Variation of Virulence and Other Properties among Sendai Virus Strains

Ryoji YAMAGUCHI,* Hiroshi IWAI,* and Katsumoto UEDA

Department of Veterinary Public Health, The Institute of Public Health, Minato-ku, Tokyo 108

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Abstract The virulence of five Sendai virus strains (MN, Z, KN, Mol, and Hm) isolated from laboratory rodents was compared, using 3-week-old female Jcl-ICR mice. The virulence of the strains was Mol, MN, KN, Z, and Hm in decreasing order. The 50% lethal dose and 50% lung consolidation inducing dose of the highest virulent strain differed by the order of more than 10^3 and 10^6, respectively, from those of the lowest virulent one. Other properties such as the growth rate in LLC-MK2 cells, neuraminidase activities, and molecular weights of structural proteins also differed among the virus strains. These results indicate that Sendai virus prevailing in laboratory rodents is not homogenous with respect to virulence and some other properties.

Sendai virus infection is one of the most prevalent infections in laboratory animals (9, 12). In mice, the virus causes overt disease in some cases (6, 8, 18) and subclinical infection in others (4, 5, 13). Factors responsible for such differences may be the age of mice at exposure (14, 18) or genetically-determined susceptibility of mouse strains (1, 12) to the virus. Besides, different virulence of the virus strains among epizootics seems to be another factor. Although a comparative study on virulence between 2 Sendai virus plaque-size mutants was reported (16), there was no report concerning the virulence of field isolates of Sendai virus. Therefore, the authors compared the virulence to mice of Sendai virus strains from different sources.

Five Sendai virus strains, MN, Z, KN, Mol, and Hm, were studied. The MN strain, originally isolated from the lung of a mouse by Fukumi et al in 1953 (5), was obtained from Dr. Fujiwara, Institute of Medical Science (IMS), the University of Tokyo in 1967. Since then, the strain has been passaged five times with limiting dilution in 10-day-old hens’ eggs. The Z strain was isolated from the brain of a mouse by Fukai and Suzuki in 1953 (3) and its plaque-cloned virus was obtained from Dr. Kanda, IMS in 1981. The preceding egg passage history was obscure for these 2 strains. The KN strain was isolated from the lung of an athymic nude mouse with wasting syndrome in 1976 and the Mol strain from the lung of a mouse native in Japan.
Table 1. Mortality and incidence of lung consolidation of mice infected intranasally with different strains of Sendai virus

<table>
<thead>
<tr>
<th>Virus dose (TCID₅₀/25 μl)</th>
<th>Virus strains</th>
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<tbody>
<tr>
<td></td>
<td>MN</td>
</tr>
<tr>
<td>10⁻¹</td>
<td>0/3</td>
</tr>
<tr>
<td>10⁻²</td>
<td>1/3</td>
</tr>
<tr>
<td>10⁻³</td>
<td>1/3</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>1/3</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>2/3</td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>2/3</td>
</tr>
<tr>
<td>LD₅₀ᵇ</td>
<td>10⁴.₂ ≤</td>
</tr>
<tr>
<td>CD₅₀ᵉ</td>
<td>≤10⁰.₅</td>
</tr>
</tbody>
</table>

Three-week-old female Jcl-ICR mice were infected intranasally with 25 μl of the virus indicated and observed for 14 days after infection.

ᵃ) n.t., not tested. ᵇ) LD₅₀, 50% lethal dose. ᵉ) CD₅₀, 50% lung consolidation inducing dose.
Mus musculus molosinus, with respiratory disease in 1979, by Dr. Itoh, Central Institute for Experimental Animals. The Hm strain was isolated from the lung of a golden hamster in 1978 by Dr. Nakayama, IMS. The last 3 strains were given to us in 1981. These three strains had been passaged in eggs 6 to 10 times by the time of the present investigation. The last three passages were made by limiting dilution. Each virus was inoculated into the allantoic cavity of 10- to 11-day-old embryonated hens' eggs and the allantoic fluids were harvested after incubation of the eggs at 34 to 36 C for 2 to 3 days. The virion was purified from infected allantoic fluid by two cycles of linear sucrose density gradient centrifugation as described previously (10). Both allantoic fluids and purified viruses were stored at -80 C before use.

In experimental infection, mice used were three-week-old female Jcl:ICR strain (CLEA Japan Inc., Tokyo) and viral material was infectious allantoic fluid, of which fifty percent tissue culture infective dose (TCID50) per 25 µl was determined using LLC-MK2 cell monolayers in microplate wells as described previously (7). Twenty-five µl of diluted allantoic fluid containing 10^1 to 10^6 TCID50/25 µl of each Sendai virus strain was intranasally inoculated using a micrometer dropper under ether anesthesia into three to four mice per group. Fifty percent mouse lethal dose (LD50) was determined on the basis of mortality during the 14 days after infection. Fifty percent lung consolidation inducing dose (CD50) was based on the gross pathology of lungs at death or at the end of the experiment. Both doses were calculated by the Behrens-Kärber method.

Mortality and incidence of lung consolidation in inoculated mice are shown in Table 1. Death occurred with the MN, KN, or Mol strains. Lung consolidation was observed in almost all mice infected with each of these 3 strains. No death occurred with the Z or Hm. Lung consolidation was observed in the mice infected with higher doses of the Z strain but not with the Hm. Mouse sera collected at the end of the experiment were examined for antibodies to Sendai virus, MN strain, by ELISA as described previously (10). All the survivors showed antibody titers ranging from 1:160 to 1:2,560 in ELISA and the titers did not differ appreciably by infecting virus strains (data not shown).

On the basis of mortality and incidence of lung consolidation, the virulence of the strains was Mol, MN, KN, Z, and Hm in decreasing order. The LD50 and CD50 of the most virulent Mol strain differed by the order of more than 10^3 and 10^6, respectively, from those of the avirulent Hm strain.

To study whether any in vitro properties or nature of the viruses might correlate with the different virulence of the strains, the growth rate in LLC-MK2 cells, virus-associated hemagglutinating (HA) and neuraminidase (NA) activities, and the molecular weight of viral proteins, were examined.

LLC-MK2 cell monolayers grown in 96-well microplates were infected in triplicate with each of the five Sendai virus strains at a multiplicity of infection (m.o.i.) of 0.01 or 1 TCID50/cell in the absence or presence of acetylated-trypsin (3 µg/ml). The culture supernatants were harvested 4, 12, 24, 48, and 72 hr after inoculation and were titrated for TCID50 using LLC-MK2 cells and for HA activity. None of the strains grew in multiple steps in the absence of trypsin (data not shown). As
shown in Fig. 1, at any m.o.i. in the presence of trypsin, kinetics of HA production was not different among the virus strains. However, infectious virus titers reached a plateau by 24 hr after inoculation at an m.o.i. of 1, which was high with the Mol and MN, low with the KN and Hm, and intermediate with the Z. At an m.o.i. of 0.01, infectious titers of the Mol, MN, and Z strains continuously increased during 72-hr period, giving the maximum titer highest for Mol, followed by MN and Z. The KN and Hm reached the maximum titers earlier but the titers were lower than those of the other 3 strains.

The HA titer was increased in proportion to the viral protein concentrations and was not different among the strains (data not shown).
NA activity was assayed principally according to the method of Tozawa et al (17). Results were expressed as optical density at 549 nm. As seen in Fig. 2, the Mol strain had the strongest NA activity, while the Hm strain had the least. The other 3 strains fell between the two.

Next, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of the structural proteins of the virus strains were examined. After SDS-PAGE was carried out principally by the methods of Laemmli (11), gels were stained with 0.2% Coomassie Brilliant Blue R for protein or with the Schiff’s reagent for sugar. Molecular weight was calculated on the basis of SDS-PAGE standards (Bio-Rad, Richmond, Calif., U.S.A.). As shown in Fig. 3, the migration patterns of the 5 major proteins varied from one strain to the other. An obvious difference was observed in P protein. The molecular weight of KN, Z, MN, Mol, and HM was 70, 77, 78, 80, and 81 K, respectively. The molecular weight of the NP and M proteins differed but only slightly among the strains. No significant difference was found in two glycoproteins, HN and F1. A notable difference in molecular weight of the inner structural proteins, P, NP, or M, and a lesser difference in HN and F, were also reported by Sugita (15) and by Etkind et al (2).

The present study revealed that a variation in virulence for mice exists among Sendai virus strains isolated from rodents. These strains showed somewhat different growth rates in cell culture as well as different NA activity and molecular weight of structural proteins especially of P protein. Whether these in vitro properties of the virus relate to the differing virulence to mouse needs further investigation. Nonetheless, the present study clearly revealed that Sendai virus prevailing in laboratory
rodents was not homogenous with respect to the virulence to mice and some other properties.

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REFERENCES


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