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Development of Zosteriform Models in Skin and Vagina of Mice using *Herpes simplex* Virus Strain McKrae

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=Abstract=

Zosteriform lesions, occurring after left flank and intravaginal inoculations of Balb/c mice with the *Herpes simplex* virus type 1 strain McKrae, developed in clinically normal skin via nerve endings. The developments of zosteriforms were standardized in 5 phases with the following references; formation of small vesicles (phase 1); occurrence of erosion and ulceration of local lesions (phase 2); occurrence of ulcerations (phase 3); occurrence of severe ulcerations (phase 4); and death (phase 5). These results provide two valuable zosteriform models to further investigate and analyze the pathological symptoms in susceptible animals infected with HSV-1 or HSV-2 and DNA vaccines.

Key Words: *Herpes simplex* virus, Zosteriform, McKrae

Herpes simplex viruses (HSV) are divided into two distinct groups designated HSV-1 and HSV-2. Both groups are morphologically identical and have approximately a 50% DNA similarity, however, there are a number of biological, biochemical, genomic and clinical differences between them [3, 9]. HSV-1 causes oral or skin infections, whereas most genital infections are caused by HSV-2. Herpesviruses travel from the inoculation site into the sensory nervous system and then return to the skin of the whole neurodermatome [15]. Recently, a zosteriform that uses several different strains of HSV-1 was produced in the skin, and has been reproduced in mice [3, 10, 15, 18]. The growth of a herpes virus at a peripheral site may have a zosteriform component superimposed on an initial local multiplication. After flank infection, this zosteriform component is clearly distinguished as being remote from the inoculation site. HSV infection in animals has been previously and experimentally investigated [12]. In particular, the mouse ear model [6] has been used to probe in detail the effects of the course of a primary infection [8, 11].

HSV-2 is sexually transmitted and undergoes replicatory infectious cycles in the epithelium of the genital tract, and genital infections caused by HSV-2 continue to be a serious health problem of epidemic proportion [2, 14]. In the guinea pig model of a HSV-2 infection, the pathogenesis of primary genital infections is similar to that seen in human diseases [17]. Gallichan and Rosenthal [5] analyzed genital pathology in female mice using HSV-2 strain 333. The development of a lesion by HSV re-

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quires the infection and destruction of epidermal cells. Skin and genital organs are the portal of entry for HSV and the development of zosteriform skin lesions indicate that the virus has spread from the ganglia to the skin. Therefore, using animals inoculated with HSV in the flank and in the genital tract provides potential models through which the development of zosteriform lesions can be investigated.

In the present study the virological pathology in mice inoculated with HSV-1 strain McKrae in the flank and in the female genital tract was observed and the importance of zosteriform systems in studying the progress of virus-infections that are active against primary and perhaps recrudescent HSV infection was also demonstrated.

MATERIALS AND METHODS

Virus and cells

Herpes simplex virus type 1 strain McKrae was obtained from B. T. Rouse, University of Tennessee, Knoxville, USA, and then grown in Vero cell (ATCC CCL81) monolayers in Eagle's minimum essential medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal calf serum (Gibco) and 1% penicillin-streptomycin.

Mice

Six to eight week old female Balb/c mice (Daehan Laboratory, Animal Research Center, Chungnam, Korea) were used throughout. They were inoculated at 6 to 8 weeks of age.

Plaque assay

A plaque assay was performed using the procedure described by Kang et al. [7].

Inoculation of virus on skin of mice

Two groups of 10 female Balb/c mice were used and inoculated with either the HSV-1 strain McKrae or a phosphate saline solution (PBS) as a negative control. The left flanks of 10 Balb/c mice were inoculated with the strain McKrae using a modified version of the procedure described by Simmons *et al.* [15]. Before the inoculation of the virus, the hair on the left flanks of twenty mice was depilated using a hair clipper and a chemical depilator kit (Il-dong Pharmaceutical Co., Kyunggido, Korea). After depilation, the mice were anesthetized with 100 μl of ketamine per 10 g of body weight, and a total of 20 scarifications were made in an approximately 4 mm^2 area of the mice. Two microliters of a 2×10^7 plaque forming unit (pfu) of the McKrae strain in PBS was inoculated into the scarifications, which were then gently massaged. The inoculated mice were monitored for a period of 10 days. Observation for skin lesions and death were made twice a day, and the degrees of the symptoms were scored. A lethal dose of the McKrae strain was used for each mouse.

Inoculation of virus in female genital tract

Two groups of 10 female Balb/c mice were used and inoculated with either the HSV-1 strain McKrae or a phosphate saline solution as a control. The mice first anesthetized and then inoculated with the McKrae strain using a modified version of the procedure described by Parr *et al.* [13]. In order to synchronize the estrus cycle at the progesterone-dominated stage, the inoculated and non-inoculated groups of mice were both injected subcutaneously with 2 mg of progesterone (Samil Pharmaceutical Co., Seoul) in 50 μl of distilled H_2O per mouse. 5 days after the administration of the progesterone, the mice of one group were inoculated with the McKrae strain in the vagina and external genital skin. One hour prior to inoculation, the vaginal closure membrane was ruptured with a saline-moistened cotton swab. The vagina and external skin were then swabbed with 0.1 N NaOH and the McKrae strain, diluted in PBS to 10^6 pfu/ml, was introduced using a cotton swab. The swab was inserted into the vagina, twisted back and forth six times, then removed

and wiped over the external genitalia. To ensure inoculation, the virus application was repeated 1 h later. The infected mice were examined daily for vaginal inflammation, neurological illness, and death, and then were scored. A lethal dose of the McKrae strain was used for each mouse. Vaginal washings were collected on days 1, 2, 3, and 4 post the intravaginal inoculation by pipetting 100 μl of PBS into the vaginal cavity. The collected samples were stored at -70℃ until used. The samples were added to 1 ml of a cell culture medium and subsequently titered on Vero cell monolayers using a plaque assay [7, 16].

RESULTS AND DISCUSSION

Development of zosteriform in the left flank of mice

A total of ten female mice were inoculated in the left flank with the HSV-1 strain McKrae and thereafter the severity of the primary disease was observed. The progress of the resulting zosteriform lesions (day 1 to 10) are illustrated in Figure 1. The zosteriform band extended from the posterior to the anterior midline and included the site (arrowed) at which the virus was initially administered. The control mice received a similar operation with a phosphate buffer solution, however none developed zosteriform rashes, and all survived. After the left flank was inoculated, the mice were observed daily for the development of a zosteriform eruption. All ten mice developed visible rashes on day 4 post-inoculation (p.i.); 8 progressed to severe lesions by day 6, and all 10 mice became paralysed in their hind limbs and died either on or before day 10. There have been several other similar reports. Sydiskis and Schultz [18] reported local skin lesions in mice using HSV-1, Constantine et al. [1] found a zoster-like HSV strain 911 in hairless mice, Dillard et al. [3] also reported a zosteriform in mice using HSV-1, Nagafuchi et al.

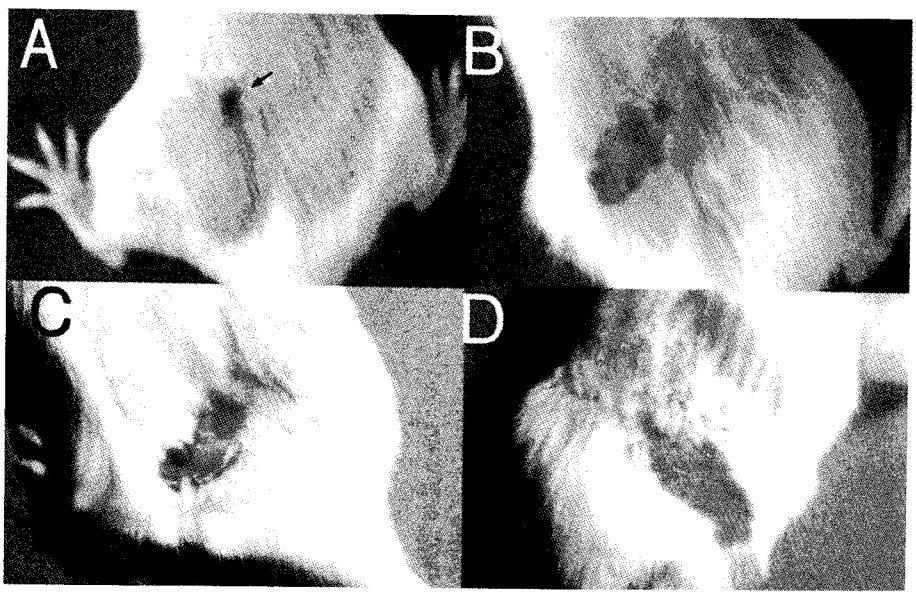

Figure 1. Development of zosteriform lesions produced by the intradermal inoculation of the HSV-1 strain McKrae in left flank into Balb/c mice. The development and severity of the skin lesions were scored on a daily basis and given numerical designations; Phase 0, no apparent infection; Phase 1 **(A):** perceptible small vesicles, erythema, or crust in local region; Phase 2 **(B):** erosion, ulceration, crust with ulceration in local region; Phase 3 **(C):** zosteriform ulceration with erosin or ulceration with crust; Phase 4 **(D):** severe zosteriform ulceration; Phase 5, death (data not shown).

(1979) scored 10 steps in nude mice using the HSV-1 strain Hayshida, and then Simmons and Nashi [15] scored 5 phases in the spread of zosteriform relative to the progress of lesions in Balb/c mice using the HSV-1 strain SC16. The current findings were standardized in 5 phases with the following references; phase 1, formation of small vesicles; phase 2, occurrence of erosion and ulceration of local lesions; phase 3, occurrence of ulcerations; phase 4, occurrence of severe ulcerations; phase 5, death. The score estimation was evaluated using the total score of all mice in each group per day divided by the number of mice in each group.

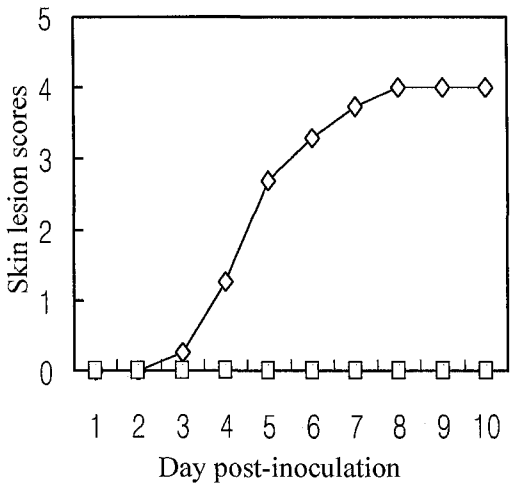

Figure 2. Scores of zosteriform lesions of mice inoculated intrasubcutaneously with the HSV-1 strain McKrae. Symbols: (◇), HSV-1 strain McKrae inoculation at a dose of 2×10^7 pfu; (□), control PBS inoculation.

Accordingly, Figure 2 was established from this calculation. These results demonstrate that the pathological symptoms were similar although the HSV strains and susceptible animals were different.

Samples of skin from the inoculation site and lower flank were separately plaque-assayed for the presence of the infectious virus (Table 1). Table 1 shows that the virus recovered from the inoculation site reached a peak titer on day 2 p.i. At this stage no zosteriform spread had occurred. However, the virus was detectable in the lower flank by day 3 p.i. in all mice. All surviving mice developed an interrupted band-like lesion on day 4 p.i., corresponding to the development of a peak titer in the skin of the lower flank. The lesions were confluent by day 5. These findings are similar to observations found in Balb/c mice when using the HSV-1 strain SC16 [15]. The inoculation site was identified by a scale produced during the scarification of the skin. This result indicates that the virus was being replicated more rapidly in the skin of the mice.

Development of zosteriform in the female genital tract of mice

A total of ten mice were infected in the vaginal tract and external genital skin. The genital zosteriform lesions (day 0 to 9) formed after the intravaginal inoculation of the McKrae strain (10^6 pfu) in Balb/c mice are illustrated in Figure 3. The control mice received a similar operation with a phosphate buffer solution,

Table 1. Infectious virus titers recovered from inoculation sites on the left flank and in the genital tract of mice

Infection sites	Log_{10} viral titer at the day post-inoculation				
	0	1	2	3	4
Left flank					
Inoculated point	7	4.1±0.1	5.2±0.02	4.5±0.08	4.3±0.05
Lower Flank	0	1.0±0.1	1.1±0.03	4.7±0.08	4.5±0.05
Genital tract	6	5.2±0.1	5.4±0.03	4.5±0.09	4.3±0.06

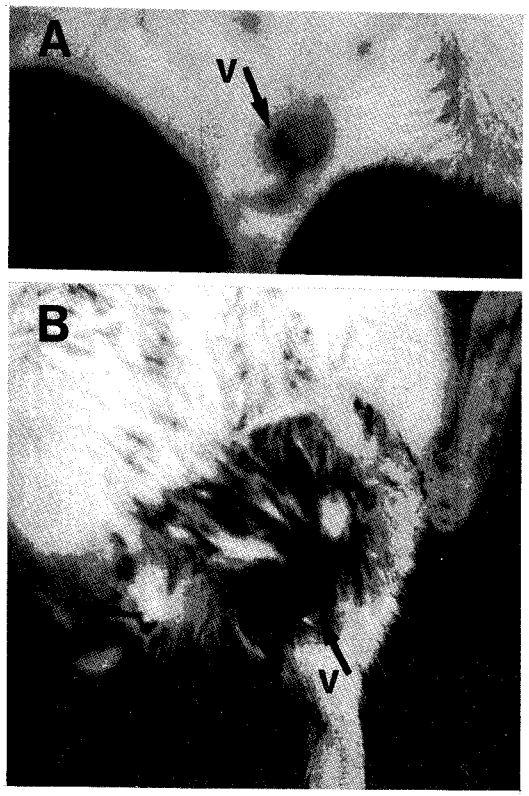

Figure 3. Zosterification steps after the intravaginal inoculation of Balb/c mice with the HSV-1 strain McKrae. Pathology was scored based on five criteria: **(A)**: Phase 0, no apparent infection. Phase 1, slight redness of external vagina; Phase 2, redness and swelling of external vagina and slight hair loss from genital tissue; Phase 3, severe redness and swelling of external vaginal (data not shown). **(B)**: Phase 4, severe genital ulceration extending to surrounding tissue; Phase 5, death (data not shown).

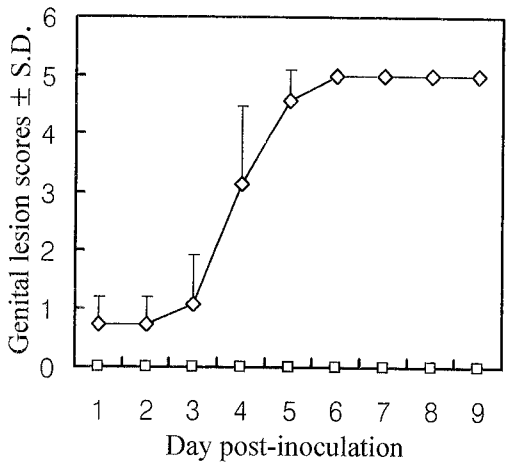

Figure 4. Genital tract lesion scores of female mice during HSV-1 strain McKrae inoculation. (◇), HSV-1 strain McKrae inoculation at a dose of 10^6 pfu; (□), control PBS inoculation.

however none developed zosteriform rashes, and all survived.

The severity of the primary disease progressed in the following order after the inoculation of the McKrae strain into the intravaginal tract: slight redness of the external vagina; redness and swelling of the external vagina, and slight hair loss from the genital and surrounding tissue; severe redness and swelling, hair loss from the external vaginal and surrounding tissue; severe genital ulceration extending to the surrounding tissue; death. These observations are exactly analogous to the findings of the lesion scoring system that used female C57BI/6 mice inoculated with the HSV-2 strain 333 [5]. All of 10 mice died on either day 5 or 6 p.i. (Figure 4). The pathology of the female Balb/c mice was scored based on five phases relative to the development of the zosteriform spread (Figures 3 and 4), and according to the following five criteria as described by Gallichan and Rosenthal [5] shown in Figure 3. The lethal dose of the McKrae strain was used for each mouse and PBS-inoculation was used as a negative control. These results demonstrate that the pathological symptoms were similar to previous findings, although the HSV strains and susceptible animals were different. Following, the intravaginal HSV-1 McKrae inoculation of the female Balb/c mice, vaginal swab samples, collected on days 1, 2, 3, and 4 post-inoculation, were plaque-assayed. The viral titers were significantly reduced (Table 1). This result indicates that the virus was being cleared more rapidly from the genital tract of the mice.

Finally, these results provide two valuable zosteriform models to further investigate and analyze the pathological symptoms in susceptible animals infected with HSV and DNA vaccines.

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