Severity of Experimentally Reactivated Herpetic Eye Disease is Related to the Neurovirulence of the Latent Virus

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The authors examined the eye diseases produced during acute and experimentally reactivated infections of rabbits intranasally inoculated with high and low neurovirulent strains of herpes simplex virus, type-1 (HSV-1). Experimental reactivation of latent trigeminal ganglionic infection was accomplished by an injection of cyclophosphamide followed by one injection of dexamethasone the next day. Neither drug, when given as a single injection, reactivated latent HSV-1 infection. During acute and reactivated phases of high neurovirulent HSV-1 strain infection, many rabbits developed very severe conjunctivitis and keratitis. Some rabbits developed hemorrhagic corneal lesions, and a few became blind. Only a few rabbits with acute and reactivated low neurovirulent virus strain infections developed mild conjunctivitis. The high neurovirulent strain was recovered from tear film more frequently than the low neurovirulent strain during reactivated infections. By use of 3H-labelled DNA prepared from purified virus to probe trigeminal ganglionic tissues in situ, both strains of virus were found to establish ganglionic latency to about the same degree. Reactivation correlated with an increase in the amount of HSV-1 RNA per ganglionic neuron and a change in subcellular location. These studies indicate that the relative neurovirulence of the infecting strain determines the ease with which it can be reactivated from latency and the severity of the reactivated ocular disease produced. Invest Ophthalmol Vis Sci 28:229-237, 1987

Inoculation of rabbit corneas with HSV-1 results in acute conjunctivitis and keratitis, and canaliculic obstruction. During the acute phase of the disease, HSV-1 is easily recovered from cell-free homogenates of acutely infected ganglia and ocular structures. After resolution of the acute infection, the virus cannot be recovered from the eye, but can be rescued from the ganglion by explanation or co-cultivation techniques. Infected rabbits occasionally spontaneously reactivate latent infections resulting in reappearance of HSV-1 in tear film.

In humans, trigeminal ganglionic infection, rather than exogenous reinfection by a new HSV-1 strain, is thought to produce recurrent eye disease. Viruses isolated from the same individual during different recurrent episodes have identical DNA patterns by restriction-enzyme analysis. Also, recent experimental studies have shown that superinfection of a previously latently infected trigeminal ganglia is inhibited, the inhibition is independent of immune factors, and localizes to the ganglion cell bodies that innervate the site of primary infection.

Herpetic eye infections of rabbits and mice have been used as models of human herpetic eye diseases, and have been used in the testing of antiviral compounds. Attention has recently focused on the relationship between the relative virulence of HSV-1 strains and development of acute and recurrent ocular diseases. HSV-1 strains are known to vary widely with regard to their neurovirulent potential in mice, and both viral genotype and mouse strain genotype appear to influence the severity of nervous system and eye involvement during acute infections. Thymidine-kinase-deficient mutants of HSV-1 replicate in the eye, have attenuated neuroinvasive potential, do not establish latency, and are not lethal, suggesting that the viral thymidine kinase gene is important in neuroinvasion. However, thymidine-kinase-negative mutants of HSV-1 cause severe ocular disease, invade, and replicate within the CNS after corneal inoculation of immunosuppressed mice.

Few studies have examined the relationship between the severity of ocular disease produced when HSV-1 strains of high and low virulence are reactivated from latency. In this report, we describe a new method of

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reactivating latent HSV-1 in the trigeminal ganglia involving the administration of both cyclophosphamide and dexamethasone. This strategy was based on the observations that dexamethasone alone can reactivate latent herpesvirus infection of rabbits and cattle and that immunosuppressive therapy can reactivate latent herpesvirus infections of mice. Using this new method, we show that severe eye disease is produced when a highly virulent strain is reactivated from latency.

Materials and Methods

Infection of Rabbits

HSV-1 strains H-129 and F were used as high and low neurovirulent strains, respectively. In mice, strain H-129 is highly lethal after both peripheral and intracerebral inoculation, whereas strain F is nearly avirulent after peripheral inoculation, and only mildly lethal after intracerebral inoculation. In rabbits, strain H-129 causes extremely severe inflammatory lesions in the brain with accompanying electroencephalographic abnormalities, whereas strain F does not. Strain H-129 was obtained from Dr. R. R. McKendall, Galveston, TX, and was originally isolated from a fatal case of human encephalitis. Strains H-129 and F were propagated in either VERO or MRC-5 cells to final titers of $10^8$ and $10^9$ TCID$_{50}$/ml, respectively, as described.

Four- to six-pound female New Zealand White (NZW) rabbits were obtained from LIT Rabbitry (Whitehall, MT) or R & R Rabbitry (Seattle, WA). Rabbits were anesthetized with sodium pentathol (Demi-Liquid®, Fort Dodge Laboratories, Inc.; Fort Dodge, IA) or tranquilized by intramuscular injection of 4 mg/kg of promazine maleate (Acepromazine®, Fort Dodge Laboratories, Inc.; Fort Dodge, IA). Rabbits were placed on their backs and 0.1 ml of virus stock diluted to contain $10^6$ TCID$_{50}$ of infectivity was instilled into each nostril. Control animals were sham inoculated. The number of animals used for each experiment is indicated in the results. Animals were cared for in strict compliance with the AVRO Resolution on the Use of Animals in Research.

Virus-Shedding in Ocular Secretions

Sterile swabs, presoaked in minimal essential medium supplemented with 100 U/ml of penicillin and 100 µg/ml of streptomycin, were used to sample tear film from the eyes at intervals after infection. Swabs were immediately placed onto fresh monolayers of VERO cells in 25 cm$^2$ flasks containing minimal essential medium supplemented with 2% fetal calf serum, 100 U/ml of penicillin, and 100 µg/ml of streptomycin. The cultures were incubated at 37°C in a humidified CO$_2$ incubator, observed for the presence of typical HSV-1 cytopathic effect (CPE) at daily intervals for up to 7 days, and then frozen at −20°C. The cultures were thawed and a portion passed onto fresh VERO or MRC-5 cells to again check for viral CPE. We did not observe any significant decrease in HSV-1 infectivity by storage at −20°C, because the interval between the freezing of the initial swab culture and repassage was relatively short. Occasionally the presence of HSV-1 in swab cultures was confirmed by testing for HSV-1 antigens using an indirect immunofluorescent assay; HSV-1 was identified in all such cases. Uninfected control rabbits maintained in the same room with infected animals were swabbed; at no time were these controls found to have HSV-1 in ocular secretions.

Eye Examination

Rabbit eyes were examined at intervals after infection for the presence of conjunctivitis, keratitis, or subcorneal hemorrhage. Conjunctivitis was graded as follows: mild: low grade of conjunctival redness and mild photophobia; moderate: more intense conjunctival redness and evidence of itching; severe: very intense conjunctival redness obliterating the cornea-sclera margin. Keratitis was graded as follows: mild: low grade of corneal redness with mild photophobia; moderate: more intense corneal redness, pain associated with swabbing, and more pronounced photophobia; severe: very intense corneal redness, more pain associated with swabbing, and severe photophobia. Slit-lamp examinations were not performed.

Reactivation of Latent Infection

On either day 72 or 76 post infection, reactivation of latent infections was accomplished by a single intravenous injection of 75 mg/kg of cyclophosphamide (Sigma Chemical Co.; Saint Louis, MO), followed by one intravenous injection of 4 mg/kg of dexamethasone (Azium®, Schering Corporation; Kenilworth, NJ) 24 hr later. Day 0 of reactivation was defined as the day cyclophosphamide was injected. Uninfected control animals were treated identically.

Isolation of HSV-1 From Nervous Tissues

At intervals throughout infection representing the acute, latent, and reactivated phases, rabbits were killed by lethal intravenous injection of sodium pentathol and perfused with phosphate buffered saline. The left trigeminal ganglion was made into a suspension in cell culture medium with a Ten Broeck homogenizer and frozen at −70°C. Homogenates were later thawed, centrifuged at 400 × g, and the supernatant added to
monolayers of VERO or MRC-5 cells. Cultures were observed for the presence of HSV-1 CPE for up to 14 days, and then repassed to again check for viral CPE.

Histology and In-Situ Hybridization

The right trigeminal ganglion from each animal killed was immersion-fixed in PLP fixative (1 mM sodium m-periodate, 75 mM lysine, 2% paraformaldehyde in 37 mM phosphate buffer, pH 7.4) for 24 hr, dehydrated in graded alcohols, and embedded in paraffin. Six, 3-μm sections from each ganglion were cut from each of three levels and deposited on treated microscope slides, pretreated for detection of RNA sequences, and hybridized in situ using a 3H-labelled DNA probe prepared by nick translation. Following hybridization, the slides were extensively washed, dehydrated, dipped in nuclear track emulsion, exposed for 3-5 wk, developed in photographic developer, stained with hematoxylin and eosin, and examined as described. At least two adjacent sections not subjected to in-situ hybridization were stained with hematoxylin and eosin for routine histological examination.

Results

Clinical Course

Strain H-129 HSV-1 infection: One group of 24 rabbits was intranasally inoculated with high neurovirulent HSV-1 strain H-129. All rabbits were quiet for 3 days post infection (dpi), and all developed nasal congestion by 4 dpi. Catarhal inflammation and congestion resolved by 15 dpi. No animals developed obvious neurologic signs, but approximately 5% died between 0 and 20 dpi. Beginning on 3 dpi, one rabbit developed mild conjunctivitis; between 5 and 6 dpi, severe conjunctivitis and keratitis accompanied by a nonpurulent mucoserous exudate was seen in 73% (16 of 22 rabbits examined). Subcorneal hemorrhage was also observed (Fig. 1). Eye inflammation healed in nearly all the rabbits by 21 dpi. On 10 dpi, 1 of 18 rabbits was noted to have opaque corneas.

On 72 dpi, a group of 14 rabbits (experiment 1) and on day 76 dpi, another group of 11 rabbits (experiment 2) had their latent HSV-1 strain H-129 infections reactivated by injection of cyclophosphamide followed by dexamethasone 24 hr later. The date at which cyclophosphamide was administered was considered day 0 post reactivation (dpr). Both drugs caused the rabbits to become quieter and unresponsive to pinching stimulus for a few hours. Cyclophosphamide caused hair loss in most of the rabbits between 4 and 10 dpi. Jaundice was noted in a few rabbits between 3 and 7 dpr. Three rabbits died between 3 and 5 dpi of the hepatotoxic effects of cyclophosphamide, which was confirmed histologically by profound hepatic congestion and necrosis. Keratitis and conjunctivitis was most severe between 5 and 7 dpr and was observed in 2 of 6 rabbits (experiment 1) and in 4 of 11 rabbits (experiment 2). In another group of 5 rabbits observed for a longer period, one examined on 80 dpi (28 dpr) had a completely opaque cornea; by about 180 dpi (128 dpr), both corneas were involved, making this animal functionally blind.

HSV-1 strain F infection: Eleven rabbits were intranasally inoculated with the low neurovirulent HSV-1 strain F. Although quiet for 3 dpi, they were more active than strain H-129-infected rabbits. Only 3% developed mild nasal congestion. Only very mild conjunctivitis was observed in 1 of 12 HSV-1 strain F-infected rabbits (data not shown). No rabbit developed neurologic signs, and none died.

On 72 dpi, 11 strain F-infected rabbits were injected with cyclophosphamide, followed by dexamethasone the next day to reactivate their latent infections. The conjunctivas and corneas of all rabbits appeared normal at all times after reactivation, and only one rabbit developed mild inflammation of the nictitating membrane. Four animals died of cyclophosphamide hepatotoxicity.

Virus Shedding in Ocular and Nasal Secretions During Latency

Latency, defined by the inability to recover virus from tear film or nasal secretions, was established in H-129- and F strain-infected rabbits by about 18 dpi. At several intervals between 14 and 70 dpi, we swabbed the eyes and noses of rabbits infected with both strains of HSV-1 to check for spontaneous reactivations. Although there was variability among rabbits as to the length of time that spontaneously shed HSV-1 could be recovered in tear film or nasal secretions, there were no differences between the number of rabbits infected with either strain that spontaneously reactivated. In one experiment, 8% (14/170 swabs were positive) of F strain-infected rabbits spontaneously reactivated in either their eyes or noses, and in another experiment 8% of strain H-129-infected rabbits spontaneously reactivated during the latent period (16/192 swabs were positive).
Table 1. Shedding of HSV-1, strain H-129, in nasal and ocular secretions in NZW rabbits following drug-induced reactivation

<table>
<thead>
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<th>Rabbit number</th>
<th>Organ swabbed</th>
<th>Day Post Reactivation*</th>
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<td>-3</td>
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<tr>
<td>Experiment 1</td>
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</tr>
<tr>
<td>65</td>
<td>N†</td>
<td>ND§</td>
</tr>
<tr>
<td>66</td>
<td>E†</td>
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<td>67</td>
<td>N</td>
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<td>69</td>
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<td>71</td>
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<td>ND</td>
</tr>
<tr>
<td>79</td>
<td>E</td>
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</table>

Experiment 2

| 102           | N   | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| 103           | N   | -  | -  | -  | -  | +  | +  | +  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| 98            | N   | -  | -  | -  | -  | -  | +  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| 100           | N   | -  | -  | -  | -  | -  | +  | +  | +  | +  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| 94            | N   | -  | -  | -  | -  | -  | -  | +  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| 97            | N   | -  | -  | -  | -  | -  | +  | +  | +  | +  | +  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| 92            | N   | -  | -  | -  | -  | -  | -  | +  | +  | +  | +  | +  | +  | +  | -  | -  | -  | -  | -  | -  | -  |
| 93            | N   | -  | -  | -  | -  | -  | -  | -  | +  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| 101           | N   | -  | -  | -  | -  | -  | -  | -  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 99            | N   | -  | -  | -  | -  | -  | -  | -  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |

* Day 0 was the day animals received cyclophosphamide.
† Nasal swab.
‡ Eye swab.
§ ND, Not Done.
| s | Animal killed and tissues processed.
† d | Animal dead for more than 24 hr, no swabs or tissue taken.
§ dp | Animal dead for less than 24 hr, no swabs taken, but tissues processed.

Virus Shedding in Ocular and Nasal Structures During Reactivated Infections

Acute infection was confirmed in all rabbits infected with both strains by demonstration of HSV-1 in eye swabs from 3 through 7 dpi.

Reactivated HSV-1 strain H-129 infections: Swabs of ocular and nasal secretions were performed on all rabbits between 2 and 4 days before drug-reactivation to determine whether any animal was spontaneously shedding virus before administration of the drugs. In experiment 1, two rabbits (No. 69, 72) and in experi-
Table 2. Shedding of HSV-1, strain F, in nasal and ocular secretions in NZW rabbits following drug-induced reactivation

<table>
<thead>
<tr>
<th>Rabbit number</th>
<th>Organ swabbed</th>
<th>Day post reactivation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nf</td>
<td>Day 0</td>
</tr>
<tr>
<td>83</td>
<td>E‡</td>
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<tr>
<td>85</td>
<td>E‡</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>N</td>
<td></td>
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<tr>
<td>89</td>
<td>N</td>
<td></td>
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<tr>
<td>81</td>
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</tr>
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<td>86</td>
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</tr>
<tr>
<td>82</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>88</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

* Day 0 was the day animals received cyclophosphamide.
† Nasal swab.
‡ Eye swab.
§, animal sacrificed and tissue processed.
# dp, animal dead for less than 24 hr, no swabs taken, but tissue processed.
‖ d, animal dead for more than 24 hr, no swabs or tissue taken.

Swabs were positive; however, this difference was not considered significant. At 2, 3, 4, and 5 days post reactivation, strain H-129 was statistically more frequently recovered than strain F (Table 3), indicating that the drug-reactivation protocol was more effective at reactivating latent trigeminal ganglionic, high neurovirulent strain infections than low neurovirulent strain infections.

Comparison of Ocular Virus Shedding Between Reactivated HSV-1 Strain H-129 and Strain F Infections

We compared the incidence of shedding of H-129 and F strains in ocular secretions during reactivated infections. The spontaneous reactivation rate for the 6 days immediately preceding reactivation of H-129 strain infections was 16% (15/96 eye swabs were positive), and for F strain infections was 0% (0/55 eye swabs were positive); however, this difference was not considered significant. At 2, 3, 4, and 5 days post reactivation, strain H-129 was statistically more frequently recovered than strain F (Table 3), indicating that the drug-reactivation protocol was more effective at reactivating latent trigeminal ganglionic, high neurovirulent strain infections than low neurovirulent strain infections.

Synergism of Cyclophosphamide and Dexamethasone in Inducing HSV-1 Ocular Shedding

Injection of dexamethasone or cyclophosphamide alone did not reactivate latent strain H-129 infections. Twelve rabbits were intranasally inoculated with strain H-129, and acute infection was confirmed by shedding

Table 3. Shedding of HSV-1 strains H-129 and F in ocular secretions after drug-induced reactivation

<table>
<thead>
<tr>
<th>Day post reactivation</th>
<th>Strain H-129</th>
<th>Strain F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of swabs positive/total</td>
<td>Number of swabs positive/total</td>
</tr>
<tr>
<td>2</td>
<td>4/19 (21%)</td>
<td>0/11 (0%)</td>
</tr>
<tr>
<td>3</td>
<td>8/11 (73%)</td>
<td>1/7 (14%)</td>
</tr>
<tr>
<td>4</td>
<td>9/11 (82%)</td>
<td>1/7 (14%)</td>
</tr>
<tr>
<td>5</td>
<td>5/9 (56%)</td>
<td>1/6 (17%)</td>
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</table>

* P values by Fisher Exact Probability Test.
Histopathology in the eye during acute and reactivated HSV-1 strain H-129 and F infections. (A) Inflammation at the cornea-sclera margin in an H-129-infected eye, 7 dpi. (B) Infiltration of the choroid (arrow) and degeneration of retinal architecture (left) in an H-129 strain-infected eye, 4 dpi. (C) Iritis in an H-129 strain-infected eye, 11 dpi. (D) Inflammation at the cornea-sclera margin in the eye of an H-129 strain-infected rabbit 7 days after drug-induced reactivation (79 dpi). (E) Inflammation at the cornea-sclera margin in an F strain-infected eye, 7 dpi. (F) Normal appearing cornea-sclera margin in an eye from an F strain-infected rabbit 12 days after drug-induced reactivation (83 dpi). H&E, bar = 10 μ.

of virus in tear film in all rabbits between 5 and 7 dpi. At 82 dpi, five rabbits were given an intravenous injection of 75 mg/kg of cyclophosphamide, and five were given 4 mg/kg of dexamethasone; none of these rabbits spontaneously shed HSV-1 for 4 days before drug injection. Eye and nasal swabs were checked for HSV-1 infectivity up to 14 days after the drugs were administered. Three percent (4/150) and 1% (2/150) of eye plus nasal swabs from the recipients of cyclophosphamide and dexamethasone, respectively, were positive for HSV-1, in contrast to the reactivation frequency of 85% observed when both drugs were injected.

Histopathology of HSV-1 Strain H-129 and Strain F Infections

Acute and reactivated strain H-129 infections: No lesions were found in the eyes from the three rabbits killed at 3 dpi. Mild to moderate inflammation of the corneal stroma at the cornea-sclera margin was found in all three animals killed 7 dpi (Fig. 2).

Two of twelve rabbits subjected to drug-induced reactivation in experiment 1 had moderate corneal stromal inflammatory infiltration (Fig. 2); one was killed 1 dpr, and the other 4 dpr. The retina and choroid of both of these rabbits were heavily infiltrated with mononuclear cells (Fig. 2). In experiment 2, 2 of 11 eyes of rabbits examined histopathologically had similar corneal lesions; one was killed 11 dpr and the other 12 dpr. Iritis was noted in 11 dpr (Fig. 2).

Sections of all eyes were carefully cut to ensure adequate examination of the optic nerves. In no instance was there inflammation of the nerve, and in sections stained for myelin, no demyelination was noted.

Acute and reactivated strain F infections: No lesions were present in the eyes of three animals killed on 3 or 5 dpi. Lesions similar in location, but less severe than strain H-129-induced lesions were present in one animal killed at 7 dpi (Fig. 2).

Of the eleven rabbits subjected to drug-induced reactivation, seven were examined for histopathologic evidence of infection. No inflammation or other abnormalities were noted in any ocular structure (Fig. 2). No histologic evidence of bacterial or fungal superinfection was observed in any F- or H-129 strain infected or control rabbit.
Trigeminal Ganglionic Latency and Reactivation Revealed by In-Situ Hybridization

We examined latently infected rabbit ganglia to determine if the distribution of HSV-1 RNA was the same as we previously identified in latently infected mouse ganglionic neurons. Sections of ganglia from ten rabbits infected with strain F and from five rabbits infected with strain H-129 were probed for HSV-1 RNA. No virus was shed in tear film at the time of death, nor was HSV-1 isolated from cell-free homogenates from the paired trigeminal ganglia. No differences in the number of autoradiographic grains representing HSV-1 RNA between strain F and strain H-129 latently infected ganglia were noted, indicating that both strains established latency within the ganglia to the same degree. As with latently infected mouse ganglia, HSV-1 RNA was detected in ganglion cell nuclei (Fig. 3).

Because we had shown that strain H-129 latent infections were more easily reactivated than strain F infections, we examined reactivated strain H-129 trigeminal ganglionic infections only by in situ hybridization. Sections from trigeminal ganglia removed from rabbits 71 and 93 on days 4 and 11, respectively, were probed for HSV-1 RNA. These two samples were chosen, because in both cases HSV-1 was actively shed in ocular secretions at the time of death (Table 1). By in situ hybridization, an increase in the number of autoradiographic grains over neurons correlated with the reactivation event (Fig. 3). In contrast to latently infected neurons, reactivated neurons exhibited both a nuclear and cytoplasmic grain distribution (Fig. 3). A few satellite cells in the ganglia were also positive for HSV-1 RNA, suggesting that viral mRNA transcripts were being transported into the cytoplasm for translation. The satellite cells were probably acutely infected, as the virus spread toward the periphery.

No hybridization was observed in five trigeminal ganglia from uninfected rabbits or in five ganglia from cyclophosphamide- and dexamethasone-treated uninfected rabbits.

Discussion

In this report we have described a model of reactivated HSV-1 infection in rabbits in which reactivation correlated with re-expression of viral mRNA, shedding of virus at the periphery, and the reappearance of ocular disease. A high neurovirulent strain of HSV-1 was more readily reactivated and had a greater potential to produce eye disease than a low neurovirulent strain. We believe that HSV-1 initially infected the trigeminal ganglion consequent to the intranasal inoculation, via the axons of the maxillary branch of the trigeminal nerve. In a previous study, we noted that mice infected by the ocular route also developed acute and latent infections of the olfactory pathway.35

Fig. 3. Localization of HSV-1 during latent and reactivated HSV-1 strain H-129 infections of the trigeminal ganglion by hybridization in situ. (A) HSV-1 RNA in a single neuron during latency (arrow). Note that the autoradiographic grains are over the nucleus. (B) HSV-1 RNA in several ganglion cells during drug-induced reactivation. Note that more grains are present in this field than in A, and that the grains are over neuronal cytoplasm and nuclei. A focus of inflammatory cells surrounds one neuron (n). H&E, bar = 6 μ.

Numerous animal models in which HSV-1 ganglionic latency was reactivated have been developed. In mice, reactivation stimuli have included epinephrine iontophoresis,36 fever subsequent to bacterial infection,37 immunosuppression,32,33 epithelial or ganglionic trauma,38,39 and neurectomy.40 In rabbits, direct ganglionic electrical stimulation,41 adrenaline injection,42 anaphylactic shock,43 and epinephrine iontophoresis44 have been used; bovine herpesvirus infection in rabbits30 and cattle1 have been reactivated by glucocorticoid injection. Rock et al reactivated latent trigeminal ganglionic bovine herpesvirus type 1 infection of rabbits using a regimen in which 4 mg of dexamethasone was injected daily for 4 days.30 In an early experiment, we found that dexamethasone alone was incapable of reactivating latent F strain infection, even when as much as 22 mg was given over a 24-hr period (Stroop, personal observations). Our finding that neither cyclophosphamide nor dexamethasone alone could reactivate strain H-129 infection suggests that these two drugs act in a synergistic manner; however, their mechanism of action has yet to be determined. The fact that bovine...
herpesvirus was easily reactivated by dexamethasone, whereas HSV-1 was not, suggests that the viral genetic elements involved in maintenance of the latent state and/or release from latency are not identical for all alpha herpesviruses.

Cyclophosphamide- and dexamethasone-induced reactivation of latent infection induced by the low neurovirulent strain F was unremarkable. Few of the animals shed virus in ocular secretions, whereas many animals with reactivated high neurovirulent strain H-129 infections shed virus. Reactivated low neurovirulent strain F infections produced no overt eye inflammation, whereas the high neurovirulent strain H-129 caused severe inflammation. One concern was the possibility that repetitive swabbing of eyes during the reactivation period rendered the animals more susceptible to microbial superinfections that could have potentially lead to misinterpretation of the cause of the gross ocular changes observed. We believe this to be unlikely. First we did not observe bacterial or fungal contamination in the cell cultures used to check the swabs for viral infectivity. Second, since the experiments were conducted at the same time, the rate of secondary infection would have been the same in both F- and H-129-strain infected animals, yet reactivated strain H-129 infections produced more severe gross and histopathologic eye disease than reactivated F-strain infections. Third, uninfected control rabbits subjected to repetitive swabbing did not develop observable eye disease.

In an effort to identify the HSV-1 gene(s) that correlate with acute ocular disease patterns, Centifanto-Fitzgerald et al.\(^\text{46}\) infected rabbits with a series of HSV-1 strains generated through intertypic recombination of MP and F HSV-1 strains (MP and F display intermediate and low neurovirulence, respectively). Strains MP and F each caused unique epithelial dendritic ulceration patterns and different epithelial and/or stromal diseases. Analysis of the disease and/or lesions produced by the recombinants identified characteristics acquired from one or the other parental HSV-1 strains. The viral function(s) derived from the virulent parent that caused severe eye disease was identified to lie between 0.70 and 0.83 map units along the HSV-1 genome.\(^\text{45}\) Thompson et al recently identified HSV-1 gene function(s), mapping between 0.71 and 0.83 map units responsible for acute encephalitogenic potential of another HSV strain.\(^\text{46}\)

The relative neurovirulence of HSV strains appears to correlate with several pathobiological features of infection. HSV-2 is more neurovirulent than HSV-1,\(^\text{47}\) and causes more severe eye lesions than HSV-1.\(^\text{48,49}\) We previously demonstrated that strain H-129 is more neurovirulent during acute and reactivated central nervous system infections in the rabbit than strain F,\(^\text{34}\) and in the present study, neurovirulence appeared to correlate with the relative ease by which the virus could be induced from latency, and with the degree of ocular pathology produced following reactivation. We are currently determining whether the neurovirulence gene function(s) and the reactivation gene function(s) co-map to the same section of the HSV-1 genome.

**Key words:** HSV-1, latency, RNA, in situ hybridization, nervous system

**References**

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