Pregnancy and Parturition of Mice Latently Infected with Pseudorabies Virus

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Abstract: In this study, the influence of pregnancy and parturition on mice in a mouse model of latent infection with Pseudorabies virus (PrV) was analyzed. Latently infected (LI) female mice were paired with mature uninfected males. The mating produced progeny without any clinical signs of Aujeszky’s Disease. At weaning, both male and female progeny of LI mice showed significantly lower weight than control mice. PrV was not detected from nasal swab specimens of the female parent mice or the trigeminal ganglia of all mice, except 3 of 50 neonatal mice. These findings demonstrate that pregnancy and parturition induce little reactivation of latent PrV, but do affect the mother’s body, as indicated by the decreased weight of progeny at weaning.

Key words: latency, pregnancy, Pseudorabies virus

The establishment of latent infections is an extremely interesting feature of all Herpes viruses. The viruses of alphaherpesviridae, one of the three subfamilies of herpesviridae, persist in an inactive state, primarily in neural tissues, mainly within the neurons of the ganglia, for varying durations [1, 13] and they avoid the host’s immune responses. Such latent viruses are often reactivated by stress [7, 8, 12]. Herpes virus infection thus results in a long-term course of recurrent disease. Animal models have provided experimental approaches for elucidating the molecular mechanisms of latent viral reactivation.

Pseudorabies virus (PrV), a member of the alphaherpesviridae, causes Aujeszky’s Disease (AD). The clinical signs of adult pigs infected with PrV include coughing, sneezing, dullness, nervous signs and lack of coordination. Infected pregnant sows suffer abortions and piglets infected with PrV die with acute symptoms within a few days. Hence, AD affects sows and piglets more severely than adults. When pigs survive AD, the virus becomes localized in the trigeminal ganglia and establishes a latent infection there [2, 3, 10, 11]. Such viruses may be reactivated by stresses such as transportation, change of food, several diseases [19, 20], pregnancy and parturition [4]. These reactivated viruses become a new source of virus for infection. Abortion and death of piglets cause large losses of production in the field. The complete clearance of AD is difficult once it invades a farm, since it is almost impossible to distinguish latently infected pigs from uninfected pigs from their outward appearance. This point makes PrV infection more serious.

Latent infection of PrV generally becomes established only in pigs because other animals die of acute infection...
when they show symptoms of AD. We have established a PrV latent infection model in mice using wild PrV, YS-81 strain [15]. Based on a procedure reported by Osorio and Rock [9], mice were pre-treated with anti-PrV swine serum and then challenged with YS-81. Almost all the mice survived, and PrV was detected and reactivated from the trigeminal ganglia (TGs) of the mice. We also reactivated PrV from latently infected mice in vivo by stimulation with acetylcholine or dexamethazone [16], as performed in pigs [14]. We are currently analyzing PrV latent infection by using this murine PrV latent infection model.

We have not yet analyzed the effect of PrV latent infection on pregnancy in mice. If PrV influences pregnancy and parturition in mice, as in pigs, it would be possible to identify the pathological mechanism of AD in pregnant animals. Therefore, we utilized our murine model, latently infected with PrV, and examined the effect of latent infection on pregnancy and parturition in this study.

The animal experiments were permitted by the Committee on Animal Experiments of Oita University and undertaken in accordance with the Guidelines for Animal Experimentation, Oita University. Five-week-old BALB/c mice (Charles River, Japan) were passively immunized, intraperitoneally (i.p.), by inoculation of 0.25 ml of anti-PrV swine serum. The final neutralization titer of this serum was 1:128. Thirty minutes later, the pre-immunized animals were infected i.p. with 100 lethal dose 50 s (LD 50 s) of YS-81, the PrV wild strain grown in porcine kidney cells (PK-15). The virus titer was assayed in cloned PK cells [5]. Mice surviving the challenge were kept for 2 months and used as latently infected (LI) mice. Latent infection was confirmed by proving the presence of PrV DNA in the TGs of these LI mice by polymerase chain reaction (PCR) described after sacrifice and the absence of virus excretion in nasal swab at day 0 in test period.

LI female mice were paired with mature uninfected males. As a control, uninfected male and uninfected female mice were paired. These mice were kept in a physically controlled area of our institute. In the first experiment, the number of progeny and clinical signs of the male, female and newborn mice were observed for 3 weeks. The weight of newborns was measured using an electronic scale, EK-120 (AND, Japan). The significance of differences in the number of newborns was analyzed by the chi-squared test. To assess differences of weight, the t-test was performed.

Nasal swabs of mothers were harvested on days 1 and 8 after delivery for detecting reactivated virus excretion. Under anesthesia, 100 μl of MEM was injected into one of the nasal cavities of each LI mouse. The wash was harvested from the other cavity and the mouth with a swab (MENTIP, Japan) as the nasal swab sample. The swab specimen was immersed in 300 μl of MEM and stored at −80°C until used for virus detection. The presence of latent PrV DNA was assessed in nasal swab specimens by PCR amplification of a 531-bp target sequence contained in the gene encoding PrV gG following the method described in our previous report [16]. The DNA was extracted from the swab specimens with ISOGEN-LS (Wako Pure Chemical Industries, Ltd., Japan). The TGs or brain was minced with a cell strainer (FALCON, USA) and the DNA was extracted with ISOGEN (Wako Pure Chemical Industries, Ltd.). The DNA samples and oligonucleotide primers were initially heated at 94°C for 2 min and denatured at 94°C for 1 min, annealed at 56°C for 1 min, and extended at 72°C for 2 min. The samples were then subjected to 30 cycles of amplification and maintained at 72°C for 7 min. The sequence of the forward primer was AGCGTAGGACACACACC, and that of the reverse primer was, AGACGAGCACGACGATGTAC. Amplification products were analyzed by electrophoresis in 1.0% agarose gels.

We obtained 5.00 progeny on average from LI mothers and 5.14 from uninfected dams. There was no significant difference in the numbers of male and female progeny by the chi-squared test (P<0.01). None of the parental or newborn mice showed clinical signs of AD. Progeny mice were weighed on days 1 and 8 after parturition and at weaning, at 3 weeks of age. The results are shown in Table 1. Both male and female progeny from LI mothers showed significantly lower weight at weaning than progeny of control mice (P<0.05 in males, P<0.01 in females by t-test). Such a difference was not seen on days 1 and 8.

Dams mice were subjected to a virus excretion test using nasal swab specimens. The swab samples were harvested on days 1 and 8 after parturition and PCR was
used to detect PrV DNA. No virus was detected from nasal swab specimens of either the LI or control groups (data not shown). This means that pregnancy and delivery did not induce reactivation of the latently infected virus in this murine model. Because the swab harvest imposes a large stress on mice, we performed nasal swabs only twice during the experimental period. It is possible that virus excretion occurs at times other than those tested. However, male parent mice and neonatal mice in the same cages as the LI mice did not show any symptoms of infection, so it is supposed that excretion, even if it occurred, was at a lower level than that which causes AD. Regarding this point, this murine latent infection model shows a difference from infection in swine.

PrV DNA was detected from TG samples of all of the latently infected mother mice. As shown in Table 2, no virus was detected from TGs of male parents or any of the negative control mice. Most of the progeny mice produced from LI mice did not show PrV in their brains; however, 3 of 50 progeny mice were PrV positive. PrV DNA was not detected in any of the progeny produced from negative controls.

As mentioned above, abortion induced by PrV infection is a problem in swine. However, we did not observe any clinical signs of AD in either parental mice or their offspring. PrV showed different kinetics of virus production in mice and swine, so the murine model of PrV latent infection probably does not perfectly reflect latent infection in swine.

On the other hand, there was clearly a significant difference in the newborns’ weights at weaning between the LI group and control group, although no difference was seen in the number of offspring or in virus excretion. It would be possible to use this latent infection model to evaluate some vaccines in pregnancy before inoculating sows by comparing weights of progeny at weaning. One limitation of this would be that it would take 3 weeks until suckling mice are weaned and we could see a difference in weight caused by PrV infection. We weighed mice on days 1 and 8 after birth and at weaning in this study and no data were obtained between these points. In future studies, we need to evaluate the weight of progeny between day 8 and weaning to minimize the time needed to see a difference in weight.

Animal models of herpes virus infection in pregnancy have been reported for cytomegalovirus [18] and equine herpesvirus-1 [6] but not for PrV in mice. Of course the swine model is the best for studying PrV, but sometimes the size of animals and duration of pregnancy is problematic for animal experiments, especially in small laboratories. It is possible to use our murine model for studying PrV infection. However, the only difference noted in this study was in body weights of progeny at weaning, which means that PrV infection in mice is not exactly the same as in swine, especially in pregnancy. Studying the mechanism of PrV infection in different animal species should clarify the reason.

It is thought the offspring received some viral protection from their mothers, and in the 3 of 50 progeny mice that showed PrV-positive in their TGs, this protection was sufficient for the inhibition of clinical symptoms but not sufficient for the protection of PrV infection in mother’s body. We did not check neutralizing antibody titer in the parents and neonates because the antibody

| Table 1. Weights of neonatal mice on Day 1 and Day 8 after birth and at weaning |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Day 1           | Day 8           | At weaning      |
|                 | Male  | Female | Male  | Female | Male  | Female | Male  | Female |
| LI mice         | 1.68 ± 0.06    | 1.49 ± 0.04    | 6.42 ± 0.28    | 6.22 ± 0.16    | 18.28 ± 0.33*  | 16.39 ± 0.31** |
| Control mice    | 1.69 ± 0.07    | 1.50 ± 0.04    | 5.70 ± 0.22    | 5.28 ± 0.38    | 20.79 ± 0.69   | 18.56 ± 0.58   |

The weight is shown as average (grams) ± standard error. The data were analyzed by Student’s t-test. * Value is significantly different compared with the control at 5% probability. ** Significant difference at 1% probability.

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<th>Table 2. Virus DNA detection from TG samples</th>
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* (No. of mice in which viral was detected) / (No. of mice tested).
titer of LI mice was shown to be low in a previous report [16]. Accordingly, we initially speculated that the neutralizing antibody was not related to the protection of the neonates against AD. However, it has been reported that even low amounts of antibody can protect neonatal mice against herpes simplex virus infection [21]. Therefore, it is also possible that in PrV infection, maternal transfer of antibody has some role in protecting neonates.

The number of female neonates was larger than that of male neonates in the LI group. Though the difference was not significant, this tendency was constantly seen in other experiments (data not shown). We do not have any evidence about whether or not there is a relationship between PrV infection and this phenomenon at this time. However, this is quite an interesting point and will become a research theme in the future.

In conclusion, pregnancy and parturition do not induce reactivation of latent PrV in mice, but latent infection does affect the mother’s body, as indicated by the decrease in weight of progeny at weaning.

References