Pathogenicity of Sendai Virus in Mice Cage-Mated with Infectors and Their Offsprings

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Abstract. Sendai virus was first isolated from the tracheas and lungs of mice 1 to 2 days after cage-mating with infectors, following by formation of lung consolidation in all mice examined 6 to 7 days. HI antibody was demonstrated in all mice 7 to 10 days, increasing in titers to 14 days. Isolation of the virus was negative from 12 days till 30 weeks when the experiments were terminated, except for some mice sacrificed 9 weeks which harbored the virus in their tracheas. HI antibody remained at high titers till the end of experiment and lung lesions persisted in more than a half of mice examined, although the affected area was localized around bronchioles in late stage of the infection. These findings were almost the same in both ddY and ICR strains of mice used, but a high mortality as 26.2 per cent was shown in ICR mice, being significantly different from 3.6 per cent in ddY mice. In offsprings from infected dams, maternal HI antibody of high titer was demonstrated at weaning, then it rapidly decreased to the lowest titer 7 to 14 weeks of age when the virus was isolated from the respiratory tracts of a few individuals. Thereafter the antibody titers increased again and isolation of the virus became negative. Detection rates of lung lesions were remarkably low in comparison with those of their dams and neither death nor such clinical signs as ruffled fur and emaciation appeared.

Natural infections of Sendai virus in mice were first reported by several Japanese researchers during 1955 and 1960 [6-8, 13, 22], and thereafter prevalence of the infection in mouse colonies was revealed mainly in Japan and U.S.A. [2, 3, 5, 9, 16-18, 20].

However, different criticisms have been given to the pathogenicity of the virus to mice according to researchers. In mice infected with the virus, many investigators observed high incidences of pneumonic lesions, poor pregnancy, prolongation of gestation period, high incidences of cannibalism by dams and increases in mortality of new-borns [2, 7, 9, 11-13, 26], but in contrast to these observations, other investigators reported neither clinical signs nor lung lesions in naturally and experimentally in-

fectured mice, indicating latent infection of the virus in mice [1, 15, 19, 25].

In the previous report in which relationships between infections of some respiratory pathogens including Sendai virus and incidences of mice with pneumonic lesions were studied in many mouse breeding colonies, we suggestively indicated the importance of Sendai virus as a cause of spontaneously occurring pneumonia [20]. The present studies are undertaken to investigate the pathogenicity of Sendai virus not only in experimentally infected mice but also in their offsprings.

Materials and Methods

Mice: Four-week-old mice of outbred ddY and ICR strains were used. They were obtained from

breeding colonies free of *Salmonella* spp., *Pasteurella pneumotropica*, *Escherichia coli* O115a, c: K(B), *Corynebacterium kutscheri*, Tyzzer's organisms, *Mycoplasma* spp., Sendai virus, mouse hepatitis virus, mouse adenovirus and reovirus type 2.

Sendai virus: Strain M-73 used in this experiment was isolated in our laboratory by inoculating a tracheal specimen of naturally infected mouse into chorioallantoic membrane of a 10-day-old chick embryo. This strain was identified as Sendai virus by agglutinability of chick red cells and cross neutralization, hemagglutination inhibition and complement-fixation tests with Sendai virus H strain which had been supplied by Dr. H. Fukumi of this institute.

For experimental infection, chorioallantoic fluid of 10-day-old chick embryos inoculated with the strain M-73 was used as challenge virus. The challenge virus suspension showed $10^{8.3}$ EID₅₀ (50% egg infecting dose)/ml and 256 hemagglutinin units/ml.

Experimental infection: Ten uninfected mice were housed in each cage of 32×22×11 cm with 2 infector mice, which had been prepared by dipping their noses into the challenge virus suspension for about 1 second, to make contact infection. The cages were placed in 3 large vinyl isolators (150×50×170 cm) throughout the experimental period to prevent infections of any pathogens other than Sendai virus. To keep continuous contamination of Sendai virus in the isolators, a cage with 10 uninfected mice and 2 infectors prepared as described above was weekly brought into each isolator, because it was known that excretion of the virus from infected mice never continued longer than 2 weeks after infection [1, 15, 19, 26].

All mice were fed gamma-irradiated pellets (Funabashi Farm) and non-sterilized tap water ad libitum. The bedding was changed twice a week.

Sampling: Ten mice of each strain were periodically autopsied for observation of lung lesions after bled by cardiac puncture under ether anesthesia. The trachea and lung specimens were separately harvested from some of sacrificed mice and stored at -80°C for virus isolation. Individual sera were obtained from blood samples and submitted to hemagglutination inhibition (HI) test.

In early stage of the experiment, a total of 40 females of each strain was mated with males in 10 cages, consisting of 4 females and one male each. Thus, offsprings obtained during the experiment were sacrificed at their various ages for the same examinations as described above.

Hemagglutination inhibition test: HI test was performed according to the method of Fukumi et al. [7] with some modifications. Antigen was the

chorioallantoic fluid of 10-day-old chick embryos inoculated with the H strain of Sendai virus, to which sterile glycerin and formalin were added at one-third volume of the fluid and 0.05 per cent, respectively. After heating at 56°C for 30 minutes, mouse sera to be tested were added with packed chick red cells, incubated at 37°C for 2 hours and centrifuged at 3,000 rpm for 15 minutes to eliminate natural hemagglutinins for chick red cells. For HI test, 0.2 ml each of diluted mouse sera in PBS and 4 units of the antigen were mixed in plastic HI plate for influenza virus use, incubated at 37°C for 40 minutes, and then added 0.4 ml of 0.5% suspension of chick red cells. Reading was made after incubation at 4°C for 1 hour. Titers of 1:16 or higher were regarded as positive.

Isolation of Sendai virus: Ten per cent suspensions of mouse trachea and lung specimens were separately prepared in Eagles M.E.M. After centrifugation at 3,000 rpm for 15 minutes, 0.2 ml of resulted supernatant were inoculated into allantoic fluid of 10-day-old chick embryos and incubated at 37°C for 5 days. The allantoic fluid was then harvested, centrifuged at 3,000 rpm for 10 to 15 minutes and examined for hemagglutinin activity (HA) for chick red cells. HA value of 1:2 or greater were regarded as positive isolation.

Examination of lung lesions: Lung lesions of autopsied mice were examined macroscopically with special reference to consolidation.

Results

1. Long term observations on experimentally infected mice

Ten mice each were sacrificed for examinations on the production of HI antibody and the formation of lung lesions every day till 14 days after contact, every week during 3 to 10 weeks and every two weeks from 12 to 30 weeks. At each sampling, 3 to 6 mice were also submitted to virus isolation. Results are shown in Figs. 1 and 2.

No remarkable differences were observed in the courses of infection between ddY and ICR mice. Sendai virus became detectable in the trachea and lung of mice 1 to 2 days after contact with infectors and was isolated from all individuals examined during 4 to 8 days in ddY mice and 2 to 9 days in ICR mice. Thereafter isolation rates of the virus

Fig. 1. Sendai virus infection in ddY mice. * Positive/examined.

Fig. 2. Sendai virus infection in ICR mice. * Positive/examined.

decreased rapidly thus no more the virus was isolated from any mice examined 12 days or later, except for a few of the both strains which were sacrificed 9 weeks of contact and found to be positive for the virus in their tracheas.

Lung lesions as consolidation appeared in some mice from 4 to 5 days of contact

and developed in almost all mice during 6 to 8 days. Maximum extent of the lesions was observed during 10 to 15 days, resulting in deaths of some mice. Thereafter, the extent of lesions decreased in course of time and localized around bronchioles, but complete recovery of the lesions was rarely observed thus, regardless of mouse strains,

more than 65 per cent of mice examined at every sampling were shown to be suffered from the lesions till the end of experiment.

HI antibody became detectable during 6 to 9 days, mostly 7 days, and increased in titers to 1:256 or higher till 12 to 14 days in most cases. Such high antibody titers were kept in many individuals till the 30th week when the experiment was terminated, though the decrease in titer was observed in some mice during the last 10 weeks.

The numbers of dead mice observed

throughout the experiment are summarized in Table 1. A high mortality was obtained in ICR mice, showing that deaths occurred in 108 (26.2%) of 412 mice examined. On the contrary, the mortality of ddY mice was so low as 3.6 per cent; only 12 deaths were detected among a total of 332 mice examined. Regardless of mouse strains, death occurred concentratively during 11 to 16 days after contact, when the lung lesions developed extensively and HI antibody rose rapidly.

Table 1. Numbers of dead mice infected with Sendai virus

Days after contact	Mouse strain	
	ddY	ICR
1-7		
8-10	3	2
11-13	6	79
14-16	2	25
17-19	1	
20-22		1
23-25		1
≥26		
No. dead	12	108
No. exam.	332	418
(%)	(3.6)	(26.2)

2. Observations on offsprings from infected dams

Forty females of each strain were mated with males after recovery from clinical symptoms, thus 139 and 207 offsprings were obtained from 23 ddY and 26 ICR dams, respectively, and submitted at their various ages to the same examinations as performed in the experimentally infected mice.

As shown in Figs. 3 and 4, similar results were obtained in the both strains of mice. The virus was never detected in weanlings younger than 8 weeks of age but isolated

Fig. 3. Sendai virus infection in offsprings from infected ddY dams. * Positive/examined.

Fig. 4. Sendai virus infection in offsprings from infected ICR dams. * Positive/examined.

from some of 9-week-old ddY mice and 8-, 12- and 13-week-old ICR mice. Isolation rates of the virus seemed to be higher in tracheal samples than in lung materials. This was true especially in ICR mice.

Development of pneumonic lesions was remarkably less frequent than in the experimentally infected mice, namely the lesions were usually detected in less than 25 per cent of sacrificed mice of each age, being significantly different from detection rates higher than 65 per cent in the experimentally infected mice. However, considerably high incidences of the pneumonic lesions were observed in 10-week-old ddY mice and 13-week-old ICR ones, these ages corresponded to 1 week older than those of mice from which the virus was isolated. Neither death nor noticeable clinical symptoms were observed in offsprings of the both strains.

HI antibody titers rapidly declined according to ages of mice from the highest (1:64 or higher) at 3 weeks of age, when the mice were weaned, to the lowest at 7 weeks in ddY mice and 10 to 11 weeks in

ICR mice. After several weeks, the titers seemed to increase again as observed in 16 to 18-week-old ICR mice.

Discussion

As regards to the pathogenicity of Sendai virus to mice, many investigators reported a high incidence of pneumonic lesions in infected infants or young mice resulting in death [2, 12, 13] or remarkably retarded growth [7, 9, 27], distinct lowering of fertilities [13], prolongation of gestation period [12] and increased cannibalism by dams [2, 9, 11, 13, 27]. On the contrary to these findings, however, some researchers indicated that neither clinical symptoms nor pneumonic lesions were observed in infected mice thus the virus isolation and detection of serum antibodies were only signs of the infection [1, 15, 19, 25]. The present study in which so young mice as 4 weeks of age were subjected to cage-mating with experimentally prepared infectors revealed that all mice of ddY and ICR strains employed were suffered from pneumonic lesions 6 to 8 days after contact, followed by severest

clinical conditions 11 to 13 days and sometimes resulted in deaths, especially in ICR mice. From these results, Sendai virus was shown to be strongly pathogenic to mice. Different evaluations on pathogenicity of Sendai virus according to investigators as mentioned above might be caused by differences of experimental conditions such as virulence of virus strains used, susceptibility of mouse strains employed [14], age of mice used [23], environmental conditions in which mice were maintained [26], inoculum sizes of virus to mice [24] and the presence of maternal antibodies from infected dams [5, 10].

The progress of infection observed in the present study were quite similar to those reported by other investigators [1, 15, 19, 26], except for the following 2 facts. One was reisolation of the virus from tracheas of some mice at the 9th week of contact after a long duration of negative isolation of the virus. This might be due to reinfection of the virus, because all mice were maintained in isolators heavily contaminated with Sendai virus and the neutralizing antibody of infected mice temporarily declined in titer at this stage of infection (unpublished data). To confirm the cause of this phenomenon, further studies are needed with special reference to the trend of neutralizing antibody in infected mice. Another exceptional fact was persistence of pneumonic lesions which appeared as linear lesions along the bronchioles as long as 30 weeks after contact in more than a half of infected mice. From these lesions, however, no causative agents including Sendai virus were detected, and thus causes of the lesions were remained unknown. Microscopical and immunochemical studies will be requested to investigate the characters of these lesions.

Lindenmann et al. [14] reported that

A2G strain of mice was more resistant to Sendai virus infection than other strains of mice, and Parker et al. [18] also observed variance in susceptibility of mouse strains to the viral infection. In our experiment, although no significant differences were observed in infection rates of Sendai virus and process of the infection between ICR and ddY mice, remarkable difference was observed in mortality between these two strains, showing that death occurred in 26.2 per cent of infected ICR mice but in only 3.6 per cent of ddY mice. The high mortality in ICR mice possibly owe to the fact that affected area of the lung was usually larger in ICR mice than in ddY mice, although scoring of the lung lesions was not made in this experiment. Such a strain difference of susceptibility in ICR and ddY mice was already known in *Mycoplasma pulmonis* infection [21].

On the other hand, many investigators have indicated the importance of maternal antibody in protection of Sendai virus infection of mice [5, 10, 12]. Namely, offsprings younger than 3 to 4 weeks of age from infected dams were protected from the virus infection by the presence of maternal antibody which mainly given through milk, while the infection usually occurred at 5 to 6 weeks of age when the maternal antibody of the offsprings declined, resulting in self antibody production [5, 12]. These phenomena were also observed in our experiments in which examinations were made on offsprings born from infected dams. However, disappearance of maternal antibody occurred at 7 to 10 weeks of age, somewhat later than that reported by other investigators, and at the same time or later than that the virus was detected from the tracheas and lungs of offsprings. Such a prolonged presence of the maternal antibody in weanlings might be due to high antibody titers

of dams.

Clinical signs and lung lesions observed in the offsprings were usually feeble and mild thus no dead cases were detected. These mild responses of the infected offsprings might be explained as follows: although the mice were infected with the virus according to decline of maternal antibody derived from their infected dams, the virus failed to multiply enough to produce overt disease, while the mice received active immunization responsible for eliminating the virus.

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要 約

ケージ内同居感染マウスおよびその出生子におけるセンダイウイルスの病原性：中川雅郎・斎藤 学・木下邦明・鈴木映子・今泉 清（国立予防衛生研究所 獣疫部）——センダイウイルスのマウスに対する病原性と感染の経過を知る目的で、ddY および ICR 系 4 週齢マウスをあらかじめ本ウイルスを感染させた infector マウスと共にケージ内で contact させ、30週間にわたって感染の推移を観察した。また同時に、これらのマウスから生まれた 1 代産子についても生後 3 週から 16~18 週にわたって同様な観察を行った。その結果、いずれの系統マウスも、contact 後 1~2 日で気管や肺からウイルスが分離され始め、6~7 日で全例に肺病変が形成された。血中 HI 抗体は 7~10 日で全例陽性に転じ、抗体価は 14 日まで上昇し続けた。ウイルスは 12 日以降分離されなくなり、9 週後に陽性を示した一部の個体を除けば、30 週まですべて陰性であった。これとは逆に、HI 抗体は 30 週まで全例が高力価を維持し、過半数のマウスでは肺病変も 30 週まで持続した。しかし、病巣部は時間の経過とともに気管支周囲に局限した。ICR 系では contact 後 11~16 日の間に高い死亡率を示したが、ddY 系のそれは低かった。一方、両系統マウスの 1 代産子は、離乳時に高い HI 抗体価を示したが、その後急速に低下し、7~14 週齢で最低となり、この時期に一部の個体からウイルスが分離された。その後抗体は再上昇し、ウイルス分離も陰性になった。また、1 代産子における肺病変発現率は感染親マウスより遙かに低く、立毛、消瘦などの臨床症状や死亡例はみられなかった。