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Encephalitis Resulting from Reactivation of Latent Herpes Simplex Virus in Mice

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Herpes simplex virus (HSV) encephalitis was produced in mice from reactivation of latent virus. Two experimental models were used: the trigeminal model after corneal inoculation of HSV, and the hypoglossal model after tongue inoculation of HSV. In the trigeminal model, cyclophosphamide treatment induced reactivation of latent virus in ganglia but not in central nervous system tissue. Spread of the reactivated virus from ganglia to brain occurred only in mice deficient in anti-HSV antibody. In the hypoglossal model, sectioning of the hypoglossal nerve provoked chromatolysis in the corresponding central nervous system motor neurons and occasionally reactivated latent HSV in the brains of mice. These results suggest that HSV encephalitis can result from the spread of reactivated virus from ganglia to brain and also from in situ reactivation in brain.

The pathogenesis of herpes simplex virus (HSV) encephalitis in humans is poorly understood. The infection is generally focal in the temporal lobes, and mortality, even with treatment, is ca. 40% (25, 26). Over two-thirds of the victims have had previous exposure to HSV, as evidenced by a history of herpes labialis or the presence of anti-HSV antibody at the time of onset of the encephalitis or both (13). In these patients, the encephalitis could result from reinfection with an exogenous HSV or reactivation of the individual's own latent virus (24). In regard to reactivation, it is well known that HSV establishes a latent infection in trigeminal ganglia of the peripheral nervous system (1, 2, 16, 21). Also, latent virus or HSV DNA has been detected in the central nervous system (CNS) in experimental animals and in humans (3, 6, 9, 12).

Work in animal models may prove helpful in clarifying some aspects of the pathogenesis of HSV encephalitis. Reactivation of latent HSV has been demonstrated in ganglia of experimental animals (8, 10, 14, 22, 23). However, encephalitis after experimental reactivation has been documented only rarely (7). The present work exploits two experimental modifications in the mouse model: antibody-deficient, latently infected animals (19) and animals with a focal infection of hypoglossal motor neurons (15). Using these models, we show that encephalitis can result (i) from reactivation of HSV in ganglia with subsequent viral spread to the CNS and (ii) from reactivation of HSV in situ in the CNS.

Female, 4- to 6-week-old BALB/c mice were inoculated by corneal scarification with 10^6 PFU of the F strain of HSV type 1. At 1 to 2 months after HSV inoculation (latent stage of the infection), the mice were treated with cyclophosphamide (Mead Johnson, Evansville, Ind.) at a dose of 200 mg/kg intraperitoneally (i.p.) on days 1 and 3 of treatment and 15 mg/kg i.p. daily beginning on day 5 of treatment. On days 7, 9, and 11 of treatment, mice were sacrificed, trigeminal ganglia and brain stem homogenates were prepared, and the homogenates were assayed for infectious HSV on rabbit kidney cell monolayers (17).

Maintenance medium consisted of Eagle minimal essential medium containing 2% heat-inactivated fetal bovine serum, 0.03% glutamine, 100 U of penicillin per ml, and 100 mg of streptomycin per ml. Without treatment, nervous tissue homogenates of latently infected mice have been invariably negative for infectious virus (14). However, as previously reported, with cyclophosphamide treatment, HSV reactivation occurs in the trigeminal ganglia (14). Table 1 shows an overall reactivation rate of 18% (5 of 28 mice) in these antibody-competent, latently infected mice. Virus was not recovered from brain tissue in these mice.

This experiment was repeated in latently infected mice that were made deficient in serum anti-HSV antibody. To obtain these mice, rabbit anti-HSV serum (microneutralization titer, 1,000) was given to BALB/c mice by i.p. injection 3, 48, 96, and 144 h after HSV inoculation. The rabbit antibody interferes with the mouse humoral immune response to HSV (19) so that by 2 months after virus inoculation, neutralizing antibody is no longer detectable in mouse serum: the microneutralization titer is less than 8, compared with 64 in antibody-competent, latently infected mice. In the neutralization test (11), 100 times the 50% tissue culture infective dose of HSV was added to duplicate serum dilutions in 0.05 ml of minimal essential medium, and HSV cytopathic effect was scored at 72 h in rabbit kidney cell monolayers. The titer was expressed as the serum dilution at which an uninfected monolayer was present in only one of the duplicate wells.

Table 1 shows the effect of cyclophosphamide treatment of these antibody-deficient, latently infected mice. The virus recovery rate in trigeminal ganglia was somewhat greater in the antibody-deficient mice: 36% (10 of 28) compared with 18% (5 of 28) of the antibody-competent mice. More significantly, virus was recovered from the CNS in 25% (7 of 28) of the antibody-deficient mice but in none of the antibody-competent mice. Overall, HSV was recovered from nervous system tissue homogenates in 10 antibody-deficient mice. Breakdown of the data shows isolates from trigeminal ganglia alone in three mice; from trigeminal ganglia and brain

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TABLE 1. Effect of cyclophosphamide treatment of mice at the latent stage of HSV infection

Days of cyclophosphamide treatment[a]	HSV isolation in tissue homogenates					
	Antibody-competent mice[b]			Antibody-deficient mice[c]		
	No. of mice	% Isolation from trigeminal ganglia	% Isolation from brain	No. of mice	% Isolation from trigeminal ganglia	% Isolation from brain
7	10	0	0	10	40	10
9	10	10	0	10	30	30
11	8	50	0	8	38	38

[a] Mice received 200 mg/kg i.p. on days 1 and 3 of treatment and then 15 mg/kg i.p. from day 5 until sacrifice.
[b] Microneutralization titer, 64.
[c] Microneutralization titer, <8.

stem in one mouse; and from trigeminal ganglia, brain stem, and cerebral hemispheres in six mice. In no case was virus recovered from the CNS and not from ganglia.

These results suggest that cyclophosphamide reactivates latent HSV in the trigeminal ganglia in both antibody-competent and antibody-deficient mice, but that subsequent spread of HSV to the CNS occurs only in antibody-deficient mice. During the 11-day course of the experiment, cyclophosphamide does not significantly reduce the serum anti-HSV antibody titer of the antibody-competent mice (14). However, cyclophosphamide would be expected to block the boost in antibody titer that normally accompanies HSV reactivation (19). Thus, the results suggest that anti-HSV antibody prevents the spread of reactivated virus from ganglia to the CNS.

In humans, it is uncertain whether a similar spread of reactivated virus occurs from ganglia to brain. An anatomical route has been suggested from trigeminal ganglia to the middle cranial fossa to explain localization of the encephalitis in the temporal lobe (5). Still, a predominant brain stem encephalitis would be expected with this mechanism since trigeminal axons synapse in the brain stem, and such a brain stem infection rarely occurs. In addition, there is no definitive evidence in humans of an abnormality in humoral immunity in HSV encephalitis, although the increase in serum antibody titer in some patients has been sluggish (13).

As discussed previously (15), the trigeminal model is a cumbersome one with which to study HSV reactivation in the CNS. With this model, it is difficult to differentiate reactivation in trigeminal ganglia and spread to the CNS from in situ reactivation in the CNS. Moreover, trigeminal neurons are widely scattered in the CNS from the pons to the upper cervical cord, so that a thorough morphological study is impractical. For these reasons, we developed a hypoglossal model in which HSV is inoculated in the tongues of mice. Besides spreading via the trigeminal route, virus from the tongue spreads directly to the CNS without an intervening ganglion in the pure motor hypoglossal nerve. A focal encephalitis ensues in the hypoglossal nucleus of the medulla, with HSV antigens demonstrable by immunoperoxidase staining in hypoglossal motor neurons during the first week after virus inoculation but not thereafter (15).

This hypoglossal model was used to reactivate latent HSV in situ in the CNS. Female, 4- to 6-week-old A/J mice were inoculated with 10^6 PFU of HSV in the tongue muscle. At 4 weeks after HSV inoculation (latent stage), mice were anesthetized with pentobarbital sodium, and the hypoglossal nerve was sectioned at the level of the carotid artery bifurcation. Neurectomy is a stimulus that is known to trigger HSV reactivation in the ganglia of humans and experimental animals (4, 23). The hypoglossal neurectomy in mice provoked chromatolysis in motor neurons of the corresponding hypoglossal nucleus (Fig. 1). Perivascular inflammatory cells were present in both neurectomized and control latently infected mice, probably as a residual sign of the acute HSV infection. HSV antigens could not be detected by immunoperoxidase staining (15, 20) in either the neurectomized or the control animals. However, infectious HSV was recovered from brain stem homogenates in 2 of 60 neurectomized mice sacrificed 3 to 10 days after surgery. Both isolates were neutralized by rabbit anti-HSV serum.

There are three possible explanations for these results: (i) reactivation of latent virus in trigeminal ganglia and spread of virus to the brain, (ii) reactivation of latent virus in brain stem neurons in the trigeminal rather than the hypoglossal system, and (iii) reactivation of latent virus in hypoglossal motor neurons of the brain stem. Although a latent infection does occur in trigeminal ganglia after tongue inoculation of HSV, hypoglossal neurectomy would not be expected to affect peripheral nervous system or CNS trigeminal neurons. If this treatment induced reactivation in the trigeminal system, one would have to assume a nonspecific effect of the anesthesia or surgery. In addition, reactivated virus did not spread from ganglia to brain in antibody-competent mice treated with cyclophosphamide (Table 1), and therefore such spread would not be expected after hypoglossal neurectomy (given the unlikely assumption that neurectomy did induce reactivation in trigeminal ganglia). In contrast, a strong argument can be advanced for HSV reactivation in hypoglossal motor neurons: neurectomy and chromatolysis have been shown to reactivate HSV in other systems (4, 23), the neurectomy used here had a specific effect of provoking chromatolysis in hypoglossal motor neurons (Fig. 1), and these motor neurons are known to be infected with HSV at the acute stage of the infection (15). Our working hypothesis is that latent HSV in brain neurons can occasionally be reactivated in the context of nerve cell damage, e.g., chromatolysis from hypoglossal neurectomy.

Similarly, cyclophosphamide may induce reactivation in ganglion cells by a direct cytotoxic effect on neurons rather than as a consequence of immunosuppression (14). In the present experiments, cyclophosphamide failed to reactivate latent HSV in the CNS. This result may reflect the low incidence of latency in the CNS (0 to 10%) compared with ganglia (80 to 100%) (3, 9, 14, 21, 23). However, the incidence of latency in the CNS may be significantly underestimated by explantation studies, since this technique appears to be more satisfactory for ganglia than for CNS tissue (3). Another possible explanation for the failure of cyclophosphamide to reactivate HSV in the CNS concerns drug

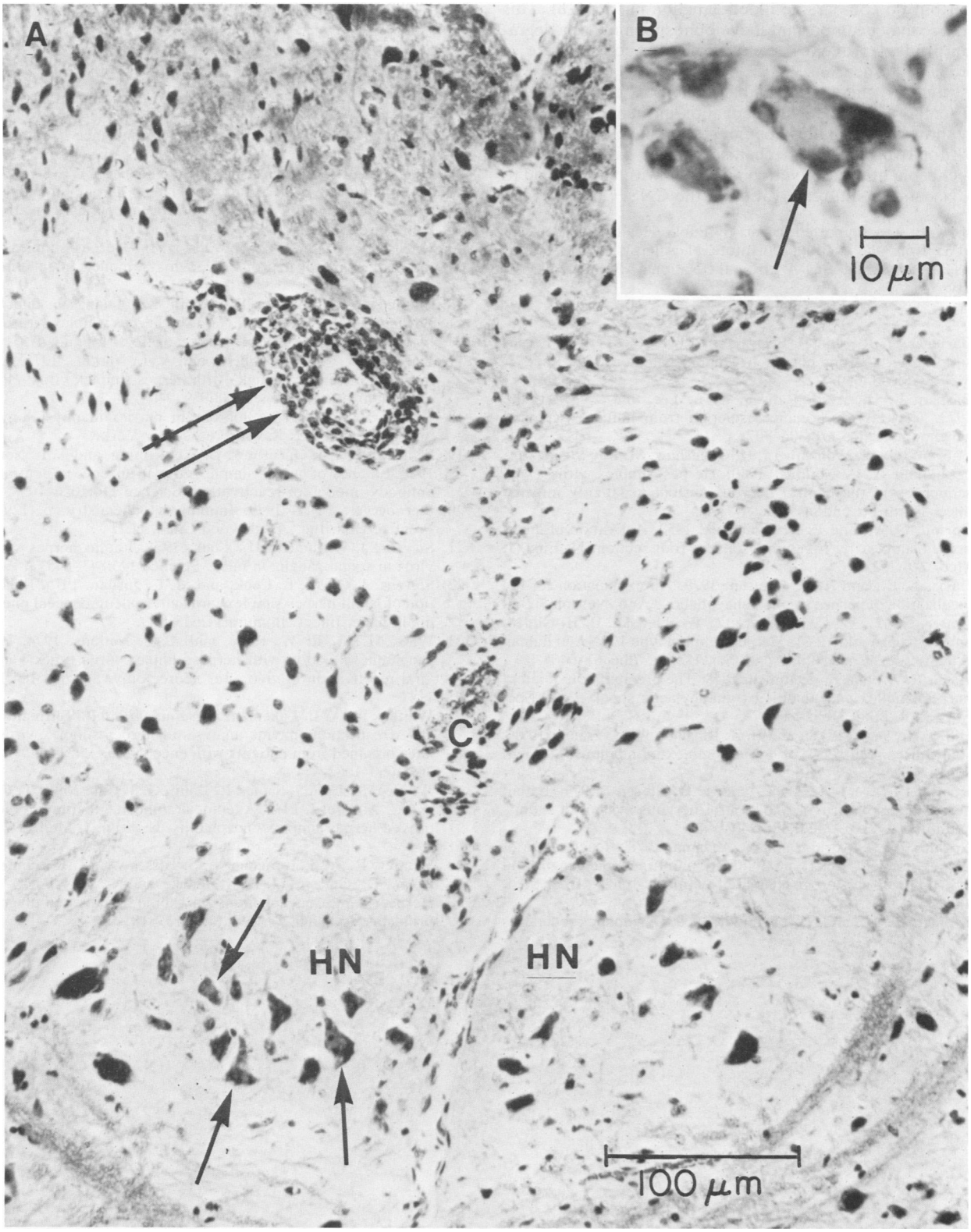

FIG. 1. Transverse section of the medulla in an A/J mouse 6 weeks after HSV inoculation by the tongue route and 7 days after hypoglossal neurectomy. The central canal (C) and hypoglossal nuclei (HN) are marked. Perivascular inflammatory cells are present (double arrows). The chromatolytic neurons (single arrows) correspond to the side in which the hypoglossal neurectomy was performed.

distribution in the nervous system. There is a blood-brain barrier but no comparable blood-ganglion barrier (18). This difference may be important if cyclophosphamide or a degradation product provokes reactivation as a consequence of cytotoxicity to neurons.

In summary, there is no animal model that corresponds to focal temporal lobe HSV encephalitis in man. The present study, however, provides documentation in an experimental model for two possible mechanisms in the pathogenesis of HSV encephalitis: reactivation in ganglia with viral spread to the CNS and in situ reactivation in the CNS.

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