Equid Herpesvirus 1 Infection in Mice
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Abstract. When the HH1 strain of equid herpesvirus 1 was intranasally inoculated to mice, the virus propagated in mouse lungs and the animals showed clinical signs such as ruffled fur, hunched posture, depression and body weight loss. Mice recovered from these signs by day 12 and cleared the virus from their lungs and produced antibody by 7th day after infection. These convalescent mice did not allow growth of the rechallenged virus. Athymic nude mice, however, failed to clear the virus from their lungs. Most of field isolates from aborted fetuses were propagated in murine lungs but attenuated strains originated from the HH1 were not.—Key words: equid herpesvirus 1, mouse model.

Equid herpesvirus 1 (EHV1) infection, which causes respiratory disease and abortion in horses, still has been one of the main problems in horse husbandry, because effective vaccine has not been available yet [1]. Immunological studies of EHV1 infection has been hampered by several limitations for experimental use of horses such as cost, space and labor, and by lack of adequate laboratory animal models. Recently, Awan et al. [2, 3] described propagation of EHV1 in lungs of adult BALB/c mice after intranasal inoculation, inducing pneumonia and abortion. This mouse-EHV1 system not only reproduced typical symptoms of the infection in horses, but also offer us availability of various experimental procedures such as adoptive immunity and use of immunodeficient mutants, since genetic and immunological characteristics of mice have been well studied.

The purpose of the present studies was to confirm multiplication of EHV1 in adult mouse lungs reported by Awan et al. [2], and to evaluate usefulness of the mouse model in studies of the pathogenicity of the virus and immunology of EHV1 infection.

The EHV1 strains used were the laboratory strain HH1 [5], which has been passaged ten times in horse kidney cell culture and three generations in equine dermal (E. Derm) cells, 7 field isolates (F1, F2, F3, F4, F6, F7, and F8 strains) from aborted fetuses, which were identified by restriction enzyme digestion patterns of viral DNA [6], and 3 clones (1E, 3F, and 3H) from an avirulent strain BK343 which was established by 343 serial passages of the HH1 virus in bovine kidney cell culture at 30°C [4].

Mice used were 4 to 5 week-old, female BALB/cA strain and its athymic mutants which were purchased from a commercial breeder (CLEA Japan Inc., Tokyo). These were infected intranasally with about 104 TCID50/25 µl of virus under ether anesthesia and were kept in an animal room with temperature of 20 to 25°C, supplying tap water and commercial pellet ad libitum. Mice were sacrificed 1 to 12 days after infection by cutting cervical vessels under ether anesthesia and the blood was collected individually in Petri dish for obtaining sera. The sera were stored at -20°C until used. Lung, heart, liver, spleen, pancreas, and kidney were aseptically removed from mice and stored at -80°C for virus titration.

Virus titration was performed on E. Derm cell monolayer by inoculation with serial ten-fold dilution of various organ suspensions (10% W/V) and viral titers (log TCID50/organ) were calculated by Behrens-Kärber's method. Antibody was assayed by conventional ELISA using purified virus as antigen, horseradish peroxidase-conjugated anti-mouse IgG rabbit IgG (Capel) as secondary antibody and ABTS (2,2'-azino-di-[3-ethylbenzthiazoline-6-sulfonic acid]) solution containing H2O2 as substrate-indicator solution. Reaction was read by ELISA reader at 405 nm and titers were expressed as reciprocal of the highest dilution over the cut off value based on reaction of negative mouse serum.

To examine the growth of EHV1 strains in mouse lungs, first, the laboratory strain HH1 and 7 field isolates from aborted fetuses were intranasally inoculated to 4-week-old, female BALB/cA mice and lung virus titers were determined 3 days after inoculation. Two mice were used for each viral strain. Among the 8 strains, the HH1 strain showed the highest lung virus titers (mean TCID50 was 105.9/ lung). Six of 7 field isolates could be recovered from most of the mice inoculated with mean titers ranging from 106 to 104.1 TCID50/lung. One field isolate F8 strain was not isolated from the inoculated mice even on the day 1 postinfection (data not shown). Next, multiplication of three clones obtained from the avirulent strain of BK343 were compared with that of the HH1 strain. In this experiment, lung viruses were examined 1 and 3 days after inoculation. Although the HH1 virus was recovered from the inoculated mice on the day 1 and 3 postinfection, the avirulent strains were not recovered even on the day 1 postinfection (data not shown).

To understand the course of EHV1 infection, 11 mice were intranasally inoculated with the HH1 virus and were observed daily. Each 2 to 3 mice were sacrificed at day 1, 4, 7 and 12 after infection and lung virus and serum antibody titers were determined. Mice showed signs of illness such as ruffled fur, hunched posture, depression and body weight loss, but recovered from these signs by day 12. Lung virus and serum antibody titers are shown in Fig. 1. Viruses were recovered 1 and 4 days after

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inoculation, however, virus was eliminated from murine lungs thereafter. Virus was not reisolated from any organs including heart, liver, spleen, pancreas, and kidney at any time after infection. Antibody was first detected at the day 7 postinoculation.

Above data show elimination of the HH1 virus from lung within one week. To determine whether the convalescent mice get immunity against the rechallenged HH1 virus, one of 2 groups of mice was infected with the HH1 virus (immunized) and the other was not (non-immunized). Ten days later, both groups of mice were challenged with the HH1 virus, and lung virus and serum antibody titers were examined from the day 1, 3, and 7 postchallenge. Results are shown in Fig. 2. Although viruses were recovered from the non-immunized on the day 1 and 3 postchallenge, no virus was recovered from the immunized mice even at day 1. Antibody titers of the convalescent mice were 1,600 or higher. Non-immunized control mice did not develop antibodies until 7 days after challenge.

Besides, to determine effect of T cells on virus clearance, 4 athymic nude mice were inoculated with $10^4$ TCID$_{50}$ of the HH1 virus and each 2 mice were sacrificed for virus titration in various organs on the day 3 and 7 postinoculation. Mice showed almost the same clinical signs as mentioned above after day 3. Virus was recovered from lungs on the day 3 and 7, and the titers were $10^4$ to $10^5$ TCID$_{50}$/lung on the day 3 and $10^4$ to $10^6$ on the day 7, indicating failure of virus clearance in nude mice (data not shown). Virus was, however, not detected in other organs including heart, liver, spleen, pancreas, and kidney.

Although the present results showed quick reduction of virus titer by 7 days after inoculation with $10^4$ TCID$_{50}$ of the HH1 strain, following facts may support that the HH1 virus was considered to grow in mouse lungs. 1) the HH1 strain was constantly recovered from the inoculated mice, while some virus strains were not recovered, despite of inoculation with the same dose as the HH1, 2) virus titer per lung 24 hours or longer after inoculation often exceeded the inoculated dose, and 3) larger amount of the virus was detected in the nude mice. Therefore, the present studies fundamentally confirmed the observation that HH1 could multiply in mouse lungs and induce clinical signs, as reported by Awan et al. [2]. The difference in lung viral growth pattern between Awan et al. and ours may be due to differences in virus dose inoculated and in virus strain used. Patel and Edington [8] and Nowotny et al. [7] showed HH1 antigen, by immunofluorescence, in lungs of suckling mice inoculated intracerebrally with some strains of HH1. The former recovered the viruses from the lungs. These and the present findings indicate HH1 can propagate in murine respiratory organs, though dependent on viral strains.

Patel and Edington [8] reported that about half of the HH1 strains tested were evaluated as pathogenic by intracerebral inoculation into mice and these pathogenic strains replicated in murine lungs after viremic spread from the brains. Nowotny et al. [7] also reported similar observations. In the present study, the HH1 strain and many field isolates from aborted fetus propagated in murine lungs, but avirulent strains did not. These findings indicate possible differentiation among HH1 strains into virulent and avirulent by intranasal inoculation of mice. However, Patel and Edington [8] described presence of one strain which was less pathogenic for mice with intracerebral inoculation, but propagated in mouse lungs. Therefore, it requires precise examination to evaluate the viral virulence by intranasal inoculation of mice.

The euthymic mice responded to HH1, eradicated the virus from their lungs and developed antibody. In addition, the convalescent mice did not allow growth of
rechallenged virus, indicating establishment of immunity as observed in experiments using horses [1]. Moreover, the athymic mice failed to clear the virus, indicating central role of T cells in recovery. Therefore, to study the mechanism for clearance of EHV1, the present results offer the system for adoptive immunity in which transfer of immune cells into infected nude mice become possible. In addition, the present results offer us additional research subjects concerning growth of avirulent strains in nude mice and evaluation of them as a candidate virus for live vaccine using this mouse-EHV1 system.

In conclusion, the present studies confirmed replication of EHV1 in murine lungs reported by Awan et al. [2] and indicate feasible usefulness of the EHV1-mouse system for studying on pathogenicity and immunology of the infection.

REFERENCES