Virus Genes 26:2, 115–118, 2003

Isolation of the Enhanced Neurovirulent HSV-1 Strains from Korean Patients

JEONG-KI KIM[1], YOUNG KEUNG KIM[1], JUNBAE HONG[1], SANG YONG KIM[1], CHONG-KIL LEE[4], CHUL JOONG KIM[2], YOUNG SANG KIM[3] & JEONG KEUN AHN[1,*]

[1]*Department of Microbiology,* [2]*Department of Veterinary Medicine,* [3]*Department of Biochemistry, Chungnam National University, Daejeon 305-764, Korea*

[4]*Department of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea*

Revised October 28, 2002; Accepted November 22, 2002

Abstract. Herpes simplex virus type 1 (HSV-1) is a neurotropic DNA virus which has latency in human. In this study, we isolated various HSV-1 strains, named KHS, from the skin lesions of Korean patients and characterized the specific features of each strain. We found that KHS strains produced small, cell associated and nonsyncycial plaques in Vero cells. We classified KHS strains into two substrains, KHS 1 which had highly condensed plaques and KHS 2 which had less condensed plaques. Since gD protein of HSV-1 plays important roles in viral plaque formation, we determined the nucleotide sequences of gD genes of KHS strains. According to deduced amino acid sequences of gD protein in KHS strains compared with prototype strains KOS and F, we found that gD of KHS strains have more putative O-glycosidic sites, serine in KHS 1 and threonine in KHS 2, respectively.

To find out the establishment of viral latency, we infected each virus strain into eyes of mice and carried out trigerminal ganglia explanting experiment. We found that both KHS strains established latent infections stably just as did the prototype KOS and F strains. The eye swab experiments were carried out to check the viral replication *in vivo*. KHS 1 exhibited a longer shedding time in eyes of mice. We also found that KHS 1 has a higher neurotropic affinity by determining the time it took for the virus to reach the trigerminal ganglia from the eyes. Currently, we are studying the possible mechanism of high neuroinvasiveness of KHS 1 strain.

Key words: gD, HSV-1, Korean patients, neurovirulence, trigerminal ganglia

HSV-1 is a neurotropic pathogenic agent infecting the terminals of sensory nerve systems [1]. During the infection of its mammalian host, HSV-1 replicates productively at peripheral sites and establishes a latent infection in sensory neurons that innervate these sites [2,3]. The viruses are localized in ganglia maintaining the latency of infection [4]. Latency is a strategy that HSV-1 has developed to maintain the presence of its genome in a nonpathogenic and non-replicate form [5]. Latent virus serves as a reservoir for the transmission to other susceptible hosts following the viral reactivation. The reactivation of latent virus is mainly dependent upon the immunological state of the host. When the reactivation occurs, the infectious virus propagates in the terminal of neuron provoking the skin lesion [6].

To isolate various Korean HSV-1 strains KHS, we collected virus samples from the skin lesions of 7 different Korean patients and repeated plaque purification steps on Vero cells. The highly purified KHS strains were infected on Vero cell monolayer to compare the plaque of each strain. Interestingly, the plaques of KHS strains were smaller than those of the prototype strains, KOS and F (data not shown). We could further classify 7 KHS strains into two groups by plaque morphology, 2 strains in one group which have highly condensed cell-associated plaques (KHS 1) and 5 strains in another group which have less condensed cell-associated plaques (KHS 2).

*Author for all correspondence.
E-mail: jkahn@cnu.ac.kr

Since it is possible that newly isolated viruses have small plaques than prototype viruses because of the lack of time for adaptation, we checked the plaque morphology of KHS strains of high passage. We figured out that KHS strains of high passage also produced condensed cell-associated plaques.

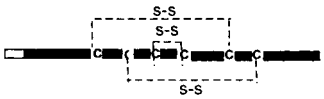
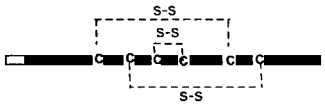
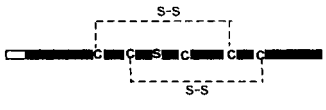
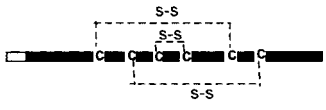
To investigate the single replication cycle for each virus strain, Vero cells were infected with each virus strain and the rate of replication of each strain was quantitated by standard plaque assay. The viral replicating rates of the KHS strains were very similar to those of prototype strains on Vero cells at MOI 1.0. Since there was a possibility for several virus particles to infect the same cell at high MOI, it might be reasonable to infect Vero cells with virus at low MOI to detect the difference of viral replication feature precisely. Therefore, we infected Vero cells with each virus strain at MOI 0.01. It turned out that KHS 1 strain showed late eclipse period compared with other prototype strains and KHS 2 strain had a similar eclipse period as prototype strains.

Since the replication features of KHS strains were a little different from the prototypes, we would like to figure out the reason why KHS strains produced small and condensed plaques. It might be possible that the small plaques were due to the differences of syncytial formation. When it comes to syncytial formation, we should consider glycoproteins that are related with cell fusion [7–9]. Among HSV-1 glycoproteins, gD is the most important protein for viral adsorption [10], penetration [11], cell-to-cell transmission [12] and cell fusion [13]. It is possible that KHS strain could result in smaller plaques on Vero cell, because of KHS gD which might have a lower affinity to cellular receptor in viral infection. Therefore, we analyzed the sequences of KHS gD genes to determine their roles in plaque formation of different morphology and late eclipse period of viral replication. We isolated pure virus particles and prepared the viral genomic DNA by CsCl gradient ultracentrifugation. The gD gene of each virus was amplified from the purified genomic DNA by PCR reaction and the whole nucleotide sequences of gD genes of KHS strains were determined. Surprisingly, among 7 KHS strains, there were only two different types of gD nucleotide sequences. The gD nucleotide sequences of 2 strains in KHS 1 which produced highly condensed cell-associated plaques were identical and the gD nucleotide sequences of remaining 5 strains in KHS 2 which produced less condensed cell-associated plaques were also same with each other. These gD nucleotide sequence data were submitted to GeneBank (BankIt449358 AF487901 and BankIt455991 AF487902). According to the deduced gD amino acid sequences of 395 residues, there was a little variations between two groups, KHS 1 and KHS 2. The amino acid similarity between two groups was about 99.5% and potential glycosylation sites, Asn, Ser and Thr, were added in gD of KHS strains (Fig. 1A). The addition of O-linked glycosylation site, Ser, to gD of KHS 1 might be possible to affect the fusion of infected cells. We also found out that there is another possible alteration in disulfide bonds in gD of KHS 1. Eisenberg et al. reported that disulfide bonds were responsible for the binding of gD to the cellular receptor HVEM and disulfide bonds consisted of cysteins between Cys 131 and 227, Cys 143 and 152 [14,15]. Since there is an alteration of Cys 143 in gD of KHS 1, there is a possibility that the configurational change of gD structure may reduce its binding affinity to the cellular receptor and specific infectivity on Vero cells (Fig. 1A).

One of the most important features of HSV-1 infection is the latency in its host cell. To test the latency characters of KHS strains in animal model, the eyes of 4 or 6 weeks old male ICR mice (Korea Research Institute of Chemical Technology) were scarified and each eye was infected with 5.0×10^4 PFU of KHS strains along with KOS and F. During 4 weeks, all the infected mice were alive and did not have any abnormal symptoms. To find out the establishment of latency, we carried out a trigerminal ganglia explanting experiment. Briefly, trigerminal ganglia from the brains of mice were surgically collected 4 weeks after virus infection, sectioned into 8 pieces and overlaid on Vero cell monolayer for 4 days. The pieces of trigerminal ganglia and Vero cells were scraped, harvested and homogenized by Douncer. Homogenates of trigerminal ganglia were overlaid on Vero cells and the plaque formation was monitored to detect the reactivated viruses. We found that both KHS strains established the stable latency just as did the prototypes, KOS and F strains (data not shown).

Since KHS 1 had a late eclipse period and slow growth pattern on Vero cells, we carried out eye swab experiments to check the viral replication in eyes. Equal amount of each virus strain (2.0×10^5 PFU) was infected into the mice eyes. At various

A

Virus strain	Amino acid change	Schematic representation of HSV gD
KOS (•)	Reference	
F (■)	No change	
	No change	
	H365R	
	P369Q	
KHS1 (▲)	D71N	
	C143S	
	R374P	
KHS2 (♦)	D71N	
	A217T	
	R374P	

B

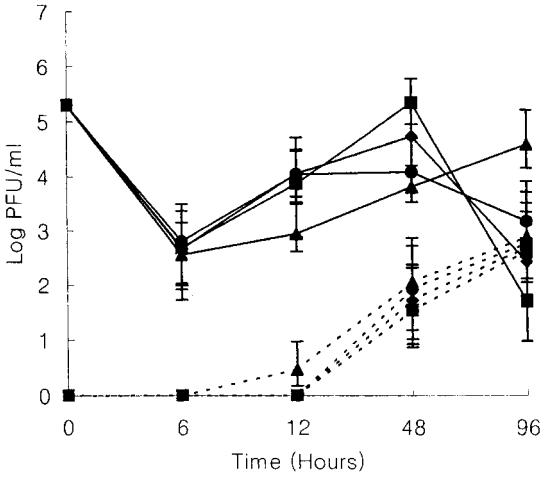


Fig. 1. (A) The alteration of amino acids in gD of various HSV-1 strains. The primary structures of disulfide bonds are schematically described. (B) *In vivo* replication and neuroinvasiveness of each virus strain. The replication rate was determined by mouse eye swab assay and the neuroinvasiveness of each viral strain was analyzed by trigerminal ganglion explanting assay. Eyes of ICR mice were infected with 2×10^5 PFU/ml of each virus. For mouse eye swab assay (—), mice eyes were wiped with cotton swabs at 0, 6, 12, 48, and 96 h post-infection and each cotton swab was suspended in media. The amount of virus was determined by standard plaque assays on Vero cells. For trigerminal ganglion explanting assay (- -), each trigerminal ganglion was removed at 0, 6, 12, 48, and 96 h post-infection. Trigerminal ganglia were homogenized and overlaid on Vero cells. The amount of virus in trigerminal ganglia was determined by plaque assay.

post-infection periods (6, 12, 48, 96 h), mice eyes were wiped with cotton swabs and virus in each cotton swab was suspended in medium. The amounts of suspended viruses were determined by plaque assays. In most of virus strains except KHS 1, virus yields were maximal at 2 days of post-infection. KHS 1 had a slow growth rate in mice eyes, the same as in Vero cells (dashed lines in Fig. 1B). We also carried out the acute latency assay to check the time for the virus to reach trigerminal ganglia from the eyes. Just as with the eye swab experiment, each virus strain was infected into mice eyes and trigerminal ganglia were surgically collected from the mice at various post-infection periods. The trigerminal ganglia were overlaid onto Vero cell monolayer and homogenized as previously described. The amount of reactivated virus was quantitated by plaque assay. We expected that KHS 1 might reach the trigerminal ganglia from the eyes much later than other strains, because KHS 1 exhibited slow growth rate in mice eyes and virus could infect neuronal cells after replicating in mice eyes. Unexpectedly, KHS 1 reached the trigerminal ganglia faster than other strains suggesting that KHS 1 is an enhanced neurovirulent strain (bold lines in Fig. 1B). We do not think the high neuroinvasiveness of KHS 1 strain is simply due to the amino acid change of gD in KHS 1 strain. Especially *in vivo*, neuroinvasiveness and latency establishment of HSV-1 are inevitably related with the host immune system. Currently, we are studying the replication features of KHS strains in neuronal cells and trying to understand the immunological interference of HSV-1.

Acknowledgement

This work was supported by the grant (R01-1999-000-00144-0) from the Korea Science and Engineering Foundation.

References

1. Shimeld C., Hill T.J., Blyth W.A., and Easty D.L., J Gen Virol *71*, 397–404, 1990.
2. Roizman B. and Sears A.E., Annu Rev Microbiol *41*, 543–571, 1987.
3. Kramer M.F., Chen S.H., Knipe D.M., and Coen D.M., J Virol *72*, 1177–1185, 1998.
4. Valyi-Nagy T., Deshmane S.L., Spivack J.G., Steiner J., Ace C.I., Preston C.M., and Fraser N.W., J Gen Virol *72*, 641–649, 1991.

5. Hill J.M., Sedarati F., Javier R.T., Wagner E.K., and Stevens J.G., Virology *174*, 117–125, 1990.
6. Stanberry L.R., Curr Top Microbiol Immunol *179*, 15–30, 1992.
7. Sodora D.L., Cohen G.H., Muggeridge M.I., and Eisenberg R.J., J Virol *65*, 4424–4431, 1991.
8. Dick J.W. and Rosenthal K.S., Arch Virol *140*, 2163–2181, 1995.
9. Bower J.R., Mao H., Durishin C., Rozenbom E., Detwiler M., Rempinski D., Karban T.L., and Rosenthal K.S., J Virol *73*, 3843–3853, 1999.
10. Brunetti C.R., Burke R., Kornfeld S., Gregory W., Masiarz F.R., Dingwell K.S., and Johnson D.C., J Biol Chem *269*, 17067–17074, 1994.
11. Johnson D.C. and Ligas M.W., J Virol *62*, 4605–4612, 1988.
12. Brunetti C.R., Burke R.L., Hoflack B., Ludwig T., Dingwell K.S., and Johnson D.C., J Virol *69*, 3517–3528, 1995.
13. Campadelli-Fiume G., Avitabile E., Fini S., Stirpe D., Arsenakis M., and Roizman B., Virology *166*, 598–602, 1998.
14. Long D., Wilcox W.C., Abrams W.R., Cohen G.H., and Eisenberg R.J., J Virol *66*, 6668–6685, 1992.
15. Whitbeck J.C., Peng C., Lou H., Rux A.H., Wills S.H., Leon P.D., Peng T., Nicola A.V., Montgomery R.I., Warner M.S., Spruce L.A., Moore W.T., Lambris J.D., Spear P.G., Cohen G.H., and Eisenberg R.J., J Virol *71*, 6083–6093, 1997.