A Low Thymidine Kinase-Producing Mutant of Herpes Simplex Virus Type 1 Causes Latent Trigeminal Ganglia Infections in Mice

By


Departments of Molecular Virology and Ophthalmology,
The Hebrew University-Hadassah Medical Center,
Jerusalem, Israel

With 6 Figures

Accepted November 30, 1982

Summary

The wild type NIH strain of herpes simplex virus type 1 (HSV-1) has a mixed plaque morphology of both large and small plaques. From this virus we selected a large plaque isolate that was a high producer of thymidine kinase (TK) activity (designated TK+) and a small plaque isolate that produced 25 per cent of the TK activity of the large plaque mutant (designated TK+/4). A TK- mutant of the large plaque virus was obtained after passage of the virus in the presence of BUdR. The pathogenicity of the TK+/4 virus strain in relation to the TK+ and TK- strains was investigated in mice after inoculation of the virus into the eyes by corneal scarification. The TK+ strain was highly pathogenic, caused encephalitis and killed most of the mice, whereas the TK- strain did not cause latent infections in the trigeminal ganglia or kill the mice. The TK+/4 virus strain replicated in the eyes within 24 hours after inoculation and entered the trigeminal ganglia, establishing a latent infection in almost all of the mice. By increasing the infectious dose tenfold, the TK+/4 virus caused an active infection in the trigeminal ganglia (ganglionitis), migrated to the brain, and killed the mice. The results indicate that not only is a low level of TK required to establish latent infections in mice, but also the degree of virulence is determined by the amount of TK produced by the infecting virus.

Introduction

Studies on the pathogenicity of herpes simplex type 1 (HSV-1) revealed that expression of the viral thymidine kinase (TK) is essential for infection of the...
ganglia and central nervous system (CNS) in mice. TK⁻ mutants which are also resistant to drugs were reported to be avirulent in adult mice and did not establish latent infections (3, 4, 8, 11, 14, 15). Not only the HSV-1 TK⁻ mutants, but also mutants that produce low levels of TK, were studied for their ability to cause latency in mice. Field and Darby (3) and Tenser et al. (15) reported that HSV-1 TK mutants that produce a low level of TK activity were able to penetrate into the ganglia and replicate; the titer of virus in the ganglia was related to the extent of TK production by the virus. Tenser et al. (15) concluded that expression of the viral TK determines its ability to enter the ganglia and establish a latent infection.

Since the low TK-producing mutants of HSV-1 constitute an important subclass among the TK⁻ mutants, we decided to study the sequence of events starting with the inoculation of the virus into the eyes of mice (10), using three mutants of HSV-1 (NIH strain): a virulent large plaque strain (TK⁺), producing a high level of TK in the infected cells, a TK⁻ mutant of this strain isolated in the presence of BUdR, and a small plaque mutant (TK 1/4) that produced 25 per cent of the TK activity of the large plaque strain but had a similar one-step growth cycle to the large plaque virus. Replication of these HSV-1 strains that differed in their ability to produce thymidine kinase was studied on a daily basis in the eyes, trigeminal ganglia, and the brains of four-week-old infected mice. Our results are compatible with those of Tenser et al. (15) in that the TK 1/4 virus can establish a latent infection in the trigeminal ganglia after an ocular infection. Moreover, we found that by increasing the initial infectious dose of the TK 1/4 mutant, and therefore the amount of TK produced, we enabled the virus to replicate and reach the CNS, causing encephalitis and death of the mice.

Materials and Methods

**Virus Strains and Cells**

The wild type (wt) virus was the NIH strain no. 11124 of HSV type 1 that had been maintained in tissue culture by multiple passages in BSC-1 cell monolayers. The cells were grown in Dulbecco's modified Eagle's medium (DMEM; GIBCO) supplemented with 10 per cent calf serum (DMEM + 10). The wt virus that produced a heterogeneous population of 79 per cent large plaque and 21 per cent small plaque morphology was diluted to yield about 10 plaque-forming units (PFU) plate, and from these, large (2 mm diameter) and small (0.5 mm diameter) plaques were isolated for plaque purification. The process was repeated many times until a small plaque strain was obtained that could be purified three times with 100 per cent small plaque purity; the large plaque derivative showed about 95 per cent plaque purity. The large plaque strain was designated TK⁺ and the small plaque strain TK 1/4.

A HSV-1 TK⁻ mutant, designated TK⁻, was isolated from the large plaque strain by incubating about 100 PFU/plate in the presence of 20 μg/ml of BUdR in the agar overlay (2). Virus plaques were isolated, passaged in the presence of BUdR and tested for TK activity.

L(TK⁻) cells were received from Dr. Hl. Cedar (Department of Molecular Biology, The Hebrew University-Hadassah Medical School, Jerusalem) and propagated in DMEM + 10.

**TK Assay**

L(TK⁻) cells were infected with identical amounts of either the HSV-1 TK⁺ or TK 1/4 mutants, incubated at 37°C, and at 10 hours postinfection (p.i.) the cells were