Fukushima J. Med. Sci.,
Vol. 62, No. 1, 2016

**[Original Article]**

# Protection from lethal herpes simplex virus type 1 infection by vaccination with a UL41-deficient recombinant strain

Tetsuo Koshizuka, Ken Ishioka, Takahiro Kobayashi,
Kazufumi Ikuta and Tatsuo Suzutani

*Department of Microbiology, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima 960-1295, Japan*

(Received October 23, 2015, accepted February 5, 2016)

**Abstract**

The UL41 gene of herpes simplex virus type 1 (HSV-1) encodes a virion host shut off protein which is involved in immune evasion. The growth and virulence of HSV-1 is markedly reduced by the deletion of UL41. In this report, the UL41-deleted recombinant HSV-1 strain VRΔ41 was evaluated as a prophylactic live attenuated vaccine against lethal HSV-1 infection in a mouse model. Intraperitoneal (i.p.) inoculation with the VRΔ41 strain clearly inhibited lethal wild-type HSV-1 (VR-3 strain) infection after both i.p. and intracerebral (i.c.) inoculations. Vaccination with the VRΔ41 strain was safer than VR-3 vaccination and was able to protect against a wild-type challenge to the same degree as VR-3 vaccination. In contrast, i.p. inoculation with ultraviolet-irradiated VR-3 induced resistance against i.p. infection, but not against i.c. infection. Although replication of the VRΔ41 strain in mice was greatly reduced compared to that of the VR-3 strain, VRΔ41 strain maintained the ability to spread to the central nervous system (CNS) from a peripheral inoculation site. These results indicated that the VRΔ41 strain evoked a potent immune reaction through viral protein expression within CNS without the induction of lethal encephalitis. The entry of antigens into the CNS was essential for the establishment of protective immunity against the lethal HSV encephalitis. We concluded that only a live attenuated vaccine is able to afford a prophylactic effect against CNS infection with HSV. In order to fulfill this requirement, UL41-deleted viruses provide a strong candidate for use as a recombinant live vaccine.

**Key words** : herpes simplex virus, UL41 gene, vaccine

## Introduction

Herpes simplex virus 1 (HSV-1) and 2 (HSV-2) are common pathogens throughout the world. They cause a variety of illnesses depending on the portal of entry and host immune status, although HSV infections are often not associated with clinically apparent disease[1]. Acyclovir (ACV) has been widely used and represents a major advance in the treatment and prophylaxis of these diseases. Even in frequently recurring genital herpes, long-term ACV therapy has been markedly effective in reducing the frequency of recurrences and shortening the duration of the few remaining recurrences[2]. Although ACV is particularly effective in improving the quality of life of these patients, HSV recurrence still occurs and is shed subclinically even during ACV treatment[3]. Moreover, HSV-2 seroprevalence has been increasing, particularly in the US, in spite of safe sex campaigns for the prevention of human immunodeficiency virus infection[4]. Thus, the need for a HSV vaccine to control diseases as well as the spread of infection has been recognized.

The immunity induced by natural infection is not sufficient to prevent recurrences. Therefore, in order to prevent recurrences throughout a life-

Corresponding author : Tatsuo Suzutani E-mail : suzutani@fmu.ac.jp
https://www.jstage.jst.go.jp/browse/fms http://www.fmu.ac.jp/home/lib/F-igaku/

time, it is necessary to induce immune responses that are more robust than those induced by natural infection through the use of a HSV vaccine. A recombinant glycoprotein D protein of HSV-2 (gD2) with alum adjuvant is able to elicit a neutralizing antibody but is not able to prevent the long term recurrence of HSV-2[5,6]. One of the more attractive strategies for meeting the need for a HSV vaccine might be to make a recombinant live attenuated vaccine by the knockout of immune evasion genes. To date, it has been revealed that several HSV genes have the ability to evade host defense systems[7]. An immediate early protein, infected cell protein 47 (ICP47) binds to the transporter associated with antigen processing (TAP) to inhibit antigen presentation on major histocompatibility complex class I (MHC-I)[8,9]. A viral protein kinase, US3, downregulates MHC-I to evade $CD8^+$ T cells[10]. Some viral glycoproteins act as decoys for cellular receptors, with glycoprotein E and glycoprotein C functioning as Fc and C3b receptors, respectively[11-13]. These genes are the targets of recombinant live attenuated vaccines.

The virion host shutoff protein (vhs) encoded by the UL41 gene is another recombination candidate for the development of a live attenuated vaccine. Packaged into HSV particles as a tegument protein, vhs is delivered into the cytoplasm of newly infected cells. Thereafter, vhs non-specifically degrades both cellular and viral mRNAs, resulting in the shutoff of the protein synthesis immediately after HSV infection. Due to this shutoff function, the expression of cytokines or MHC molecules is markedly suppressed[14,15]. In this manner, vhs ensures the evasion of the host defense mechanisms. Therefore, vhs-deficient HSV strains should evoke the host defense machinery, leading to a reduction in virulence through the rapid clearance of the virus from the host animal[15-17]. The deletion of vhs induces the expression of SOCS3, a suppressor of cytokine signaling, in HSV-1 infected cells[18], and cytokine production has been shown to be increased in vhs-deficient HSV-infected monocytes *in vitro*[15]. Although vhs is classified as non-essential for viral growth *in vitro*[7], vhs might be a causative gene for immune evasion. It has been clearly demonstrated that vaccination with a vhs-defective HSV-1 strain effectively suppresses virus replication and clinical symptoms associated with wild-type HSV-1 reinfection in a mouse ocular model[19]. Further, virus shedding in recurrent infections is reduced by vaccination with this strain[20]. However, it has not yet been clarified whether vhs-deficient vaccines induce an effective defense against lethal HSV infection. The relationship between the site of vaccination and preventive effect against HSV diseases is also unclear. Therefore, in this study, we examined whether a vhs-deficient HSV-1 vaccine administered by different routes had a protective effect against lethal infections.

## Materials and methods

### *Cells and viruses*

Human embryo lung (HEL) fibroblasts were prepared in our laboratory and used for experiments at the $10^{th}$ to $18^{th}$ passage. African green monkey (Vero) cells were obtained from Flow Laboratories, Inc. (McLean, VA). These cells were cultured in Eagle's minimum essential medium (MEM) supplemented with 10% newborn calf serum. The wild-type HSV-1, strain VR-3, was supplied by the American Type Culture Collection (Rockville, MD). A thymidine kinase (TK)-deficient strain, $VRTK^-$, and a UL41-deficinet recombinant strain, VRΔ41, were described previously[15,21,22]. Both recombinant strains were isolated from the VR-3 strain. The vhs activity was completely abolished in the VRΔ41 strain by the replacement of green fluorescent protein (GFP) with the major part of the UL41 gene[15].

The repair virus, ULΔ41R, was described previously[15]. These virus strains were grown in HEL cells, titrated in Vero cells and stored in small portions at −80°C.

To prepare an inactivate vaccine, the VR-3 strain was irradiated with ultraviolet radiation at a wavelength of 254 nm using a UV-linker (Funakoshi Co. Ltd., Tokyo, Japan) to give a final exposure of 2 J $cm^{-2}$.

### *Animal Procedures*

The animal experiments were carried out under the oversight of the Animal Care and Use Committee in accordance with guidelines for the Animal Experiments of the institution.

The virulence of HSV-1 strains was analyzed in a mouse model based on the survival rate for the 50% lethal dose ($LD_{50}$). Groups of 10 or more female BALB/c mice were anesthetized with ethyl ether and inoculated in the right cerebral hemisphere with 3 μl of a serial 10-fold dilution of one of the HSV-1 strains, or in the abdominal cavity with 10 μl (for 3-day-old mice) or 200 μl (for 4- and 8-week-old mice) of the virus suspension. The survival rate was evaluated daily for 4 weeks and the $LD_{50}$ was

determined graphically.

The protective effect of the vaccine against lethal HSV-1 infection with the HSV-1 strains was evaluated as follows. Groups of 10 or more 4-week-old mice were injected intracerebral (i.c.) or intraperitoneal (i.p.) with 3 or 200 μl of the virus suspension containing 5, 10, 100 or 1,000 plaque forming units (pfu) of HSV-1 strains VR-3, VRΔ41, VRTK$^-$ or UV-irradiated VR-3 (uvVR-3) as a vaccine. At 30 days post-vaccination, these mice were challenged i.p. or i.c. with $1\times10^6$ pfu (220 $LD_{50}$ for 8-week-old mice) or 120 pfu (20 $LD_{50}$ for 8-week-old mice) of the VR-3 strain, respectively, and the survival rates were calculated.

To analyze the spread and replication of the HSV-1 strains *in vivo*, mice were inoculated i.p. with 1,000 pfu of HSV-1. At one to seven days post-infection, mice were decapitated under ether anesthesia and the peritoneal cavity was washed with 2 ml of MEM. The stomachs and cerebra were removed and homogenized in MEM using a homogenizer. After centrifugation at 3,000 rpm for 10 min at 4°C, the virus in the supernatant was titered on Vero cells. The peritoneal lavage fluids were centrifuged at 1,500 rpm for 5 min at 4°C and the supernatant containing the cell-free virus and the pelletized peritoneal cells were separated. The HSV-infected peritoneal cells were counted by infectious center assay as follows. The cells were washed three times with MEM, and then suspended and serially diluted with MEM. The cell suspensions were inoculated on Vero cell monolayers in 24-well tissue culture plates and incubated overnight in a $CO_2$ incubator. The culture was refed with MEM supplemented with 2% newborn calf serum and 0.5% methylcellulose 4,000 (Nacalai Tesque, Inc., Kyoto, Japan) and incubated for 4 days.

## Results

### *Virulence of HSV-1 strains in mice*

In order to carry out the vaccine study on HSV-1 infection in a mouse model, the virulence of the HSV-1 strains used in this study was evaluated by i.c. and i.p. inoculation into 3-day-, 4-week- and 8-week-old BALB/c mice (Table 1). Mice developed natural resistance to all the HSV-1 strains with age, but the difference was much more marked in the i.p. than in the i.c. inoculated mice. The mutant HSV-1 strains (VRΔ41 and VRTK$^-$) were less virulent than the wild-type strains (VR-3 and VRΔ41R). In addition, VRTK$^-$ was more attenuated than VRΔ41. VRTK$^-$ infection did not induce any symptoms, either after i.c. or i.p. injection, in 4- and 8-week-old mice. On the other hand, a high-dose i.c. injection of the VRΔ41 strain induced a lethal infection in 4-week-old mice, whereas an i.p. injection induced no symptoms.

### *Effect of vaccination with HSV-1 strains*

The protective effects of i.p. or i.c. vaccinations with VR-3, VRΔ41, VRTK$^-$ and uvVR-3 against lethal VR-3 infections are shown in Fig. 1. The VR-3 strain titer for i.p. and i.c. challenges was approximately 220 and 20 $LD_{50}$ for 8-week-old mice, respectively. In all cases, the vaccines took effect in a dose-dependent manner, with the VRΔ41 vaccine having the greatest effect, followed by the VR-3, VRTK$^-$ and uvVR-3 vaccines. Due to the virulence of the VR-3 strain, approx. 40% of 4-week-old mice died after vaccination with 10 pfu of VR-3 via the i.p. route. Mice vaccinated with VRΔ41 via the i.p. route showed slightly better defense than did VR-3-vaccinated mice against both i.p. and i.c. challenges (Fig. 1B and C). VRΔ41-vaccinated mice, in particularly, did not show any clinical symptoms due to vaccination. Intraperitoneal inoculation with the uvVR-3 vaccine induced an adequate protective

Table 1. The $LD_{50}$ values of HSV-1 strains in BALB/c mice[a]

| Virus | Intracerebral (i.c.) inoculation | | | Intraperitoneal (i.p.) inoculation | | |
|---|---|---|---|---|---|---|
| | 3-day-old | 4-week-old | 8-week-old | 3-day-old | 4-week-old | 8-week-old |
| VR-3 | 0.21[b] | 2.0[b] | 6.0 | 0.37 | 27 | $4.5\times10^3$ |
| VRΔ41 | 1.0[b] | $3.6\times10^3$ [b] | N.T.[c] | 10 | $> 10^6$ | $> 10^6$ |
| VRΔ41R | 0.23 | 1.1[b] | N.T. | 0.63 | $1.0\times10^2$ | N.T. |
| VRTK$^-$ | 79[b] | $> 10^5$ [b] | $> 10^5$ | 25 | $> 10^6$ | $> 10^6$ |

[a]The 50% lethal dose ($LD_{50}$) is expressed as pfu/mouse.
[b]These data are cited from a previous report [Suzutani *et al*., 1995 ; 2000].
[c]Not tested.

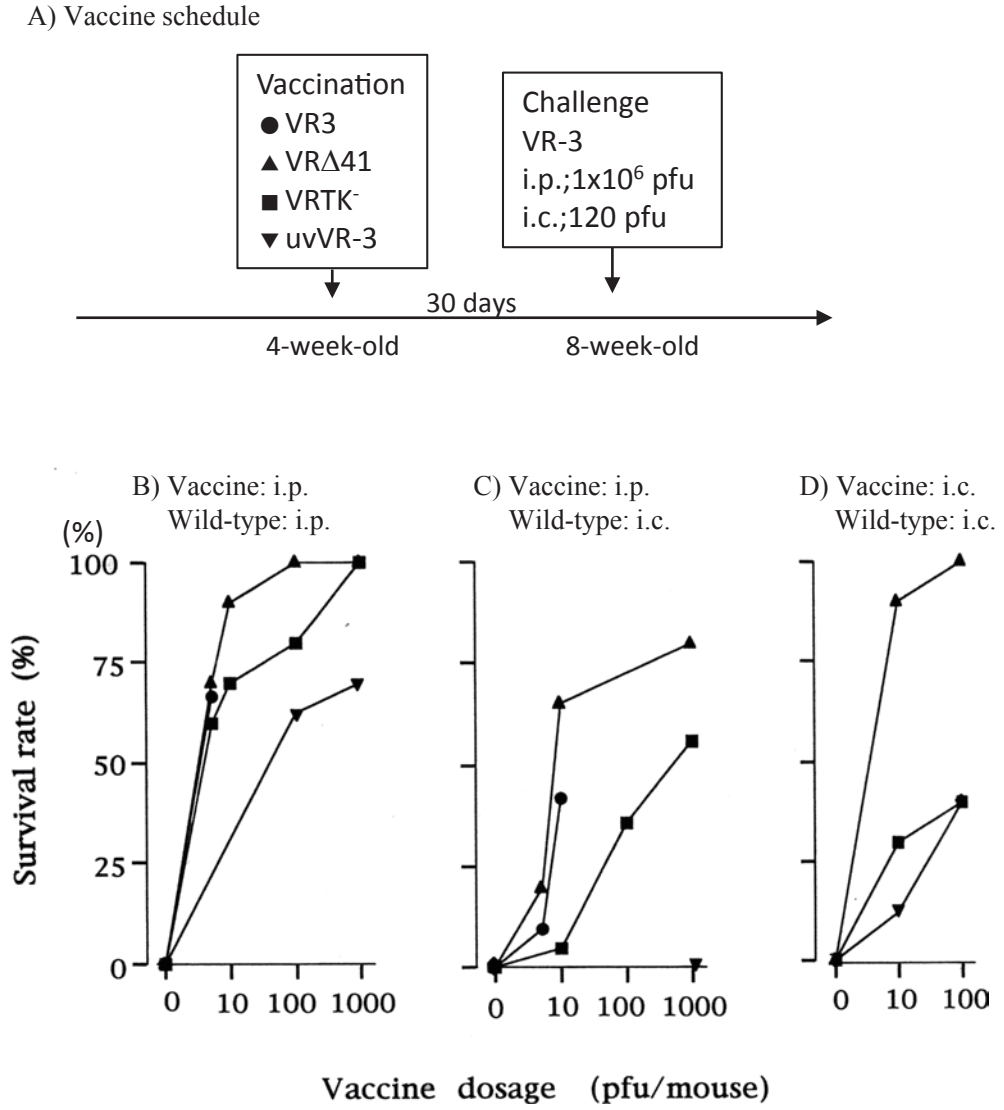


Fig. 1. Preventive effect of HSV vaccines against lethal infections. The vaccine and challenge schedule was undertaken as shown in schema (A). The HSV-1 strains VR-3 (●), VRΔ41 (▲), VRTK$^-$ (■) or UV-inactivated VR-3 (uvVR-3 ; ▼) were inoculated to 4-week-old BALB/c mice as a vaccine via the intraperitoneal (i.p.) (B, C) or intracerebral (i.c.) (D) route. At 30 days post-vaccination, a VR-3 challenge of $10^6$ or 120 pfu was introduced via i.p. (B) or i.c. (C, D) inoculation, respectively.

effect against i.p. infection with the VR-3 strain but no effect was observed against i.c. infection. However, i.c. inoculation with the uvVR-3 virus produced a weak protective effect against lethal i.c. infection. The effect of i.c. inoculation with the VR-3 vaccine was not evaluated because of the high virulence of VR-3 strain ; the $LD_{50}$ value was 2.0 pfu for 8-week-old mice.

*The replication and spread of HSV-1 strains in mice*

In the case of live vaccines, the ability of the vaccine virus to replicate and spread *in vivo* affects the viral load in organs and thus alters the site of the immune response. Therefore, the organ tropism of the vaccine virus is expected to influence the quantity and quality of the immune response. To understand the potent protective effect induced by the VRΔ41 vaccine, particularly against lethal encephalitis, we examined whether VRΔ41 is able to reach the brain after i.p. inoculation. The titer of HSV-1 strains in various organs after i.p. inoculation with $10^3$ pfu was evaluated. As shown in Fig. 2A, the VR-3 strain replicated in the peritoneal lavage and cells in a bimodal manner. The VR-3 strain was detected in the brain at 4 days post-inoculation, by which time it had increased to $4\times10^3$ pfu. The mice died from encephalitis after the 7th day. Compared to the VR-3 infection, the replication of VRΔ41 was markedly restricted (Fig. 2B). Only a low VRΔ41 strain titer was detected in an abdominal

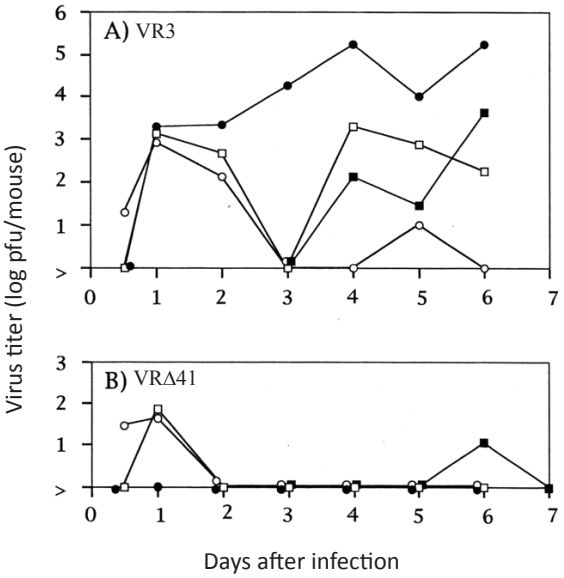


Fig. 2. Replication and spread of HSV-1 in mice. Mice were injected with $10^3$ pfu of the VR-3 (A) and VRΔ41 (B) strains intraperitoneally and the virus yield in the stomach (●), brain (■), and peritoneal lavation as cell-free virus (□) and infected cells (○) was determined. The results are expressed as the means of triplicate experiments.

lavation on the first day post-inoculation. Thereafter, the VRΔ41 load was suppressed to undetectable levels in all of the organs tested. At day 6, the virus was detected in the brain with a low titer, indicating that the VRΔ41 strain was less able to spread and replicate in the CNS than was VR-3. Therefore, VRΔ41 was not able to induce lethal encephalitis. The VRTK$^-$ strain was not detected in any samples (data not shown).

## Discussion

The necessity for and availability of a vaccine against HSV infection has been debated since the 1920s[23]. The very nature of HSV infections has made the debate difficult to resolve ; HSV, and particularly HSV-1, infections are common in humans and in most cases induce only self-limiting diseases or asymptomatic infections. In the last three decades, the need for a HSV vaccine has become accepted because of the increasing seroprevalence of HSV-2, particularly in the US[4], with increases in the number of cases of recurrent genital herpes and lethal congenital herpes. Moreover, cases of acute retinal necrosis syndrome (ARN) caused by HSV-2 have been reported in anti-HSV-2 antibody-positive and anti-HSV-1 antibody-negative patients in Japan[24]. It should be mentioned that preexisting anti-HSV-1 antibodies can prevent the severe diseases associated with subsequent HSV-2 infection[25] ; however, the seroprevalence of HSV-1 has been decreasing in some advanced countries[26,27]. These epidemiological and etiological changes have resulted in a growing need for a HSV vaccine.

In previous studies, the vhs-deficient live vaccine was shown to be effective as both a therapeutic and prophylactic vaccine, although it could not prevent reinfection or eliminate latent infection[19,20]. To clarify its effectiveness in greater detail, we examined the effect of the prophylactic vhs-deficient vaccine by quantitative analysis. The VRΔ41 strain showed a potent effect against lethal i.p. and i.c. infections equal to wild-type infections and induced no clinical symptoms. Although VRΔ41 was able to replicate *in vitro*[15], its growth *in vivo* was severely impaired, as shown in Fig. 2. Previously, we reported that HSV evades the non-specific host defense mechanism via vhs function through the inhibition of interleukin production and the antiviral effect of interferon[15]. The results of this study indicated that vhs function also plays an important role in the evasion of the immune system, with the loss of vhs-function inducing potent immunity. Part of this evasion of the immune system is thought to be due to the suppressed expression of MHC[14] and some adhesion molecules on the plasma membrane of infected cells (unpublished result). In addition, the induction of SOCS3 is impaired by the deletion of vhs[18]. The expression of cytokines in the CNS leads to the migration of immune cells into the CNS[28]. TNF-α and IL-1β production are important in the prevention of HSV encephalitis in mice[29]. Although the precise cytokine profiles were not elucidated, alterations in cytokine signaling in the CNS might contribute to the attenuation of the vhs-deficient virus.

A TK-deficient virus is another candidate for the live attenuated vaccine. As TK generates the substrates for viral DNA replication in infected cells, TK function is very important to viral growth both *in vivo* and *in vitro*[7]. As shown in Fig. 1, vaccination with VRTK$^-$ was effective in preventing wild-type infection although VRTK$^-$ was less effective than VRΔ41. Together with the results regarding viral spread, these findings suggest that the replication and spread of VRTK$^-$ is severely restricted *in vivo*. Therefore, the limited spread of VRTK$^-$ might be less effective in eliciting a local immune response, particularly within the CNS.

In recurrent infections, HSV can reactivate, replicate and spread to the mucous membrane or skin despite the existence of anti-HSV antibodies[25].

It appears that the antibodies might be induced by inoculation with uvVR-3 via the i.p. route but be unable to protect against infection via the i.c. route (Fig. 1C). One important consideration regarding recurrent infection is that as HSV-1 replicates even in the presence of antibodies, the protection afforded by antibodies might not be sufficient to prevent HSV infection. The development of an anti-HSV vaccine must, therefore, take this into account.

Several vaccines have been proposed on the basis of recombinant DNA technology, and some of them, mainly recombinant subunit vaccines, have been evaluated in clinical trials[1,6,30-33]. These vaccines are intended for the prevention of genital herpes, but one important drawback is that the component vaccine is not able to prevent other HSV diseases, particularly HSV encephalitis, meningitis and ARN, as these vaccines only induce antibodies. In this study, the uvVR-3 vaccination injected through the i.c. route, but not the i.p. route, induced a weak preventive effect against lethal encephalitis by VR-3 challenge via i.c. inoculation (Fig. 1C and D). The exposure of antigens in the CNS, probably to microglia cells, appears to be essential to the prevention of infectious disease in the CNS[34]. Based on the presented results, in order to prevent CNS infection, it is necessary that the antigen is transported into CNS. As shown in Fig. 1, vaccination with VRΔ41 afforded defense against lethal infection more efficiently than did vaccination with VRTK$^-$, which was not detected in the CNS. As it is unrealistic to administer a vaccine into the brain for prophylactic purposes, it is better to use a live attenuated vaccine which is able to reach CNS to prevent lethal encephalitis but not induce any symptoms. Therefore, only a live attenuated vaccine is suitable for prophylaxis against HSV encephalitis. Many problems with regard to live HSV vaccines remain, including their safety and the possibility of using a recombinant virus. However, work toward the development of a live attenuated HSV vaccine should be continued and UL41-deleted viruses represent attractive candidates for the development of such recombinant live vaccines.

## References


1. Stanberry LR, Cunningham AL, Mindel A, *et al*. Prospects for control of herpes simplex virus disease through immunization. Clin Infect Dis, **30** : 549-566, 2000.
2. Mindel A, Faherty A, Carney O, Patou G, Freris M, Williams P. Dosage and safety of long-term suppressive acyclovir therapy for recurrent genital herpes. Lancet, **1** : 926-928, 1988.
3. Wald A, Zeh J, Barnum G, Davis LG, Corey L. Suppression of subclinical shedding of herpes simplex virus type 2 with acyclovir. Ann Intern Med, **124** : 8-15, 1996.
4. Fleming DT, McQuillan GM, Johnson RE, *et al*. Herpes simplex virus type 2 in the United States, 1976 to 1994. N Engl J Med, **337** : 1105-1111, 1997.
5. Straus SE, Corey L, Burke RL, *et al*. Placebo-controlled trial of vaccination with recombinant glycoprotein D of herpes simplex virus type 2 for immunotherapy of genital herpes. Lancet, **343** : 1460-1463, 1994.
6. Straus SE, Wald A, Kost RG, *et al*. Immunotherapy of recurrent genital herpes with recombinant herpes simplex virus type 2 glycoproteins D and B : results of a placebo-controlled vaccine trial. J Infect Dis, **176** : 1129-1134, 1997.
7. Knipe DM, Howley PM. Fields virology, 6th ed. Wolters Kluwer/Lippincott Williams & Wilkins Health, Philadelphia, PA., 2013.
8. Fruh K, Ahn K, Djaballah H, *et al*. A viral inhibitor of peptide transporters for antigen presentation. Nature, **375** : 415-418, 1995.
9. York IA, Roop C, Andrews DW, *et al*. A cytosolic herpes simplex virus protein inhibits antigen presentation to CD8+ T lymphocytes. Cell, **77** : 525-535, 1994.
10. Imai T, Koyanagi N, Ogawa R, *et al*. Us3 kinase encoded by herpes simplex virus 1 mediates down-regulation of cell surface major histocompatibility complex class I and evasion of CD8+ T cells. PLoS One **8** : e72050, 2013.
11. Frank I, Friedman HM. A novel function of the herpes simplex virus type 1 Fc receptor : participation in bipolar bridging of antiviral immunoglobulin G. J Virol, **63** : 4479-4488, 1989.
12. Friedman HM, Cohen GH, Eisenberg RJ, Seidel CA, Cines DB. Glycoprotein C of herpes simplex virus 1 acts as a receptor for the C3b complement component on infected cells. Nature, **309** : 633-635, 1984.
13. Para MF, Baucke RB, Spear PG. Glycoprotein gE of herpes simplex virus type 1 : effects of anti-gE on virion infectivity and on virus-induced fc-binding receptors. J Virol, **41** : 129-136, 1982.
14. Hill AB, Barnett BC, McMichael AJ, McGeoch DJ. HLA class I molecules are not transported to the cell surface in cells infected with herpes simplex virus types 1 and 2. J Immunol, **152** : 2736-2741, 1994.
15. Suzutani T, Nagamine M, Shibaki T, *et al*. The role of the UL41 gene of herpes simplex virus type 1 in evasion of non-specific host defence mecha-

nisms during primary infection. J Gen Virol, **81** : 1763–1771, 2000.
16. Becker Y, Tavor E, Asher Y, Berkowitz C, Moyal M. Effect of herpes simplex virus type-1 UL41 gene on the stability of mRNA from the cellular genes : beta-actin, fibronectin, glucose transporter-1, and docking protein, and on virus intraperitoneal pathogenicity to newborn mice. Virus Genes, **7** : 133–143, 1993.
17. Strelow LI, Leib DA. Role of the virion host shutoff (vhs) of herpes simplex virus type 1 in latency and pathogenesis. J Virol, **69** : 6779–6786, 1995.
18. Yokota S, Yokosawa N, Okabayashi T, *et al*. Induction of suppressor of cytokine signaling-3 by herpes simplex virus type 1 contributes to inhibition of the interferon signaling pathway. J Virol, **78** : 6282–6286, 2004.
19. Walker J, Leib DA. Protection from primary infection and establishment of latency by vaccination with a herpes simplex virus type 1 recombinant deficient in the virion host shutoff (vhs) function. Vaccine, **16** : 1–5, 1998.
20. Walker J, Laycock KA, Pepose JS, Leib DA. Post-exposure vaccination with a virion host shutoff defective mutant reduces UV-B radiation-induced ocular herpes simplex virus shedding in mice. Vaccine, **16** : 6–8, 1998.
21. Suzutani T, Koyano S, Takada M, Yoshida I, Azuma M. Analysis of the relationship between cellular thymidine kinase activity and virulence of thymidine kinase-negative herpes simplex virus types 1 and 2. Microbiol Immunol, **39** : 787–794, 1995.
22. Suzutani T, Machida H, Sakuma T. Efficacies of antiherpesvirus nucleosides against two strains of herpes simplex virus type 1 in Vero and human embryo lung fibroblast cells. Antimicrob Agents Chemother, **32** : 1046–1052, 1988.
23. Bernstein DI, Stanberry LR. Herpes simplex virus vaccines. Vaccine, **17** : 1681–1689, 1999.
24. Itoh N, Matsumura N, Ogi A, *et al*. High prevalence of herpes simplex virus type 2 in acute retinal necrosis syndrome associated with herpes simplex virus in Japan. Am J Ophthalmol, **129** : 404–405, 2000.
25. Hashido M, Lee FK, Nahmias AJ, Kawana T. Prevalence of herpes simplex virus type 1- and 2-specific antibodies among the acute, recurrent, and provoked types of female genital herpes. Microbiol Immunol, **41** : 823–827, 1997.
26. Hashido M, Lee FK, Nahmias AJ, *et al*. An epidemiologic study of herpes simplex virus type 1 and 2 infection in Japan based on type-specific serological assays. Epidemiol Infect, **120** : 179–186, 1998.
27. Nahmias AJ, Lee FK, Beckman-Nahmias S. Sero-epidemiological and -sociological patterns of herpes simplex virus infection in the world. Scand J Infect Dis Suppl, **69** : 19–36, 1990.
28. Sonar S, Lal G. Role of Tumor Necrosis Factor Superfamily in Neuroinflammation and Autoimmunity. Front Immunol, **6** : 364, 2015.
29. Sergerie Y, Rivest S, Boivin G. Tumor necrosis factor-alpha and interleukin-1 beta play a critical role in the resistance against lethal herpes simplex virus encephalitis. J Infect Dis, **196** : 853–860, 2007.
30. Belshe RB, Leone PA, Bernstein DI, *et al*. Herpevac Trial for W. Efficacy results of a trial of a herpes simplex vaccine. N Engl J Med, **366** : 34–43, 2012.
31. Krause PR, Straus SE. Herpesvirus vaccines. Development, controversies, and applications. Infect Dis Clin North Am, **13** : 61–81, vi., 1999.
32. Langenberg AG, Burke RL, Adair SF, *et al*. A recombinant glycoprotein vaccine for herpes simplex virus type 2 : safety and immunogenicity [corrected]. Ann Intern Med, **122** : 889–898, 1995.
33. Coleman JL, Shukla D. Recent advances in vaccine development for herpes simplex virus types I and II. Hum Vaccin Immunother, **9** : 729–735, 2013.
34. Yang I, Han SJ, Kaur G, Crane C, Parsa AT. The role of microglia in central nervous system immunity and glioma immunology. J Clin Neurosci, 17 : 6–10, 2010.