

## Pathogenesis of Sendai Virus Infection in the Central Nervous System of Mice

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Received for publication 15 December 1975

The present study was aimed to clarify the pathogenesis of Sendai virus infection to the central nervous system (CNS) of mice. One- to 2-day-old suckling and 4-week-old mice were inoculated intracerebrally with the virus. The virus multiplied higher in sucklings than in adults. Immunofluorescent studies in sucklings revealed that the viral antigens appeared initially in ependyma, choroid plexus epithelium, and meninges. Subsequently they spread to subependymal cells and finally were found in neurons of hippocampus for as long as 4 months postinfection. In adults, however, the viral antigens rapidly disappeared in the early stage. Most mice inoculated intracerebrally with Sendai virus appeared healthy, although hydrocephalus developed in a few mice. Virus-specific antibody and interferon production seemed to have no influence on the persistent infection of Sendai virus in the CNS of mice. One of the most significant findings may be that the viral antigens persist in the brain for as long as 4 months in a latent form. This may offer a useful model for the study of latent CNS infection of paramyxoviruses.

Paramyxoviruses are known to cause latent infections in susceptible animals. Recent evidence for etiological implication of measles virus in subacute sclerosing panencephalitis strongly supports a concept that some of the diseases of the central nervous system (CNS) of hitherto unknown etiology might be caused by persistent viral infections. A recent report on isolation of an agent resembling type 1 parainfluenza virus from the brain tissue of a patient with multiple sclerosis also supports this concept (16). That isolate was indistinguishable from Sendai virus in its antigenic and total polypeptide compositions, although it differed in certain phenotypic properties (8).

It is well known that Sendai virus belonging to type 1 parainfluenza viruses causes respiratory infections in mice (1, 13, 17). However, its neurotropic property has not been elucidated except for the studies reported by Mims and Murphy (11).

The present study was undertaken to reveal the pathogenesis of Sendai virus infection to the CNS of mice.

### MATERIALS AND METHODS

**Virus.** The Nagoya strain of Sendai virus was inoculated in the allantoic cavity of 11-day-old embryonated eggs. The allantoic fluid was harvested after incubation for 3 days at 37 C and stored at -70 C until use.

**Mice.** Male and female dd mice, 1 to 2 days old and 4 weeks old, were used. Hemagglutination inhibition tests for antibody against Sendai virus performed beforehand in these mice were negative. Virus inoculation of suckling and adult mice was done by injecting 0.02 ml of infected allantoic fluid, properly diluted by phosphate-buffered saline (PBS), into the right cerebral hemisphere.

**Virus titration and interferon assay.** Virus titration and interferon assay were carried out on extracts of pooled brains obtained from groups of three mice. Brains were aseptically removed, and a 20% (wt/vol) tissue homogenate was prepared in Eagle minimum essential medium. After centrifugation at 3,000 rpm for 15 min, the supernatant was used for virus and interferon assays.

Infectivity of Sendai virus was titrated by intrallantoic inoculation of 11-day-old embryonated eggs with 0.1 ml of each of 10-fold dilutions of the supernatants in PBS.

For interferon assay, brain extracts were further centrifuged at 20,000 rpm for 60 min to eliminate virus particles. The supernatant was then dialyzed overnight at pH 2 and then dialyzed back to pH 7.4 before assay. Interferon assay was carried out according to the plaque reduction method using mouse L cells and vesicular stomatitis virus as a challenge virus, and the titers were expressed as the reciprocal of the highest dilution of the sample causing 50% plaque reduction. In this assay method one interferon unit was equal to 2.4 NIH international standard units.

**Antibody titration.** Hemagglutination inhibition antibody of pooled sera obtained from groups of

three mice was titrated according to a microtechnique method (15).

**Virus recovery.** Brains were aseptically removed, minced with scissors, and then trypsinized for 30 min at room temperature. Brain cell suspension thus obtained was overlaid on a monolayer culture of PS cells, a continuous cell line derived from porcine kidney cells, in Leighton tubes with cover slips at 34 C. After 1 to 5 days of incubation at 34 C, a hemadsorption test and an immunofluorescent study of the cover slip specimen were performed.

**Histology.** Brain tissues for routine histology were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin. The Kluever-Barrera method was used for demonstrating demyelination.

**Immunofluorescent techniques.** For preparation of anti-envelope rabbit antiserum, viruses were grown in the allantoic cavity of 10- to 11-day-old embryonated eggs. Infected allantoic fluid was cleared by centrifugation at 3,000 rpm for 30 min. Viruses were then pelleted by centrifugation at 20,000 rpm for 60 min, resuspended in PBS, and used for immunization.

For preparation of antinucleocapsid rabbit antiserum, a purified nucleocapsid suspension was prepared from Sendai virus-infected BHK cells by the method reported previously (12).

Anti-envelope and antinucleocapsid sera were raised in rabbits by injecting these antigen preparations first intramuscularly and then intravenously. Antisera thus obtained were conjugated with fluorescein isothiocyanate.

For the immunofluorescent study, frozen sections of 10  $\mu$ m in thickness were made on a Lipshaw cryostat, dried, fixed in acetone for 15 min, covered with fluorescein isothiocyanate-labeled rabbit antiserum, and incubated overnight at 4 C. The sections were then washed three times with PBS, mounted in buffered glycerol, and observed under an Olympus fluorescent microscope.

## RESULTS

### Multiplication of Sendai virus in the CNS.

Figure 1 demonstrates the changes of titers of Sendai virus recovered at intervals after infection from the brains of mice inoculated intracerebrally with  $10^6$  50% egg infective doses ( $EID_{50}$ ) of virus. After an initial drop at 4 h after infection, titers increased rapidly, reaching the maximum on day 1; thereafter they declined rapidly, being undetected on day 5. The viral growth in the brain of the suckling mice appeared to be more vigorous than in adult mice, as indicated by a much higher titer in sucklings on day 1.

Infective viruses could be recovered from brain tissues cocultivated with PS cells on day 14 postinfection but not on day 79.

Mice given  $10^6$   $EID_{50}$  of Sendai virus showed no signs of illness throughout the study except for occipital prominence seen in some mice, as will be described below.

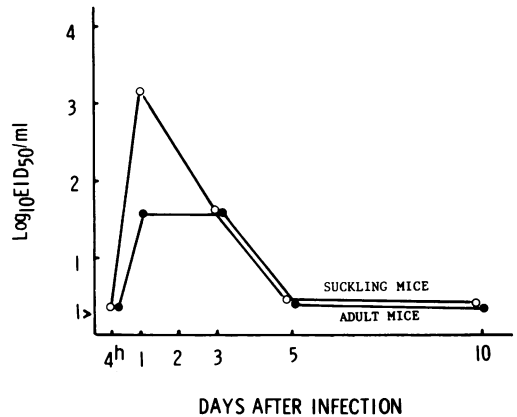


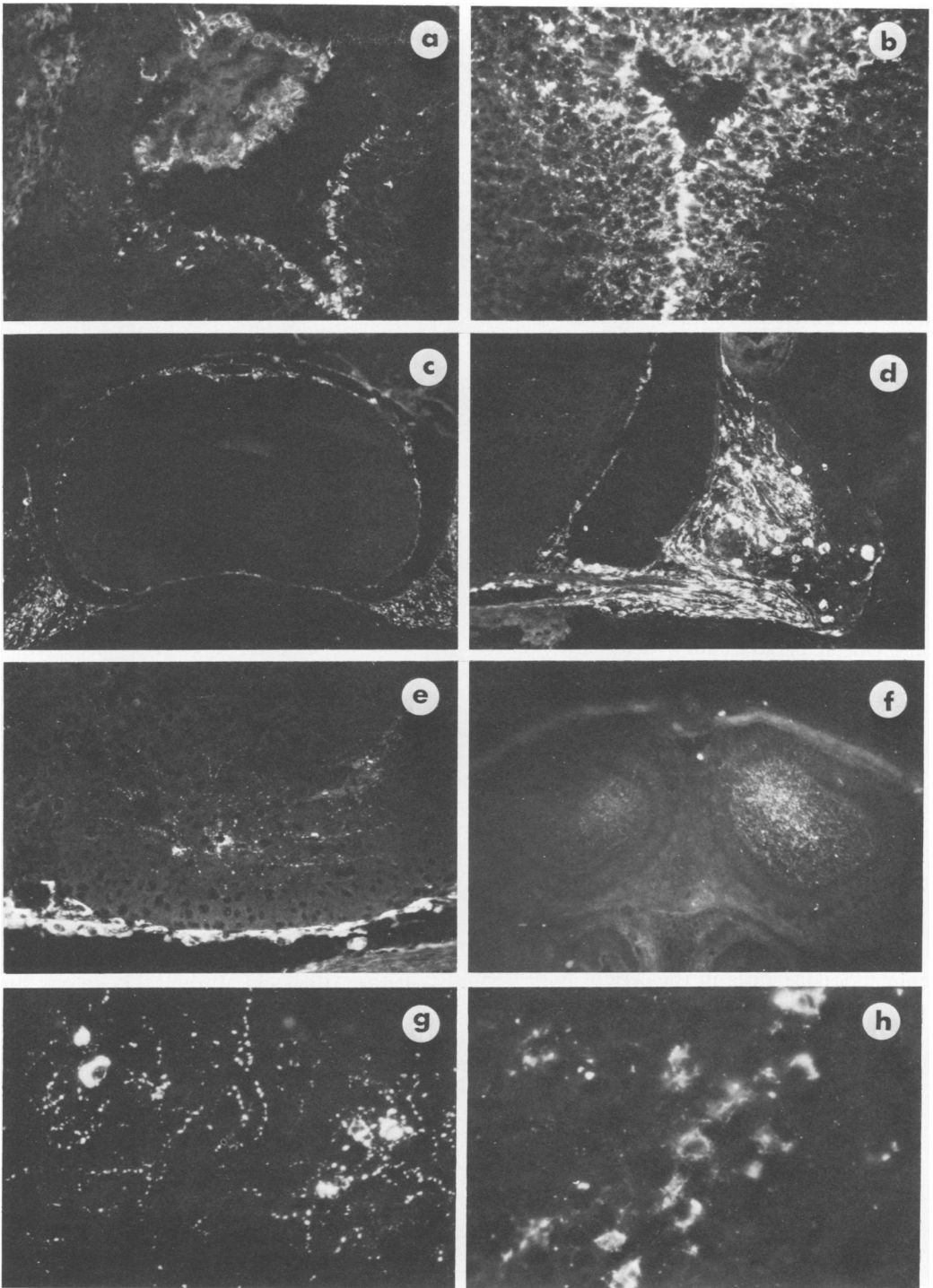
FIG. 1. Virus titers in brain extracts of suckling and adult mice inoculated intracerebrally with  $10^6$   $EID_{50}$  of Sendai virus at various times after infection. Pools of three brains were tested on the days indicated.

### Site of growth of Sendai virus in the CNS.

The brain and spinal cord from suckling and adult mice inoculated intracerebrally with  $10^{7.6}$   $EID_{50}$  of Sendai virus were examined by the direct immunofluorescent technique using fluorescein isothiocyanate-labeled antinucleocapsid and anti-envelope antiserum. Specific fluorescence by both staining methods was seen in the cells lining the cerebrospinal fluid spaces, that is, ependyma, choroid plexus epithelium, and meninges on days 1 to 2 postinfection (Fig. 2a and 2c). On day 2, the cells in subventricular regions began to show pronounced fluorescence (Fig. 2b). This contiguous spread of viral infection of the brain substance via the ependyma and meninges was more prominent in sucklings than in adults.

The posterior root ganglions were also involved in suckling mice in early stage. On day 2, nucleocapsid and envelope antigens were present in cytoplasm of the neurons of the posterior root ganglion (Fig. 2d), in the cells belonging to the anterior horn of the spinal cord (Fig. 2e), and in the olfactory bulb in sucklings (Fig. 2f). There were also viral antigens detectable in the inner ear of suckling mice (data not shown), and the details will be presented separately.

Consequently, these viral antigens gradually disappeared, and no fluorescence was seen in the cells lining the cerebrospinal fluid spaces at the end of week 2 in sucklings or in adults. In suckling mice, however, viral antigens could be detected in the neurons, especially of the hippocampus, on day 7 and persisted for as long as 4 months without causing any clinical disorders, whereas no viral nucleocapsid and envelope an-



**FIG. 2.** *Fluorescent antinucleocapsid antibody-stained sections of the CNS of suckling mice inoculated intracerebrally with  $10^{7.6}$  EID<sub>50</sub> of Sendai virus are presented. (a) Ventricular profile with fluorescent ependyma and choroid plexus epithelium 1 day after infection. (b) Fluorescence is beginning to extend from the ependymal to subependymal cells 2 days after infection. (c) Fluorescence is observed in meninges and posterior ganglions in a cross-section of spinal cord, (d) in neurons of posterior ganglion in a high-power view, (e) in meninges and anterior horn cells of spinal cord, and (f) in olfactory bulbs 2 days after infection. In hippocampal neurons viral antigens are detectable (g) 3 weeks and (h) 3 months after infection. The same fluorescent patterns were observed in the sections stained by anti-envelope antiserum.*

tigens could be detected in adult mice after 2 weeks. Figures 2g and 2h demonstrate nucleocapsid antigens in the neurons of the hippocampus in suckling mice at 3 weeks (Fig. 2g) and 3 months (Fig. 2h) after infection. Figure 2 shows the presence of the nucleocapsid antigens of Sendai virus; however, envelope antigens were also observed within the same area (data not shown).

In the case of adult mice, nucleocapsid and envelope antigens of Sendai virus were detected in the same area as in the case of suckling mice. However, the antigens in the adult mice brain became undetectable by day 14 postinfection.

**Histological observations.** Suckling and adult mice inoculated intracerebrally with  $10^6$  EID<sub>50</sub> of Sendai virus were sacrificed at various times after infection, and their brains were histologically examined after hematoxylin and eosin staining and/or myeline staining.

In the early stage, the principal changes observed in suckling and adult mice were periventricular and perivascular mononuclear cell infiltration. The infiltration in periventricular regions gradually subsided; however, perivascular cuffing persisted for a month or more. No demyelination foci were seen in suckling mice by month 4 postinfection.

Some suckling mice inoculated intracerebrally with  $10^6$  EID<sub>50</sub> of Sendai virus developed occipital prominence of the skull by week 4 postinfection. In these mice, a marked ventricular dilatation (Fig. 3) was invariably noted. Control suckling mice inoculated intracerebrally with PBS did not show any abnormal dilatation of ventricles.

**Antibody response.** Prior to infection with  $10^{6.8}$  EID<sub>50</sub> of Sendai virus, there was no detectable hemagglutination inhibition antibody against Sendai virus in the sera of mice used

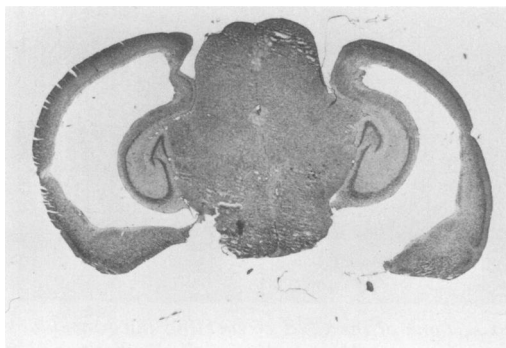


FIG. 3. Coronal section of mouse brain 4 weeks after intracerebral inoculation with  $10^6$  EID<sub>50</sub> of Sendai virus neonatally. Marked dilatation of lateral ventricles are shown.

throughout the present study. This may exclude a possibility of natural contamination with Sendai virus in these mice.

In suckling mice a slight increase in the antibody level was evident by day 5, followed by an appreciable increase by days 14 and 21. In contrast, the antibody response in adult mice was remarkable by day 5 (Fig. 4). It is worthy to note that there was no significant rise in antibody level until day 5 of infection, that is, the day when the virus titer became undetectable. No detectable antibody response against Sendai virus was observed in control suckling and adult mice inoculated intracerebrally with PBS.

**Interferon production in the brain.** Mice inoculated intracerebrally with  $10^{6.8}$  EID<sub>50</sub> of Sendai virus were sacrificed at various times after infection, and interferon activity in the brain was assayed. The results are shown in Fig. 5. Maximum interferon titer was reached on day 1. There was no difference in modes and extents of interferon production between suckling and adult mice.

## DISCUSSION

To our knowledge there has been no report of a quantitative study on Sendai virus growth in the CNS. In the present study, it was shown that Sendai virus could multiply in the brain of suckling and adult mice when the virus was inoculated intracerebrally. Suckling mice were more susceptible to intracerebral Sendai virus infection than adult mice and yielded higher virus titers. A relationship between age and susceptibility to the virus will be discussed later.

The present immunofluorescent observations confirmed and extended previous studies reported by Mims and Murphy (11). In our stud-

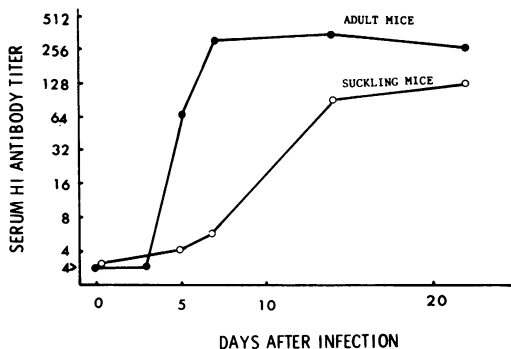


FIG. 4. Hemagglutination inhibition (HI) titer in sera of suckling and adult mice inoculated intracerebrally with  $10^{6.8}$  EID<sub>50</sub> of Sendai virus.

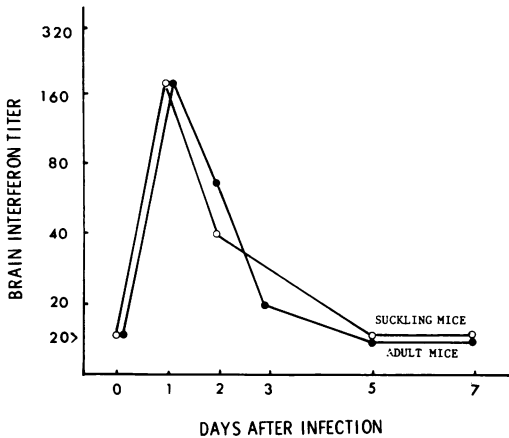


FIG. 5. Interferon titers in brain extracts of suckling and adult mice inoculated intracerebrally with  $10^{6.8}$  EID<sub>50</sub> of Sendai virus. Pools of three brains were tested on the days indicated. Titers are shown as the reciprocal of the dilutions of brain extract causing 50% reduction in the virus plaque counts.

ies, in addition to involvement of ependyma, choroid plexus epithelium, and meninges in the early stage of infection, simultaneous involvement of the spinal cord and posterior ganglion was evident. These observations, in accordance with the findings of intracerebral influenza virus inoculation (9), indicate that the cells lining the cerebrospinal fluid spaces would be initial sites of virus growth in the CNS. About 7 days after infection, viral antigens were found in the neurons, especially of the hippocampus, whereas only a small amount of viral antigens could be detected in the initial site of virus infection. A precise route of the virus spread from the cells lining the cerebrospinal fluid spaces to the neurons of the hippocampus remains to be disclosed, although various hypotheses have been proposed for the spread of viruses within the brain (2).

One of the most interesting findings in the present study seems to be a long-term persistence of viral antigens in the brain, mainly the hippocampal area, for as long as 4 months post-infection without causing any clinical signs of illness. To our knowledge, this constitutes the first example of a long-term persistent infection in the CNS of experimental animals by Sendai virus.

Mumps, parainfluenza-2, and influenza A virus in suckling hamsters and influenza A virus in suckling mice were shown to cause hydrocephalus (7). In the present study, Sendai virus in suckling mice was also shown to cause hydrocephalus on occasion in contrast to the finding reported by Mims and Murphy (11), who

detected hydrocephalus at no time in adult mice.

It may be pointed out that the strain of Sendai virus used in the present study seems to be of a lower virulence in mice in comparison with that used by Mims and Murphy (11). Therefore, the development of hydrocephalus in mice in response to Sendai virus infection might be influenced, in part, by viral strain variations or animal age.

The antibody and interferon responses observed in this experiment closely resemble those observed by others who studied Sendai virus infections in the mouse lung (13). Upon an intracerebral inoculation of Sendai virus, serum hemagglutination inhibition antibodies become apparent by day 5. This may be due to virus leak as a consequence of blood brain barrier destruction (10). It was observed that infectious virus became undetectable in the infected brain before hemagglutination inhibition antibody could be detected in the serum and that virus titer began to fall immediately after interferon reached its maximum titer. The role of interferon in recovery from the viral infections was emphasized (6), and the brain was reported to be a rich source of mouse interferon (4). Therefore, it is inferred that interferon production might be involved in the elimination of the virus from the infected brain.

It is well known that newborn mice are more susceptible to viral infections than adult mice (5). We also found, by using virus titers and virus persistence in the CNS as parameters for susceptibility, that sucklings were more susceptible than adults to an intracerebral inoculation of Sendai virus. There are many factors influencing susceptibility to virus infections in suckling and adult mice. One of the factors that has been implicated is ability to produce interferon (14). In our study, however, no difference in interferon production was observed between sucklings and adults. Therefore a relationship between age and susceptibility to virus infection cannot be explained by differences in their abilities to produce interferon.

The CNS of newborn mice is known to be nonmyelinated. This may facilitate direct spread of virus infection and account for a high susceptibility of newborn mice to viral infections (3).

The present study offers a useful model for analysis of the pathogenesis of chronic diseases of the CNS in humans due to latent viral infections.

#### ACKNOWLEDGMENTS

We thank S. Yamaguchi, T. Hattori, and E. Iwata for their excellent technical assistance. We are also very grate-

ful to A. Kunii, J. Kitoh, Y. Matsuoka, and Y. Sugiura for their thoughtful suggestions.

This work was supported in part by grants from the Ministry of Education and the Ministry of Welfare.

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