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Latent Infections Induced by Herpes Simplex Viruses[1]

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Introduction

Herpesviruses, particularly the herpes simplex viruses, have long been considered classic examples of agents that induce latent infections in man. Until our recent work, all supportive evidence for this concept was indirect, the most convincing being the observation that the appearance of facial herpetic lesions commonly followed section of the trigeminal nerve root.[3] We found that herpes simplex virus type 1 can induce a long-standing latent infection in sensory ganglia of both rabbits and mice (8, 10). Our findings have been extended to include herpes simplex type 2 in mice (unpublished observations) and, using similar methods, we are now studying the virological state of appropriate human ganglia.

Pathogenesis of Recurrent Herpetic Infection

Our results were predicted by a general hypothesis [discussed in detail elsewhere (6, 7)] for the pathogenesis of recurrent herpetic disease in humans. To summarize, primary infection in man is postulated to result initially in viral replication in epithelial cells, with subsequent invasion of superficial nerve endings. The virus would then travel centripetally in sensory nerves, ultimately reaching ganglia, where it would induce a latent infection. Upon "activation," the virus would travel centrifugally in the nerve trunk to the epithelium where vesicles are produced. Between exacerbations of clinically apparent disease, the virus could be maintained in ganglionic cells by alternative mechanisms which Roizman (7) has designated the "dynamic state" and the "static state" hypotheses. According to the dynamic state hypothesis, latent infection would be maintained by continued replication of a small but constant amount of infectious virus in one or several types of ganglionic cell. Extension of the infection would be inhibited by the presence of circulating antibody, specific immune lymphocytes and, possibly, interferon. In the static state hypothesis, herpes simplex virus would be maintained in "virogenic" cells, with complete viral replication reversibly interrupted at an early stage in the cycle. As is noted below, our present results are consistent with the static state hypothesis.

Latent Herpetic Infection in Mice and Rabbits

In the murine model, animals are inoculated intradermally in a rear footpad. Virus then replicates locally, travels up the sciatic nerve, and establishes a productive infection in sacrosciatic spinal ganglia, spinal cord, and brain. Nearly all mice develop posterior paralysis in about 7 days after infection, and some subsequently die with acute encephalitis. About one-half of the paralyzed mice recover in an additional 2 weeks, and spinal ganglia from these animals are used for studies of viral latency. At the time ganglia are taken, neither infectious virus nor viral-specific products detectable by immunofluorescent or ultrastructural techniques can be found in ganglionic cells. However, as is shown in Table 1, infectious virus is induced in the ganglia derived from these mice when ganglia are explanted and cocultivated with monolayers of RK_{13} cells. As can be seen, the animals harbor latent virus for at least 4 months (the maximum time interval that we have examined), and virus can be detected after ganglia have been in culture for about 1 week. An electron micrograph of a neuron and an adjacent satellite cell in a ganglion explanted from a latently infected mouse and maintained for 4 days *in vitro* is presented (Fig. 1). Morphologically complete virions can be seen between the cells. Finally, the latent infection appears to be selective for sensory ganglia; other parts of the peripheral and central nervous system do not replicate infectious virus when maintained in the same fashion.

In rabbits, the latent infection is maintained in trigeminal ganglia after the animals have been inoculated on the cornea. Again, infectious virus is induced when the ganglia are cocultivated *in vitro* with RK_{13} cells. The model in rabbits is of particular interest because these animals undergo recurrent eye infections, and latent virus is present in sensory ganglia supplying this organ.

More recently, we have attempted to reactivate infectious virus and clinically demonstrable neurological or cutaneous disease in latently infected mice, to determine the cell types involved in maintenance of the latent infection, and to define the mechanism by which the infection is maintained. Despite the use of a wide variety of pharmacological, immunological, and surgical procedures which are known to reactivate active herpetic infections in humans and experimental animals, we have been unable to reactivate either infectious virus or clinical evidence of disease in mice (9). We were unable to perform direct experiments involving purified cell types derived from latently infected ganglia, but indirect morphological and immunological experiments (9) have led us to the tentative conclusion that latent virus is selectively harbored in neurons rather than in supporting

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[3] Three comprehensive reviews concerning the natural history of herpes simplex virus have been published (4, 6, 7). Details concerning these findings are given therein.

Table 1

Recovery of infectious herpes simplex virus following in vitro cocultivation of latently infected sacrosciatic spinal ganglia of mice

Sacrosciatic spinal ganglia were explanted and cocultivated with monolayer cultures of RK_{13} cells *in vitro*. Virus-producing ganglia were detected between 5 and 11 days after explantation, when cytopathic effects were produced on the RK_{13} cell monolayers.

Experiment	Mice positive/ mice tested	Duration of latent infection *in vivo* (mo.)
1	6/6	2.5
2	5/5	3
3	5/5	4

cells. In this regard, it is important to note that during the initial paralytic infection, many neurons undergo a productive infection, and cytological techniques indicate that they do not survive. Thus, there appear to be at least 2 neuronal responses to infection. In one, virus is replicated and the cell is killed; in the other, the cell survives and latent virus persists.

As to the state of the virus in latently infected ganglia, all evidence is consistent with the static state hypothesis as an explanation for maintenance of the infection (9). The bulk of this information derives from our inability to detect either infectious virus or viral-specific products in ganglia at the time of explantation. Determination of the precise molecular mechanism by which the virus is maintained depends, first, upon the ability to detect the viral genome in ganglionic cells. The method can then be used to determine the intracellular site at which the genome persists. Using radioactive, viral-complementary RNA (made *in vitro*) and ganglionic cell DNA in hybridization procedures on nitrocellulose filters (sensitivity = 10 to 20 viral genome equivalents per ganglionic cell), we have been unable to detect viral DNA in latently infected ganglia (J. G. Stevens, unpublished results). Attempts to improve this sensitivity and to use *in situ* hybridization methods are now underway.

Generalizations and Conclusions

Our results with model systems can be generalized to a consideration of neoplastic conditions in 2 ways. First, it is clear that the herpes simplex viruses can persist (probably in a nonreplicating state) in nervous tissues for extended periods of time, and they appear not to be detectable by the standard techniques which are now in use. The possible extensions of these observations to other tissues, including cervical epithelium, are immediately obvious. In particular, the persistence of at least some viral genetic information in neoplastic cells transformed by viruses (3) [including herpes simplex type 2 in an experimental system (2)] is presently the rule. If, as now seems likely, this persistence should be a necessity for maintenance of the transformed state induced by viruses, the requirement has been fulfilled in our model systems.

Second, from these experiments and those of others using herpesviruses which induce lymphoproliferative diseases, it is clear that a most efficient way to detect the presence of latent herpesviruses is to culture *in vitro* the cells containing repressed virus. Infectious virus is then induced and replicated (5). Of particular importance is the fact that, in at least 1 instance, such an induction from cells in a human cervical carcinoma has been reported (1). If this phenomenon could be extended to a greater percentage of cultures derived from carcinomas, a much stronger case for an etiological role for herpes simplex virus type 2 in cervical carcinoma could be made. Finally, it would be of interest to know if herpes simplex type 2 induces a latent infection of sacrosciatic spinal ganglia following intravaginal inoculation. Such a phenomenon could be important in the genesis of cervical carcinoma.

In conclusion, we have shown that herpes simplex viruses can induce long-standing latent infections of the nervous system *in vivo*. In such infections, the entire viral genome is conserved and can later be detected as infectious virus when appropriate tissues are cultivated *in vitro*. Since viral genetic material is also conserved in most if not all tumors known to be induced by viruses, application of the basic principles learned in these neural systems will most certainly be of significance in the assessment of any important role played by herpes simplex virus in cervical carcinoma.

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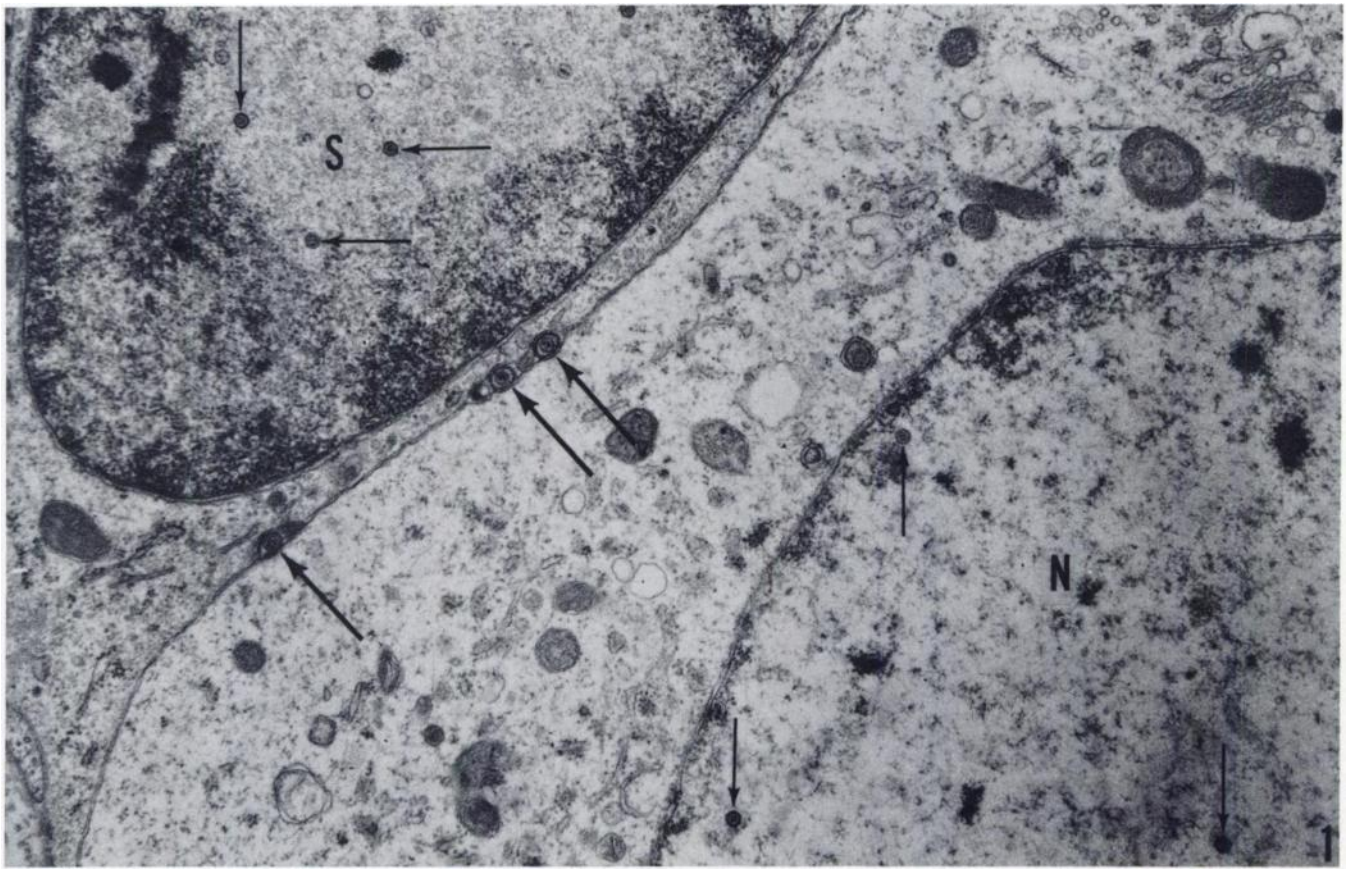

Fig. 1. Electron micrograph of a spinal ganglion from a mouse with latent herpes simplex virus processed after 4 days in culture. Part of a neuron (*N*) and an adjacent satellite cell (*S*) can be seen. The nuclei of both cells contain herpesvirus capsids (*small arrows*), and 3 mature virions are lying between the cells (*large arrows*). × 21,000.