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Neurovirulent strains of herpes simplex virus type 1 are not necessarily competent for reactivable latency.

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Abstract

Ability of two neurovirulent strains (F and +GC (LPV) Miyama) of herpes simplex virus type 1 (HSV-1) to establish and maintain reactivatable latency in trigeminal ganglia (TG) was compared after intranasal inoculation of mice. The +GC (LPV) Miyama strain showed a very low rate of virus reactivation in explant cultures of TG, while the F strain showed a high rate of reactivation. These data indicate that neurovirulent strains of HSV-1 are not always competent for reactivatable latency, although most virulent strains of HSV-1 thus far reported were competent for reactivatable latency.

KEYWORDS: herpes simplex virus type 1, neurovirulence, latency, reactivation

Neurovirulent Strains of Herpes Simplex Virus Type 1 are not Necessarily Competent for Reactivable Latency

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Ability of two neurovirulent strains (F and +GC (LPV) Miyama) of herpes simplex virus type 1 (HSV-1) to establish and maintain reactivable latency in trigeminal ganglia (TG) was compared after intranasal inoculation of mice. The +GC (LPV) Miyama strain showed a very low rate of virus reactivation in explant cultures of TG, while the F strain showed a high rate of reactivation. These data indicate that neurovirulent strains of HSV-1 are not always competent for reactivable latency, although most virulent strains of HSV-1 thus far reported were competent for reactivable latency.

Key words : herpes simplex virus type 1, neurovirulence, latency, reactivation

Several animal models have been used to investigate the mechanism of latent infection of humans with herpes simplex virus (HSV) (1, 2). Among them, we have preferentially used a mouse latency model after intranasal inoculation (3-5), because it closely simulates the natural portal of HSV. Viruses grow in nasal mucosal epithelial cells, travel through trigeminal nerve and replicate in trigeminal ganglia (TG). Subsequent viral spread to the brain and its replication there cause fatal encephalitis in mice. Difference in neurovirulence was observed among strains of HSV type 1 (HSV-1) (3, 6, 7). The latent herpetic infection was established and maintained in TG of mice which survive the acute phase of infection. Reactivation of latent HSV can be induced artificially (reactivable) by excising TG

and explanting them onto Vero cells (*In vitro* reactivation) (8). Differences in ability to establish and maintain reactivable latency were also observed among strains of HSV-1 (9-18). In the present study, we attempted to elucidate the relation between neurovirulence, and the ability to establish and maintain reactivable latency shown by each strain of HSV-1. Our question is whether or not neurovirulent strains are always competent for reactivable latency.

There is, however, a practical difficulty in investigating reactivable latency of virulent strains of HSV-1, because ability of these strains to establish and maintain reactivable latency can be examined only for the mice which survive the acute phase of infection. Most mice inoculated with a higher dose of a virulent strain are killed and only mice inoculated with a lower dose are available for the assay. To solve this problem,

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some procedure which protects mice against death in the acute phase is necessary. We reported that mice infected with an avirulent strain could survive superinfection with lethal dose of a virulent strain (4, 5, 7, 19). Accordingly, this procedure was applied to the present study. Two (virulent and avirulent) strains reactivated from TG of dual-infected mice were successfully distinguished by the differences in cytopathic effects, and the electrophoretic patterns of their DNA after digestion with a restriction endonuclease.

Two virulent strains, F and +GC (LPV) Miyama, and an avirulent strain, -GCr Miyama, were used (4, 5, 7, 20-23). Preparation of virus samples and plaque assay for infectivity were performed as described previously (7, 22). Five-week-old ICR outbred mice (Charles River Breeding Laboratories) were used throughout this study. Intranasal inoculation of mice was performed as described previously (4, 5).

In the first experiment (Table 1), mice were inoculated intranasally with various doses of each strain ranging from $10^{2.5}$ to $10^{6.5}$ PFU/mouse. Appearance of neurological symptoms and death of these mice were observed daily during 4 weeks

after infection. Animals which survive the acute phase of infection were used for the assay of virus reactivation as described previously (20). Briefly, TG of these mice were excised out aseptically and explanted onto Vero cell cultures in 24 well microplates. Cultures were maintained for 5 weeks and the appearance of cytopathic change specific for HSV was observed.

The F and +GC (LPV) Miyama strains were both virulent as shown by increasing mortality of infected mice, while -GCr Miyama was attenuated as shown by constantly low mortality even at the highest dose tested. These data obtained by this study were not in conflict with the average 50% lethal dose (LD_{50}) determined in several previous experiments ($10^{3.3}$ PFU/mouse for the F strain, $10^{4.1}$ PFU/mouse for the +GC (LPV) Miyama strain and $>10^{6.5}$ PFU/mouse for the -GCr Miyama strain) (4, 5). The rate of reactivation of the F strain increased in a dose-dependent manner, while the rate of reactivation of the +GC (LPV) Miyama strain, as well as that of the -GCr Miyama strain, remained at a low level even at the highest dose tested. Therefore, the F strain was virulent and compe-

Table 1 Mortality and reactivatable latency shown by three strains of HSV-1

Strains	Virus titers inoculated (PFU/mouse)	No. of mice tested	No. of dead mice	Mortality ^a (%)	No. of TG tested	No. of TG yielding virus	Rate of virus reactivation ^b (%)
F	$10^{2.5}$	10	0	0	10	0	0
	$10^{3.5}$	15	5	33	20	1	5
	$10^{4.5}$	65	60	92	10	7	70
	$10^{5.5}$	66	63	95	6	6	100
	$10^{6.5}$	10	10	100	0	— ^c	— ^c
+GC(LP) Miyama	$10^{2.5}$	10	0	0	10	1	10
	$10^{3.5}$	10	3	30	8	1	13
	$10^{4.5}$	20	7	35	24	2	8
	$10^{5.5}$	30	19	63	22	4	18
	$10^{6.5}$	15	11	73	8	1	13
-GCr Miyama	$10^{4.5}$	20	0	0	34	0	0
	$10^{5.5}$	10	1	10	16	1	6
	$10^{6.5}$	20	3	15	32	3	9

a : Mortality = [(No. of dead mice) / (No. of mice tested)] × 100

b : Rate of virus reactivation = [(No. of TG yielding virus) / (No. of TG tested)] × 100

c : —, Not tested

tent for reactivatable latency, while the +GC (LPV) Miyama strain was virulent but was limited in its ability to establish reactivatable latency.

In the second experiment, the ability of two virulent strains to establish and maintain reactivatable latency was further examined under conditions where most mice inoculated with lethal dose of a virulent strain could survive. This was accomplished by the inoculation with an attenuated strain at 24 hours prior to the challenge of virulent strains. In this way, the rate of virus reactivation could be examined in a greater number of mice after inoculation with a higher dose of a virulent strain, making a more precise analysis possible.

Mice were infected intranasally with $10^{6.5}$ PFU/mouse of the attenuated -GCr Miyama strain, or the same amount of Eagle's minimum essential medium (negative control) 24 hours before inoculation with virulent strains. Then, the mice were infected with either of the two virulent strains at a dose equivalent to 10 LD₅₀. As shown in Table 2, the preinoculation with the attenuated strain reduced the mortality of mice infected with either of two virulent strains from 90% to 10% and 90% to 0%, respectively. Drastic reduction of the mortality enabled us to examine the rate of virus reactivation in TG of a majority of the infected mice.

Virus reactivation was observed in 57% of TG of mice dually infected with -GCr Miyama and F. All eight virus isolates reactivated from these TG were identified as the F strain by

analysis of viral DNA digested with restriction endonuclease, *Bam*HI (Fig. 1). Viruses were reactivated from 2 out of 14 TG (14%) of mice dually infected with -GCr Miyama and +GC (LPV) Miyama. One of these was the +GC (LPV) Miyama strain and the other was the -GCr Miyama strain, as judged from the difference in the ability to induce syncytium between the two variants of the Miyama strain

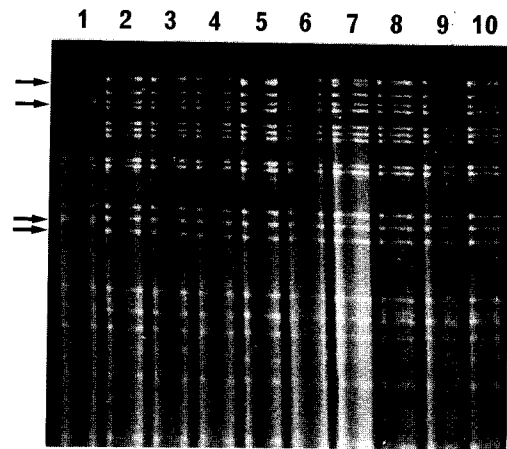


Fig. 1 Restriction endonuclease analysis of viruses reactivated from 8 explant cultures of TG of mice dually infected with the -GCr Miyama and F strains of HSV-1. Viral DNA was purified by the method of Hirt (24) and digested with *Bam*HI. Restricted viral DNA was electrophoresed in 0.6% agarose gel with a tris-borate buffer (25). Lane 1, -GCr Miyama; lane 2-9, viruses reactivated from 8 explant cultures of TG; lane 10, F. Arrows indicate differences in electrophoretic patterns between -GCr Miyama and F.

Table 2 Mortality and reactivatable latency shown by two virulent strains of HSV-1 in mice preinoculated with the -GCr Miyama strain of HSV-1

Strains	Preinoculation of the -GCr Miyama strain	No. of mice tested	No. of dead mice	Mortality ^a (%)	No. of TG tested	No. of TG yielding virus	Rate of virus reactivation ^b (%)
F	-	10	9	90	— ^c	— ^c	— ^c
	+	10	1	10	14	8	57
+GC(LP) Miyama	-	10	9	90	— ^c	— ^c	— ^c
	+	10	0	0	14	2	14

a : Mortality = [(No. of dead mice) / (No. of mice tested)] × 100

b : Rate of virus reactivation = [(No. of TG yielding virus) / (No. of TG tested)] × 100

c : —, Not tested

(22, 23). These results confirmed that the ability of the +GC (LPV) Miyama strain to establish and maintain reactivatable latency was very restricted.

In the present study, we compared the ability of the two virulent strains of HSV-1 to establish and maintain reactivatable latency in two separate experiments. One was done by examining the mortality of mice and the rate of virus reactivation after intranasal inoculation with various doses of either strain ranging from $10^{2.5}$ to $10^{6.5}$ PFU/mouse. The other was done by examining the rate of virus reactivation in TG of mice which survived 10 LD₅₀ equivalent of either of the two virulent strains by preinoculation with an attenuated strain at 24h before the challenge of the virulent strain. Although this approach is rather artificial, it is worth trying because most of the primary infections of humans with HSV are subclinical or self-limiting and not fatal. In addition, we have the evidence that dual infections do not affect the rate of virus reactivation of each strain (manuscript in preparation).

The wild strains of HSV were neurovirulent and competent for reactivatable latency. On the other hand, all mutants which were limited for reactivatable latency were also avirulent (9-17). Therefore, it was speculated that the decrease in neurovirulence for any virus strain is prerequisite for its limited ability to establish and maintain reactivatable latency. As far as we know, the +GC (LPV) Miyama is the only strain which is neurovirulent but has a limited ability to establish reactivatable latency.

Mutations in several genes of HSV including thymidine kinase, ribonucleotide reductase and immediate early gene ICP0 were reported to be responsible for both reduction of neurovirulence and restriction of reactivatable latency of the virus (9-17). Mutations in the other genes including DNA polymerase were reported to reduce neurovirulence, but not affect the ability of reactivatable latency (12). In conjunction with these reports, two possible genetic explanations for the unique phenotype of the +GC (LPV)

Miyama strain can be considered. One is that a single mutation which restricts reactivatable latency, but does not affect the neurovirulence, might have occurred on a locus quite different from the gene thus far reported. The other possibility is that the +GC (LPV) Miyama strain has experienced two mutations. The first mutation in a gene such as thymidine kinase, ribonucleotide reductase and ICP0 might have reduced both neurovirulence and reactivatable latency. The second mutation which somehow restores neurovirulence alone might occur within or outside of these genes.

In conclusion, we demonstrated that neurovirulent strains of HSV-1 are not always competent for reactivatable latency. Further study is necessary to define the mutations responsible for the unique phenotype of the +GC (LPV) Miyama strain and this study will provide new information about viral factors which are essential for acute and latent infections of HSV-1.

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