2 (HSV-2) in both experimental animals (23, 25) and humans (1, 3), the pathogenesis of recurrent herpetic disease remains poorly understood. Yet to be established is whether disease production, e.g., the common cold sore or recurrent genital eruption, depends primarily on events within ganglia or on conditions at the epithelial surface (4, 20). In addition, studies of experimental infection in mice raise the question of whether autonomic as well as sensory ganglia may be involved in HSV latency and reactivation (16, 17, 24). Likewise, the role of host immune defenses in the pathogenesis of recurrent herpetic eruptions remains uncertain. Although it is perhaps generally conceded that immune mechanisms confine the epithelial lesion to the circumscribed cold sore, less certain is the role of immunity in determining whether or not an epithelial lesion is initiated (14). Furthermore, if virus is reactivated in the ganglion, the immune mechanisms involved in successfully restricting intraganglionic infection have yet to be defined.

To approach these problems experimentally, we have turned to a model of HSV latency in the superior cervical ganglion (SCG) of the peripheral autonomic nervous system in mice. Autonomic ganglia probably share a common embryological origin with sensory ganglia (26), and previous studies have shown that infection in the SCG parallels that produced in sensory ganglia of mice: in both cases, inoculation of peripheral target tissue innervated by ganglionic neurons leads to an acute lytic infection that is followed by prolonged latency (16). We now report that latent infection of the SCG can be reactivated in vivo by postganglionic neurectomy and that reactivation of virus is enhanced when neurectomized animals are treated with cyclophosphamide or antithymocyte serum (ATS).

MATERIALS AND METHODS

Tissue culture and media. Rabbit kidney (RK) cells, prepared as described earlier by Gallagher (9), were used either as primary cell monolayers or after one or two passages. Whole human embryo cells (Flow 5000) were obtained commercially (Flow Laboratories, Inc., Rockville, Md.). The basic medium used for all cultures was Eagle minimum essential medium supplemented with 0.5% lactalbumin hydrolysate, 50 μg of gentamicin per ml, and 100 U of nystatin per ml; for maintenance of cultures, 2% heat-inactivated fetal bovine serum (Microbiological Associates, Rockville, Md.) was added, whereas for cell growth 10% fetal bovine serum was used. Cultures were maintained at 36°C in an atmosphere containing 5% CO_2.

Virus. The F strain of HSV-1 (obtained from American Type Culture Collection, Rockville, Md.) was used in these studies. The stock virus pool for inoculation contained 2×10^8 plaque-forming units (PFU) of

HSV per ml and was prepared and assayed on RK cells (10).

Animals. Four- to six-week-old BALB/c female mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were used for all studies. New Zealand white rabbits (West Jersey Biologicals, Wenonah, N.J.), 3 to 5 pounds (ca. 1.36 to 2.27 kg), served as a source of RK cells.

Virus inoculation. For intraocular (i.o.) virus inoculation, mice were briefly anesthetized with ether and, with the aid of a dissecting microscope, 4 μl of the stock virus pool was injected into the anterior chamber of the right eye with a 100-μl syringe and repeating dispenser (The Hamilton Co., Reno, Nev.). For all studies of in vivo viral reactivation, mice received an intraperitoneal (i.p.) injection of 2×10^6 PFU of live HSV 24 to 48 h before i.o. virus challenge. This pre-inoculation immunization was carried out because in previous studies with nonimmunized mice we noted that the infected ipsilateral SCG was partially destroyed during the acute phase of the infection and was often atrophic when animals were later sacrificed. Because preservation of the integrity of the ganglion would seem important in studies of viral reactivation, particularly by neurectomy, we attempted to reduce the degree of ganglion destruction during the acute phase of infection by immunizing mice shortly before i.o. virus challenge. We found that when mice were actively immunized with i.p. virus within 3 days before i.o. injection, the prevalence of latent infection in the SCG was as high as, or higher than, that in nonimmunized animals and that, furthermore, ganglion atrophy was largely prevented.

Assay of ganglia for virus. Mice were sacrificed by exposure to 100% CO_2, and the right SCG, i.e., the SCG ipsilateral to the infected eye, was removed under sterile conditions with the aid of a dissecting microscope. The ganglia were then washed three times in Dulbecco phosphate-buffered saline (PBS) containing antibiotics (50 μg of gentamicin per ml and 100 U of nystatin per ml) and assayed by one of two methods: homogenization or explantation.

Homogenization was carried out in a Ten Broeck tissue grinder (Bellco Glass Inc., Vineland, N.J.) with individual ganglia suspended in 1.0 ml of PBS containing antibiotics and supplemented with 1% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.). The homogenates were rapidly frozen and thawed three times and assayed for HSV on RK monolayers. For initial reactivation studies, only the presence or absence of virus in homogenates was determined as follows. Samples were divided into two aliquots and placed on duplicate 30-mm plastic petri dishes containing RK monolayers, and after rocking at 36°C for 2 h, maintenance medium was added. The cultures were then monitored for HSV-induced cytopathic effect (CPE) over the next 7 days; the specificity of CPE in random samples was tested by neutralization of culture supernatants with rabbit anti-HSV serum. For studies of the acute phase of infection and in later studies of virus reactivation, viral titers in ganglion homogenates were determined. For this quantitative assay, undiluted homogenates, and at times 1:10 and 1:100 dilutions as well, were placed on duplicate RK monolayers and rocked for 2 h at 36°C as described above; after this incubation, samples were aspirated, and an overlay medium was added in which the fetal bovine serum of maintenance medium was replaced with 2% pooled human serum (Grand Island Biological Co., Grand Island, N.Y.) cont[illegible]ing anti-HSV antibody to prevent secondary plaque formation (10). After 48 h, plates were fixed with 95% ethanol and stained with Giemsa; viral plaques were then counted by using a dissecting microscope.

For the explantation assay, washed ganglia were placed on human embryo cell monolayers in 30-mm petri plates. For the first 18 h, a low level of medium was retained in the dishes to allow adherence of the ganglia, but thereafter 2 ml of maintenance medium was placed in each plate and changed twice weekly. Cultures were kept for 3 weeks and monitored for the appearance of CPE, which first develops at the perimeter of the explant and subsequently spreads over the remainder of the indicator cell monolayer.

Surgery. All neurectomies were performed aseptically under pentobarbital anesthesia with a dissecting microscope.

Serum antibody titers. Blood was obtained from the left (noninjected side) retro-orbital plexus, and serum anti-HSV neutralizing antibody titers were determined by using a plaque-reduction method (24). Serum titers were expressed as the reciprocal of the highest serum dilution producing a 50% reduction in viral plaques.

RESULTS

Acute and latent infection of the SCG with the F strain of HSV-1. Base-line studies were carried out to define the character of SCG infection with the F strain of HSV-1. Figure 1

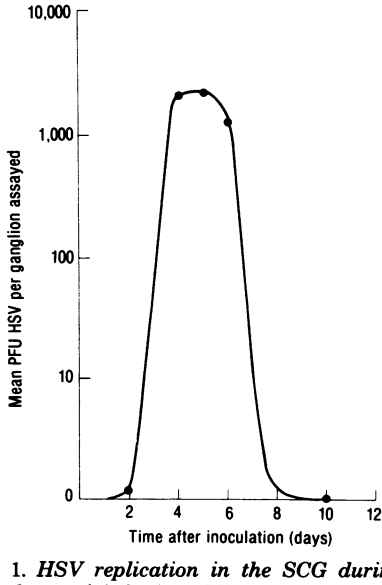

FIG. 1. *HSV replication in the SCG during the acute phase of infection. Mice were sacrificed at intervals after right-sided i.o. virus injection, and viral titers of homogenates of the ipsilateral SCG were determined. Each point represents the mean of determinations from at least 10 animals.*

shows that after i.o. virus injection low levels of HSV were detected in SCG homogenates by day 2 after inoculation and that virus titers then increased sharply, reaching peak levels at day 5; the amount of infectious virus then fell off rapidly, so that by day 10 HSV was no longer detected in homogenates of the ganglia.

The presence of HSV in the SCG was also readily detected during the acute phase of infection when the explantation method of virus assay was used. Moreover, in contrast to results with homogenization, explantation allowed detection of virus in ganglia after the acute phase of infection had subsided. Thus, in the same experiment from which the data in Fig. 1 were derived, not only were 10 of 10 ipsilateral ganglion explants from mice sacrificed on day 5 after inoculation positive for HSV, but, in addition, HSV was recovered from 8 of 12 SCG explants from animals sacrificed 33 days after inoculation (data not shown). We have now recovered virus from explanted autonomic ganglia over 1 year after inoculation.

The profile of HSV recovery from the SCG by the two assay methods allows latency to be operationally defined as that period of infection after the acute phase has subsided during which ganglion homogenates are negative while explants are positive for HSV. In the studies of reactivation to be described below, at least 3 weeks intervened between i.o. virus inoculation and efforts to reactivate virus.

Reactivation of latent HSV by postganglionic neurectomy: homogenate-positive ganglia as an index of reactivation. Our first efforts at HSV reactivation have focused on the use of postganglionic neurectomy as a reactivating stimulus because: (i) neurectomy is probably the most consistent stimulus for HSV reactivation in humans (5) and (ii) neurectomy has been successful in reactivating HSV from sensory ganglia in experimental animals (25). To evaluate whether virus was reactivated in the ganglion, two methods were used: (i) detection of virus in ganglion homogenates and (ii) acceleration of in vitro HSV expression in ganglion explants. The second of these methods will be discussed below. The detection of virus in ganglion homogenates as an index of reactivation has proved useful in previous studies of sensory ganglia, and the rationale of this method has been discussed (25). In brief, because latency is characterized by the inability to detect virus in ganglion homogenates from mice inoculated at least 3 weeks earlier, a stimulus applied during the latent phase that causes HSV to again be detectable by homogenization is considered effective in reactivating virus.

The SCG of the mouse receives input from neurons of the intermediolateral grey matter of the cervicothoracic spinal cord via preganglionic fibers that enter the ganglion caudally and synapse on ganglionic neurons. The axons of the ganglionic neurons, in turn, leave the SCG in three or more definable postganglionic nerves. The most rostral of these nerves probably carries the majority of fibers innervating the ipsilateral iris, whereas the other two postganglionic nerves supply other autonomic target organs in the head as well as those in the neck and forequarter (8). With a dissecting microscope, the ganglion and nerves are easily located adjacent to the carotid artery bifurcation in the neck. Table 1 shows that when the postganglionic nerves of latently infected ganglia are cut, virus is reactivated in vivo. In the first experiment, only the rostral postganglionic nerve was cut ("simple postganglionic neurectomy"), whereas in the second experiment all three major postganglionic fibers were severed ("compound postganglionic neurectomy"). In both experiments, virus was detected by the homogenization assay only on day 4 after operation; homogenates of ganglia from unoperated controls and from neurectomized mice assayed before or after day 4 were all negative for HSV. In all subsequent studies of neurectomy-induced reactivation, therefore, mice were assayed 4 days after operation. Also, because a larger proportion of ganglia were positive in the compound neurectomy group, this more extensive operative procedure was employed in subsequent experiments. The results of explantation of ganglia from mice in the two groups (total of 31 positive out of 39 assayed)

TABLE 1. *Timing of HSV reactivation in the SCG after ipsilateral postganglionic neurectomy*[a]

Operative procedure	Homogenization of SCG; assay after neurectomy on day:						Explantation of SCG: unoperated
	Unoperated	1	2	3	4	5	
Simple postganglionic neurectomy	ND[b]	0/14	0/13	0/15	1/14	0/13	11/15
Compound postganglionic neurectomy	0/19			0/12	3/12	0/12	20/24

[a] Results are expressed as the ratio of the number of ganglia positive for HSV to the number of ganglia assayed.

[b] ND, Not done.

provide an index of the prevalence of latent infection in the SCG of these animals.

We next compared animals subjected to ipsilateral postganglionic neurectomy not only with unoperated control animals but also with animals subjected to two types of control operation—contralateral postganglionic neurectomy and ipsilateral preganglionic neurectomy. These studies were undertaken in order to determine whether HSV reactivation by postganglionic neurectomy was reproducible and also to assess whether the nonspecific stress of surgery, rather than the disruption of postganglionic fibers emanating from the latently infected ganglion, could be implicated in causing the reactivation of virus. In these, as in subsequent studies, latent infection was established by i.o. HSV infection at least 3 weeks before attempts at reactivation; animals were sacrificed for ganglion assay by homogenization 4 days after surgery. The results of four experiments are shown in Table 2. In these studies, HSV was consistently detected by homogenization in ganglia from the ipsilateral postganglionic neurectomized animals. Although the incidence of detectable reactivation was low, with only approximately 10% (total, 6 of 57) of the ganglia from the ipsilateral postganglionic neurectomy group positive for virus, these results nonetheless contrast with the uniform failure to detect HSV in homogenates of ganglia from mice subjected to either contralateral postganglionic (0 of 53) or ipsilateral preganglionic (0 of 44) neurectomy ($P < 0.05$ by chi-square contingency analysis when totals of the ipsilateral postganglionic neurectomy group are compared with totals of either of these operative controls).

Reactivation of latent HSV by postganglionic neurectomy: acceleration of virus expression in ganglion explants in vitro as an index of viral reactivation in vivo. In previous studies, we noted that when ganglia were assayed by explantation during the acute phase of infection, CPE developed rapidly in the indicator cell monolayer surrounding the explant. In contrast, when ganglia of latently infected mice were explanted, a delay in virus expression was seen (unpublished data). In addition, expression of HSV in vitro in a given experimental group was noted to be remarkably synchronous. This suggested that the timing of virus expression of explants in vitro could be used as an index of acute, latent, or reactivated infection in vivo. We therefore studied these stages of HSV ganglion infection by monitoring the timing of HSV expression from ganglion explants in vitro. After explantation and cocultivation of the SCG on Flow 5000 monolayers, the cultures were checked daily, and when viral CPE could be detected in at least five of the indicator cells surrounding the explant, the cultures were scored as positive. In each case, the specificity of the early cytopathic change was confirmed by the continued progression of typical CPE over the remainder of the monolayer in the ensuing 1 to 2 days.

Figure 2A shows that ganglia removed from animals 5 days after HSV inoculation expressed virus rapidly when placed in culture. In this acute-phase experimental group in which mice were not preimmunized before i.o. virus challenge, all 10 of the ipsilateral ganglia were positive for HSV. Of particular note, initial expression of virus was detected on day 2 in vitro in seven of the cultures, and the remaining three cultures turned positive over the next 2 days. In contrast, when ganglia of preimmunized mice were explanted during the latent phase of infection, the first evidence of virus expression was delayed until day 6 in vitro (Fig. 2B). The same results were obtained when mice challenged i.o. without pre-immunization were assayed during the latent phase of infection (data not shown).

To determine whether timing of HSV expression in SCG explants would prove useful in evaluating reactivated virus, we subjected latently infected mice to ipsilateral postganglionic neurectomy and explanted ganglia 3, 4, and 5 days later. Figure 2C shows that in animals sacrificed 3 days after operation, two of the five ganglia positive for HSV expressed virus on day 5 in vitro, i.e., 1 day before expression in unoperated controls. In cultures of ganglia from mice sacrificed 4 days after postganglionic neurectomy, virus could be detected as early as day 3 in vitro, with one-half of the positive explants expressing virus before in vitro day 6 (Fig. 2D). Ganglia of mice sacrificed on day 5 did not exhibit early HSV expression in vitro (data not shown). These results indicate that postgan-

TABLE 2. *Reactivation of HSV in the SCG after ipsilateral postganglionic neurectomy assessed by homogenization: comparison with unoperated and operated controls*[a]

Expt	Ipsilateral postganglionic neurectomy	Unoperated control	Contralateral postganglionic neurectomy	Ipsilateral preganglionic neurectomy
1	1/10	ND[b]	0/9	0/10
2	1/10	0/13	0/13	0/10
3	1/12	0/11	0/8	ND
4	3/25	ND	0/23	0/24

[a] Results are expressed as the number of ganglia positive for HSV per the number of ganglia assayed by homogenization.

[b] ND, Not done.

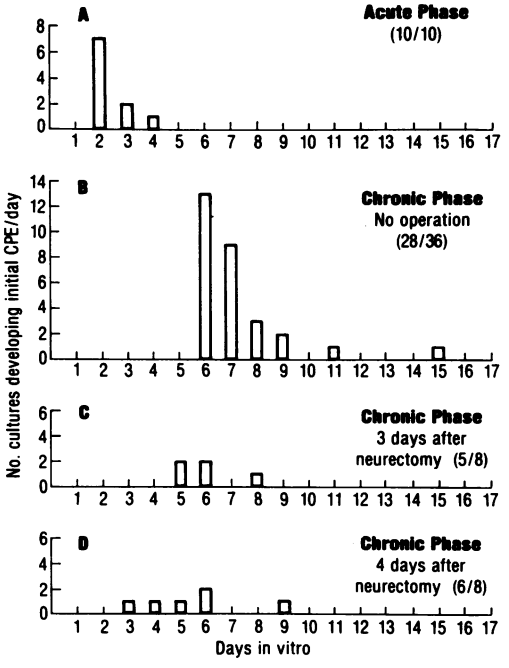

FIG. 2. *Timing of HSV expression in vitro of SCG explants as an index of neurectomy-induced viral reactivation. Ganglia ipsilateral to the side of i.o. injection were cocultivated in vitro and observed daily for the appearance of viral CPE. The bars depict the number of ganglia showing initial CPE on each day. The numbers in parentheses give the total number of ganglion explants positive for virus per the number of ganglia assayed in each experimental group. In group A, mice were not preimmunized and were sacrificed 5 days after i.o. injection. In groups B through D, mice were preimmunized, challenged i.o. with HSV, and sacrificed 25 days later. In group B, no operation was performed, whereas in group C, ipsilateral postganglionic neurectomy was done 3 days before sacrifice, and in group D, ipsilateral postganglionic neurectomy was done 4 days before sacrifice.*

glionic neurectomy leads to an acceleration of expression of HSV from SCG explants in culture. The finding that this early expression was maximum in ganglia removed on postoperative day 4 confirms the temporally circumscribed reactivation of HSV that was noted when homogenization of ganglia was used as the index of reactivation.

Effect of cyclophosphamide treatment on neurectomy-induced HSV reaction. To determine if immune defenses contribute to the low incidence and circumscribed time course of virus reactivation induced by neurectomy, we next tested the effect of cyclophosphamide treatment on HSV reactivation. Two regimens of cyclophosphamide were used initially: a single i.p. dose of 150 mg/kg given on the day of operation and a 100-mg/kg dose administered i.p. daily over 5 days beginning 1 day before operation and continuing through postoperative day 3. Figures 3A and B show that SCG explants

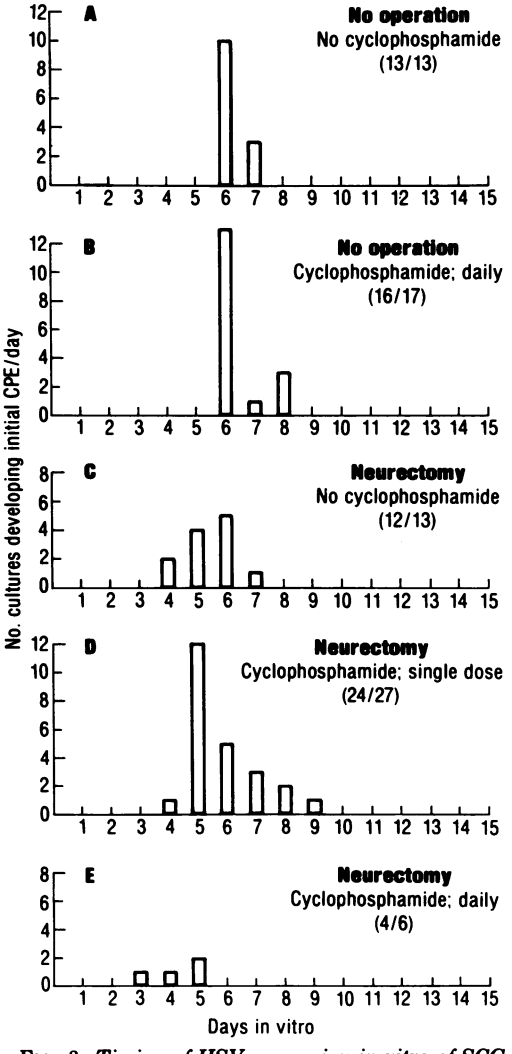

FIG. 3. *Timing of HSV expression in vitro of SCG explants: effect of postganglionic neurectomy and cyclophosphamide treatment on viral reactivation. Ganglia ipsilateral to the side of i.o. injection were cocultivated in vitro and observed daily for the appearance of viral CPE. The bars depict the number of ganglia showing initial CPE each day. The numbers in parentheses give the total number of ganglion explants positive for virus per the number of ganglia assayed in each experimental group. All mice were preimmunized before i.o. virus injection and sacrificed 25 days later. Cyclophosphamide was given either daily at a dose of 100 mg/kg (groups B and E) or as a single dose of 150 mg/kg (group D; see text). Postganglionic neurectomy was performed 4 days before sacrifice (groups C, D, and E).*

from unoperated mice did not express virus until day 6 in vitro whether the animals were treated daily with cyclophosphamide or with PBS on a similar schedule. When animals were subjected to postganglionic neurectomy and sacrificed 4 days later, virus was detected before day 6 in vitro in one-half of the positive ganglion cultures (Fig. 3C). When postganglionic-neurectomized mice were treated with a single injection of cyclophosphamide, similar results were noted: virus was detected before day 6 in vitro in 13 of the 24 ganglia positive for virus (Fig. 3D). However, when neurectomized mice were treated with daily cyclophosphamide, virus was detected in all positive ganglia before day 6 in culture, indicating enhancement of HSV reactivation by daily drug treatment (Fig. 3E).

Enhancement of HSV reactivation was also found when homogenization was used to assess the effect of daily cyclophosphamide treatment in combination with neurectomy. Mice were treated daily with cyclophosphamide while control animals received PBS on a similar schedule; drug- and saline-treated mice were then further divided into unoperated, contralateral postganglionic neurectomy, ipsilateral preganglionic neurectomy, and ipsilateral postganglionic neurectomy groups. As shown in Table 3, homogenates of ganglia from the unoperated and the contralateral postganglionic neurectomy groups were negative for virus whether or not the animals received cyclophosphamide. Similarly, in none of the eight mice subjected to ipsilateral preganglionic neurectomy without cyclophosphamide was HSV detected, although in one of seven preganglionic-neurectomized mice treated with cyclophosphamide, reactivated HSV was found. As in earlier experiments, ipsilateral postganglionic neurectomy without cyclophosphamide induced a low level of virus reactivation; in this instance, 2 of 31 ganglion homogenates were positive for HSV. In contrast, in 17 of 45 ganglion homogenates from mice treated with cyclophosphamide and subjected to ipsilateral postganglionic neurectomy, HSV was detected ($P < 0.01$ by chi-square contingency). Furthermore, in this group viral titers in the ganglia were high, with a mean of greater than 168 PFU/assayed ganglion, and in several of the individual ganglia viral titers of over 1,000 PFU were detected. (The viral titers presented for this drug-treated group are below the actual values because in several instances sample dilution was not carried sufficiently high.) This contrasts with mean titers of 15 and 10 PFU/assayed ganglion in the mice subjected to postganglionic neurectomy without cyclophosphamide and preganglionic neurectomy with cyclophosphamide.

Effect of ATS on neurectomy-induced HSV reactivation. In an initial effort to assess the influence of thymus-dependent (T) lymphocytes on HSV reactivation, latently infected mice were given three every-other-day i.p. injections of 0.2 ml of rabbit ATS (Microbiological Associates, Rockville, Md.); control mice received normal rabbit serum on the same schedule. On day 3 after the last serum injection, a group of mice were subjected to ipsilateral postganglionic neurectomy, while control animals were either left unoperated or subjected to contralateral postganglionic neurectomy. Mice were then sacrificed on postoperative days 4, 5, and 6 for ganglion assay by homogenization. Table 4 shows that ATS treatment alone or in combination with contralateral neurectomy failed to effect viral reactivation. Moreover, in contrast to cyclophosphamide treatment, when ATS-treated mice were subjected to ipsilateral postganglionic neurectomy and assayed on postoperative day 4, no enhancement of HSV reacti-

TABLE 3. *Effect of cyclophosphamide treatment on postganglionic neurectomy-induced reactivation in the SCG*

Operative procedure	Drug treatment[a]	Assay of SCG by homogenization	
		Ratio[b]	Mean viral titer[c]
None	PBS	0/20	0
None	Cyclophosphamide	0/21	0
Contralateral postganglionic neurectomy	PBS	0/9	0
Contralateral postganglionic neurectomy	Cyclophosphamide	0/9	0
Ipsilateral preganglionic neurectomy	PBS	0/8	0
Ipsilateral preganglionic neurectomy	Cyclophosphamide	1/7	10
Ipsilateral postganglionic neurectomy	PBS	2/31	15
Ipsilateral postganglionic neurectomy	Cyclophosphamide	17/45	>168

[a] Both PBS and cyclophosphamide (100 mg/kg per day) were given i.p. daily for 5 days beginning the day before operation.

[b] Number of ganglia positive for HSV per number of ganglia assayed.

[c] Total PFU of HSV in positive ganglia per number of ganglia assayed.

TABLE 4. *Effect of ATS treatment on neurectomy-induced reactivation in the SCG*

Operative procedure	Serum treatment	Assay of SCG by homogenization after neurectomy on day: 4 Ratio[a]	4 Mean viral titer[b]	5 Ratio[a]	5 Mean viral titer[b]	6 Ratio[a]	6 Mean viral titer[b]
Unoperated	NRS[c]	0/20	0	ND[d]		ND	
Unoperated	ATS	0/21	0	ND		ND	
Contralateral postganglionic neurectomy	ATS	ND		0/9	0	0/9	0
Ipsilateral postganglionic neurectomy	NRS	5/30	40.3	0/9	0	0/9	0
Ipsilateral postganglionic neurectomy	ATS	4/37	2.5	3/18	141	2/16	>88

[a] Number of ganglia positive for HSV per number of ganglia assayed.
[b] Total PFU of HSV in positive ganglia per number of ganglia assayed.
[c] NRS, Normal rabbit serum.
[d] ND, Not done.

vation was noted as judged either by the percentage of animals with positive ganglion homogenates or by the viral titers detected in the positive ganglia.

However, ATS treatment was not without effect. When ganglia of ipsilateral postganglionic-neurectomized mice were assayed after postoperative day 4, a potentiating effect of ATS was evident. Thus, as also shown in Table 4, 3 of 18 and 2 of 16 ganglion homogenates were positive for HSV on days 5 and 6 after surgery, with the positive ganglia exhibiting high viral titers. This contrasts with the failure to detect virus beyond postoperative day 4 in neurectomized mice either in this or in previous experiments in the absence of ATS or cyclophosphamide treatment.

Serum antibody titers at the time of virus reactivation. To determine whether neurectomy-induced HSV reactivation occurred in the presence of anti-HSV antibody, serum titers of neutralizing antibody were determined at the time when reactivated virus was detected, i.e., 4 days after postganglionic neurectomy. Figure 4 shows that serum antibody titers were high not only in those mice subjected to postganglionic neurectomy alone but also in the animals given either the daily dose cyclophosphamide regimen or ATS treatment in conjunction with neurectomy. In addition, reactivated virus was detected in mice with high antibody levels (between 128 and 512), and no correlation between individual serum titers and detectable HSV reactivation was evident.

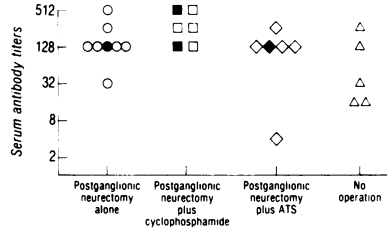

FIG. 4. *Serum-neutralizing antibody titers at the time of virus reactivation. Serum was obtained from mice at the time of sacrifice 4 days after neurectomy. Closed symbols in each group represent antibody titers of mice in which homogenates of ganglia were positive for HSV, whereas open symbols depict titers in mice with negative ganglia. Unoperated mice were sacrificed at the same time as neurectomized mice, i.e., over 25 days after initial i.o. inoculation.*

DISCUSSION

Experimental HSV infection of the SCG in mice can be divided into three distinct phases: acute, latent, and reactivated. During the acute phase, HSV replicates actively in the ganglion and is detected in high titer in ganglion homogenates. As the host immune response develops, acute infection subsides, giving way to the latent phase, during which HSV can no longer be detected in homogenates of ganglia, although the persistence of virus is revealed when explantation and cocultivation methods are used. Finally, HSV can be reactivated when the postganglionic nerves emanating from latently infected ganglia are cut.

The reactivation of HSV seen in these experiments was highly specific. After ipsilateral postganglionic neurectomy, productive virus replication, similar to that seen during the acute phase of infection, was induced in the ganglia of latently infected mice as judged by both the return of homogenate-detectable virus and the acceleration of virus expression from ganglion explants. The magnitude of this replication in non-immunosuppressed mice, as indicated by viral titers in ganglion homogenates, fell short

of that seen during the acute phase of infection, although as much as 600 PFU of virus per ganglion was detected on postoperative day 4. Similarly, expression of virus from ganglion explants was accelerated in neurectomized mice and approached the rapid appearance of HSV cytopathology seen in ganglia explanted at the peak of acute infection. This accelerated virus expression presumably reflects the induction of active intraganglionic virus replication in vivo that then continues in culture, causing cytopathology to appear earlier than in control cultures of ganglia from latently infected nonneurectomized mice where an additional period of time is required for initiation of de novo virus induction (in vitro virus reactivation). Indeed, the in vivo reactivation brought about by neurectomy raises the question of whether in vitro reactivation can be attributed, at least in part, to the interruption of ganglionic nerves that necessarily accompanies ganglion removal. The two methods of evaluating in vivo HSV reactivation have proved to be complementary. The early expression of virus in explants is perhaps a more sensitive indicator of reactivation, whereas homogenization holds the advantage that plaque counts provide a more direct and more easily quantified indicator of productive virus infection.

The mechanism by which cutting the axons of ganglionic neurons, i.e., postganglionic neurectomy, leads to HSV reactivation within the SCG is uncertain. However, the specificity of postganglionic nerve section as a reactivating stimulus is clearly demonstrated in these studies in which reactivated virus is detected only in those animals undergoing postganglionic neurectomy and never in unoperated controls or in mice undergoing contralateral neurectomy. The one possible exception noted in these experiments, the induction of virus production in a mouse subjected to ipsilateral preganglionic neurectomy and also treated with cyclophosphamide (Table 3), can be reconciled with the specificity of the operative procedure when it is considered that a small portion of postganglionic fibers from the SCG may travel caudally in the preganglionic nerve trunk (8). These results in the SCG are also in accord with observations on sensory ganglia of humans and mice in which section of sensory nerves central or peripheral to sensory ganglia leads to HSV reaction (5, 25). Common to each of these situations is the interruption of axons originating from neurons of latently infected ganglia.

The present understanding of the virus-host cell relationships underlying HSV latency and reactivation is fragmentary, and, therefore, the mechanism by which neurectomy induces virus reactivation remains speculative. Indeed, the cell type harboring latent virus in the ganglion is uncertain, although present evidence probably favors the neuron (6). If, in fact, latent HSV does reside in ganglionic neurons, it may be speculated that reactivation may: (i) represent an epiphenomenon of the axon reaction, (ii) be triggered by alteration in trophic influences on the neuron, or (iii) reflect an agonal disorganization of cellular control mechanisms. The axon reaction (retrograde response, chromatolysis) follows axotomy and can, in general, be considered an anabolic response in which there is not only an overall increase in ribonucleic acid and protein synthesis but also a redirection of macromolecular synthesis characterized by a decline in neurotransmitter production and an increase in the manufacture of structural macromolecules needed for regeneration (12). These profound metabolic changes in the neuron may provide cell-coded functions that augment productive HSV infection. These same metabolic steps may be switched on or off under other, less drastic, circumstances although not with such regularity or intensity as after nerve section. Alternatively, neuronal permissiveness may be influenced by contact with peripherally innervated target organs; when disconnected from the target organ by axotomy, the neuron soma may be deprived of trophic factors (15) that act to inhibit virus expression. Because a number of neurons die after their axons are interrupted, it is possible that a premorbid disorganization in the regulation of cellular metabolism is involved in HSV induction. On the other hand, if the satellite cell, which has been reported to be relatively nonpermissive for virus (7, 11), is the cellular locus of latent HSV, the signal to satellite-cell deoxyribonucleic acid synthesis and cell division that accompanies the axon reaction (12) may also serve to induce HSV replication.

Whatever the mechanism triggering HSV reactivation, these studies clearly demonstrate that the permissiveness of autonomic ganglia for viral replication, like that of sensory ganglia, can be varied in vivo. Furthermore, this variable permissiveness, manifested as a switch from latent infection detected only by explantation to reactivated infection in which fully infectious virus is detected by homogenization, can be influenced by exogenous factors that perturb the metabolism of ganglionic cells. The present observations, therefore, provide evidence supporting the theory that events within the ganglion can determine whether or not virus is clinically reactivated.

Just as reactivated HSV appears predictably and regularly on day 4 after postganglionic neurectomy, the virus is quickly and efficiently eliminated from non-immunosuppressed mice, fall-

ing to undetectable levels within 24 h. Although the circumscribed time course of viral reactivation could, in theory, reflect an underlying transience in the permissiveness of ganglion cells, host immune defenses are most probably critical in eliminating reactivated virus. It is likely that multiple aspects of the host immune system cooperate in this process. Thus, although high serum antibody titers clearly do not prevent reactivation, this does not preclude an important role for antibody in neutralizing extracellular virus or eliminating infected cells in concert with complement or antibody-dependent cytotoxic cells (19, 21). However, the experiments with ATS in which reactivated virus could still be detected on days 5 and 6 after neurectomy suggest that T-lymphocytes are necessary for the final elimination of infectious HSV from the ganglion. Moreover, the marked effect of cyclophosphamide treatment, which resulted in both a higher percentage of detectable viral reactivation and higher viral titers in ganglia after postganglionic neurectomy, raises the question of whether an additional population of cells is also important in confining and eliminating reactivated virus. Additional studies are necessary to more clearly assess which of the immune mechanisms that have been shown to operate in vitro (13) or in experimental animals during primary infection (18, 27) are important to the host in dealing with reactivated ganglionic virus in vivo.

The similarity of experimental autonomic and sensory ganglion infections with HSV with respect to acquisition, latency, and, as shown in the present studies, reactivation by axotomy lends support to the hypothesis that autonomic ganglia may serve as reservoirs for latent HSV in humans (17). If in humans, as in mice, there exists little difference between the capacity of sensory and autonomic ganglia to sustain viral latency and reactivation, autonomic infection may contribute directly to human illness and to dissemination of virus in the community. By analogy with sensory ganglion infection, it can be speculated that HSV, reactivated in the autonomic nervous system, will travel down postganglionic nerves by centrifugal axoplasmic transport to be released at nerve terminals and thereby be made available to seed autonomic target organs. This may result in focal destruction of target organ cells and, in the instance where the target organ is an exocrine gland, local replication may lead to release of HSV into external secretions with resultant spread to susceptible contacts.

However, whether or not autonomic infection with HSV proves to be of importance in human disease, experimental infection of the SCG provides a useful model of latent and reactivated herpesvirus infection. Because the SCG is eminently accessible to surgical and pharmacological manipulation, and because extensive background information concerning the biology of this autonomic ganglion is available, detailed investigation of this model may allow insight not only into the contributions of the immune system to acute, latent, and reactivated herpetic infection but also into the intrinsic cellular mechanisms governing the changes in ganglionic cell permissiveness.

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