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Herpes simplex virus (HSV) is a common ocular infection. Approximately 400,000 persons in the United States have experienced ocular HSV disease and there are approximately 50,000 new and recurrent cases each year. Although only approximately 20% of people develop disease from HSV type 1, 60% to 80% are infected, have antibodies, and have sensory ganglia from which virus can be isolated. Even though an individual may not have had clinically apparent disease, high fever, immunosuppression, and sometimes surgery can reactivate latent herpes.

Our work on herpes began 41 years ago in a condemned building at the Massachusetts Eye and Ear Infirmary in Boston (Fig. 1). The animal room on the second floor was easy to recognize, because it was the only air-conditioned room in the building. Emily Varnell, then a young chemical engineer, came to work with me and has worked with me ever since in trying to treat and prevent recurrences of herpes.

My initial theory was that viral disease could be treated by identifying a drug that would bind to DNA polymerase, a virus-specific enzyme responsible for the final assembly of viral DNA. The first candidate was idoxuridine, which had been synthesized as a potential anticancer agent by William Prusoff at Yale 8 years before and was the only drug at that time known to react with DNA polymerase.1 To test my unsupported theory, eye drops containing idoxuridine were administered to herpes-infected rabbits every hour during the day and every 2 hours during the night. To maintain this round-the-clock schedule, Emily Varnell, Anthony Nesburn, and I took turns sleeping on a cot in the animal room and waking up every 2 hours throughout the night. These experiments demonstrated that the treatment was successful, and the results of these and subsequent experiments established that it is possible to treat virus disease, that nucleosides are effective for this treatment, and that nucleosides can achieve this effect by binding to the DNA polymerase.2 In fact, most of the antivirals developed thereafter including trifluridine—which we introduced3 and which is still used as the drug of choice to treat ocular herpes in the United States—were nucleosides that interfered with viral DNA polymerase. Nucleoside antivirals fall into two categories. Nucleosides with abnormal bases, such as idoxuridine and trifluridine, form a DNA code that cannot be read by the viral enzymes, so that the virus cannot reproduce. Nucleosides with abnormal sugars, such as acyclovir and famciclovir, bind to viral DNA polymerase or act as terminators of the sugar backbone of DNA, resulting in incomplete DNA chains that prevent replication of the virus.

Eventually, it became apparent that although some ocular viral disease is caused predominantly by multiplying virus, other ocular disease is largely a hypersensitivity reaction to viral antigens. To treat the hypersensitivity component, the combination of corticosteroids and antivirals was introduced,4 and this combination is still in use today.

The next question we asked was, why do only some people get disease, and why is some disease severe and some very mild (i.e., some develop skin disease, some necrotizing keratitis, and some superficial dendritic keratitis). To shed light on the role of the genetic heterogeneity of HSV in causing different types of disease, we isolated viruses from many different patients and used the virus isolates to infect rabbits. We found that some isolates caused mild disease, some caused moderate disease, and some caused severe disease, but each isolate had its own characteristics and was different from other isolates (Fig. 2). For example, some isolates caused long dendrites, others middle-sized dendrites, and still others small punctate lesions (Fig. 3). With Bernard Roizman of the University of Chicago, we fragmented the DNA and made recombinants that had unique properties.5 We found that we could isolate a portion of the viral genome that specified the morphology of the epithelial dendrite. We also found that the genes causing stromal disease segregated separately from those causing epithelial disease.

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FIGURE 1. This decrepit, condemned building in downtown Boston on the campus of the Massachusetts Eye And Ear Infirmary housed our laboratory. Our space consisted of three tiny rooms on the second floor, formerly the apartment of the superintendent of the hospital cleaning staff. There was no elevator in the building, and all animals and equipment had to be carried up the steps. Reproduced from Kaufman HE. Introduction: The first effective antiviral. In: Adams J, Merluzzi VJ, eds. The Search for Antiviral Drugs. Boston: Birkhauser Boston; 1993:1-21. © Springer-Verlag New York, Inc.
Thus, it was shown that the character of disease is determined, at least in part, by the infecting virus. At the time of initial infection, HSV-1 travels to the neural ganglion and becomes latent, conferring a lifetime infection. We also know that once a ganglion is infected by one strain of the virus, it is generally resistant to superinfection, even though peripheral infection by other virus strains may occur. The resistance of neural cells to superinfection is not well understood and certainly requires further study. It seems clear, however, that the initial infecting virus is most often responsible for recurrent infections throughout life. If the initial virus causes mild disease or no disease and tends not to recur, it is a "good virus," and the patient can generally lead a trouble-free life unless immunosuppressed. If it is a "bad virus" that tends to recur and cause severe disease, lifelong morbidity may result.

Because no vaccine to date has been shown to be sufficiently effective to prevent recurrences of herpes in those already infected, it may be that using our new DNA technology, we should consider the infection of children with "good viruses" in an attempt to prevent later herpetic morbidity.

A major question regarding herpes therapy concerns the role of systemic antiviral treatment of ocular disease, especially now that topical antiviral agents permit the rapid treatment of epithelial disease. Using acyclovir as the prototypical systemic antiviral agent, the Herpetic Eye Disease Study (HEDS) asked several questions. The first was, does acyclovir effectively treat stromal disease and iritis? The disappointing answer was that it does not. The second was, does acyclovir, when given early, prevent stromal disease? Again, the answer was no. The third question was, do steroids reliably make symptoms of stromal disease...
disease resolve early? Here, the answer was yes: Symptoms disappeared and vision improved more rapidly, although the final outcomes in terms of visual acuity and recurrences after treatment was discontinued were similar, whether the patients received steroids or not.10 The fourth question was, does the combination of steroids and antiviral eye drops result in increased complications, risks, or recurrences? The answers were no, no, and no, and, furthermore, no increases in recurrences were observed in patients followed for 6 months after steroids were discontinued.10 The final question was, with some real ability to treat both epithelial disease and stromal disease, would a systemically administered antiviral such as acyclovir clearly reduce the recurrence rate? Unfortunately, however, the reduction was only approximately 40%. Even with systemic acyclovir treatment, more than half of the patients with ocular herpes had recurrences, and it is the problem of continued recurrences that leads to continued morbidity and blindness. Similarly, we know that, in genital herpes and other herpetic diseases as well, acyclovir provides incomplete protection against recurrences, does not prevent shedding of virus in bodily fluids, and does not prevent the spread of disease.11–14

Over the years, we have examined a variety of possible approaches to the prevention of recurrent disease. The first began with the finding of Bobek and Cheng that it is possible to synthesize an inhibitor of viral thymidine kinase: The result was ethynylthymidine.15,16 I recognized that thymidine kinase occurs in non-neural cells, so that a selective inhibitor of viral thymidine kinase would not inhibit viral multiplication in these cells because the cellular enzyme would be available. I knew, however, that the central nervous system did not normally contain thymidine kinase and that viral mutants deficient in thymidine kinase generally do not recur frequently. We found that, in fact, ethynylthymidine reduced the recurrence rate of herpes, but it is insoluble and difficult to administer, and the reduction in recurrence rate, although significant, would not be clinically useful.17

Next, Gebhardt refined a mouse heat stress model18 in which mice that had been infected with herpes and allowed to recover were immersed in warm water for a period of 10 minutes, producing reactivation of herpes (Fig. 4). In this model, he found that acyclovir was relatively effective in inhibiting viral multiplication on the surface of the cornea, but had little effect in inhibiting viral reactivation in the trigeminal ganglion, even when given before heat stress was applied (Gebhardt BM, unpublished data, 2001). We thought that epinephrine, which Kwon and Hill have shown to cause reactivation of herpes in rabbits (Kwon et al.19,20 and Shimomura et al.21) might be a mediator of heat-stress-induced recurrences. We found that propranolol reduced reactivation of herpes in mice, but this reduction, although significant, did not appear sufficient to justify the use of drugs that had significant side effects22 (Table 1).

Two years ago, a clinical link between prostaglandins and recurrences of herpes keratitis appeared in the literature with the publication of a report of three patients in whom recurrent herpes appeared to be related to treatment with the antiglaucomatous agent latanoprost, a prostaglandin F2α analogue that binds to prostaglandin receptors (Fig. 5).23 Subsequently, Peter Racciato saw 10 more patients with recurrences during latanoprost therapy (Racciato P, written communication, 2001). In a controlled rabbit study, we found that latanoprost made herpes keratitis worse and increased recurrences of herpes significantly.24 The effect differed with different strains of virus, but was particularly evident when the rabbits were infected with a strain of virus causing keratitis that was also made very much worse by corticosteroids. We also found that unoprostone (Fig. 6), a molecule that is derived from prostaglandin F but is a docosanoid rather than an eicosanoid and has little affinity for prostaglandin receptors, did not make herpes keratitis worse.25

There was a flurry of interest in a connection between prostaglandins and herpes approximately 20 years ago, at which time prostaglandins were shown to enhance the spread of herpes virus in cell culture26,27 and to induce interferon production27,28 and were implicated in the reactivation of herpetic skin lesions.20 Also, inhibitors of prostaglandin synthesis were reported to inhibit herpes virus replication29 as well as the reactivation of latent herpes from murine trigeminal ganglia.30 An uncontrolled study of the treatment of cutaneous herpes with nonsteroidal anti-inflammatory agents revealed a possible beneficial effect.31 Hill and Blyth32 suggested that the induction of prostaglandins in the skin might potentiate local susceptibility to virus and be more responsible for recurrences than activation in the ganglion alone. More recently, Athar et al.33 reported increased cyclooxygenase (COX)-2 expression in
the skin of UVB-exposed mice and suggested that COX-2 inhibitors might have an anticarcinogenic effect on the skin.

All these findings suggest that the prostaglandin system may play a critical role in both the multiplication and reactivation of herpes. It may even account for the reduction of recurrences we observed after propranolol treatment in mice, because as a treatment for glaucoma, epinephrine is known to induce the secondary formation of prostaglandins, and COX inhibitors that prevent prostaglandin synthesis also prevent the ocular hypotensive effect of epinephrine in both animals and humans. It seems reasonable that activation of a COX-2 gene may well be a cellular mechanism important in the induction of herpetic recurrences. Clearly changes within the cell occur that stimulate the virus to reproduce. Gebhardt and I (unpublished data) and later Hill et al.34 looked at the changes in host gene expression in the ganglia of mice subjected to heat stress and found that, in fact, an increase in COX-2 gene transcription occurs. In mice examined within an hour after the application of heat stress alone, only COX-2 and heat shock protein genes were activated (Fig. 7). If, in fact, these heat-stressed animals that are prone to herpes recurrences require the induction of the COX-2 gene to induce the recurrences, perhaps agents that inhibit this process would also be effective in preventing the recurrences. Gebhardt found that aspirin was effective in inhibiting recurrences of herpes (Gebhardt BM, unpublished data, 2000) and that the highly specific COX-2 inhibitor 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2(SH)-furanone (DFU)35 is even more effective. DFU resulted in a 68% reduction in recurrences when given before the stress was applied and a 50% reduction when given after the stress was applied (Gebhardt BM, unpublished data, 2001). In both cases, the inhibition of recurrences was accompanied not only by a reduction of virus on the corneal surface, but also by a reduction of virus DNA in the ganglia. These and other studies from our laboratories suggest that there are significant differences in cyclooxygenase inhibitors. For example, celecoxib caused a 40% reduction in recurrences, less than that seen with DFU. Very recently, Shenk36 reported that a COX-2 inhibitor can inhibit cytomegalovirus (CMV) multiplication in culture, and Bazan has suggested that there may be inflammatory pathways occurring after COX-2 that are as important as the initial COX-2 induction37 (Bazan NG, oral communication, 2001).

**Future Possibilities**

This research, although early and in many ways preliminary, suggests for the first time that the induction of a cellular gene may cause viral recurrence. If so, it may be possible to prevent and treat viral disease by inhibiting a cellular gene pathway induced by specific stimuli, and the relevant drugs are available and safe. It is likely that this pathway is important and that inhibitory drugs will be effective against labial herpes, HSV-2, perhaps CMV, and varicella zoster virus. In fact, the drugs may be therapeutic as well as preventive, in that latanoprost makes infection worse and COX-2 inhibitors reduce CMV multiplication. It is possible that COX-2 inhibition will be topically effective in the eye (the latanoprost effect was topical), even though an initial trial with the nonsteroidal anti-inflammatory drug ketorolac did not show therapeutic efficacy. It is also likely that these agents will be additive to antivirals and may help prevent spread of the virus if they prevent recurrences or viral reactivation in the ganglion. Although much of this approach is based on recent work in a single species and is therefore speculative, nevertheless the implications of being able to inhibit a cellular gene whose induction produces recurrences of viral disease are exciting.

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