INFECTION AND IMMUNITY, Feb. 1983, p. 879–888
0019-9567/83/020879-10$02.00/0

Vol. 39, No. 2

Pneumotropism of Sendai Virus in Relation to Protease-Mediated Activation in Mouse Lungs

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Received 7 June 1982/Accepted 29 October 1982

The pneumotropism of Sendai virus in mice was studied in relation to the activation and replication of the virus in the lung. Inactive Sendai virus grown in LLC-MK_2 cells, which possessed an uncleaved precursor glycoprotein, F, and was noninfectious to tissue culture cells, neither grew nor caused pathological changes in the lung of mice. When trypsin treatment was made which cleaved F into F_1 and F_2 subunits, the virus became activated so that it could initiate replication in the bronchial epithelium of the lung. In this case, the progeny virus was produced in the activated form and multiple-cycle replication occurred successively. A parallel relationship was found between the degree of the viral replication and that of clinical signs of the respiratory disease, body weight loss, and histopathological changes in the lung. A protease mutant, TR-2, which was able to be activated only by chymotrypsin but not by trypsin, could also initiate replication in the bronchial epithelium, when activated by chymotrypsin before inoculation into mice. The progeny virus, however, remained inactive, and the replication was limited to a single cycle, which resulted in the limited lung lesion. The overall results suggest that some activating mechanism for the progeny virus of wild-type Sendai virus exists in the lung of mice and the priniciple (activator) responsible for this phenomenon has a character similar to trypsin. The possible location of the activator is discussed.

Sendai virus is a widespread contaminant in the respiratory tract of mice and often causes outbreaks of death by pneumonia in mouse colonies (24). Experimental infection in laboratory mice has been studied as a model for respiratory viral infection (1–6, 8, 28, 34–38, 46), but information about the pneumotropism of the virus has not been available.

Organ tropism of enterovirus is determined by the distribution of receptors for the virus on host cells (13), whereas the presence of receptors is not a sufficient guarantee of the multiplication of Sendai virus, since its penetration of the cells can be achieved by the fusion of the viral envelope with the host cell membrane (21, 29), for which is responsible a proteolytic cleavage of an envelope glycoprotein F into disulfide-linked glycoprotein subunits, F_1 and F_2 (18, 33, 39). Sendai viruses grown in many established lines of tissue culture cells possessed the uncleaved F precursor and were shown to be inactive; they lacked the ability of envelope fusion and then showed neither infectivity nor hemolysis and cell fusion activities, although they possessed full hemagglutinating and neuraminidase activities (17, 22, 23, 27). These activities were fully restored by mild treatment with trypsin, which was accompanied by the specific cleavage of F (14, 15, 18, 19, 33, 39, 40). On the contrary, the viruses grown in either embryonated chicken eggs or certain primary tissue culture cells were already activated, and multiple-cycle replication of the virus could occur in these hosts (9, 32, 41–43). The cleavage of F glycoprotein was accomplished by some protease(s) in the chorioallantoic fluid (30) or in the tissue culture cells (43). On the basis of these observations, we and other authors indicated that the presence of the activating enzyme(s) for Sendai virus determines the host range and organ tropism of this virus (17, 24, 43). A similar view has been presented about the virulence of Newcastle disease virus (11, 31).

One of us (M.H.) has made a preliminary observation that an inactive Sendai virus grown in L cell culture loses the pathogenicity for mice, whereas an active Sendai virus grown in eggs is pathogenic (16). In the present study, we extend the above observation and examine the pneumotropic mechanism of Sendai virus in mice in relation to the activation and replication of the virus in the lung.

(This paper has been submitted by M.T. as a thesis for the degree of Doctor of Medicine at Yamagata University.)

MATERIALS AND METHODS

Seed virus and cells. The Fushimi strain of Sendai virus grown in the chorioallantoic cavity of 10-day-old embryonated chicken eggs was used as the wild-type seed virus. A monolayer culture of LLC-MK_2 cells (an established cell line of rhesus monkey kidney) was grown in Eagle minimum essential medium (MEM) supplemented with 5% bovine serum.

TR-2. A trypsin-resistant mutant, TR-2, was isolated by infecting LLC-MK_2 cells with the wild-type seed virus in the presence of 1 μg of chymotrypsin per ml and passaging the virus five times in the same system. TR-2 was resistant to trypsin but able to be activated by chymotrypsin, which was accompanied by the cleavage of glycoprotein F into F_1 and F_2 subunits (Table 1 and Fig. 1). No difference was found between the wild-type seed virus and TR-2 in gel patterns of the polypeptides, neuraminidase activity, buoyant density (1.182), or one-step growth profile in LLC-MK_2 cells (data not shown). TR-2 could grow in the chorioallantoic cavity of chicken eggs only when 10 μg of chymotrypsin per egg was added. TR-2 could be favorably compared with pa-cl, a protease-sensitive mutant isolated by Scheid and Choppin (40) whose amino acid sequence was changed so that it was resistant to trypsin but not to chymotrypsin.

Infectivity assays. Two different methods for infectivity assay were used depending on whether the virus was active or inactive. The plaque assay, which could detect both active and inactive viruses, essentially followed the method described by Sugita et al. (44), in which LLC-MK_2 cells were used as host cells, with the agar overlay medium containing 5 μg of trypsin or chymotrypsin per ml. The immunofluorescent-cell-counting assay followed the method described previously (25), except for the use of LLC-MK_2 cells as host cells. This method could detect only activated virus (14).

Preparation of inactive Sendai virus. A monolayer culture of LLC-MK_2 cells was inoculated with egg-grown wild-type seed virus or TR-2 at a multiplicity of infection of 10 PFU/cell. After adsorption for 60 min at 37°C, the cells were washed and fed again with MEM without serum. After incubation for 48 h, the culture fluid was harvested and clarified by a low-speed centrifugation. The infectivity titer of the wild-type seed virus was 4.8×10^9 PFU/ml, and that of TR-2 was 3.8×10^9 PFU/ml when determined in the presence of trypsin and chymotrypsin, respectively. However, when infectivity was determined by the immunofluorescent-cell-counting method, in the absence of the proteases, the titer was 6.9×10^5 cell-infecting units per ml with the wild-type seed virus and 1.7×10^5 cell-infecting units per ml with TR-2. No hemolytic activity was detectable with both viruses.

In vitro activation of inactive Sendai virus. LLC-MK_2 cell-grown wild-type seed virus in MEM was incubated with 4 μg of trypsin per ml for 10 min at 37°C (pH 7.2), and the trypsin action was stopped by adding 8 μg of soybean trypsin inhibitor per ml. Similarly, LLC-MK_2 cell-grown TR-2 was activated by 25 μg of chymotrypsin per ml and the enzymatic action was stopped by 50 μg of the inhibitor per ml. Detailed procedures were described previously (14). The biological activities of these viruses before and after activation by the proteases are shown in Table 1. The restoration of the biological activities by the proteases was accompanied by the specific cleavage of glycoprotein F into F_1 and F_2 (Fig. 1).

UV irradiation of Sendai virus. The virus suspension of the wild-type seed virus in MEM at 1,000 hemagglutinating units (HAU) per ml was activated by trypsin, and samples of 0.5 ml of the virus were poured into petri dishes of 3.5 cm in diameter. UV irradiation was done with a sterilizing lamp (Toshiba GL-15) at a distance of 1 m (3.5×10^{-7} J/mm^2 per s) for various periods.

Hemolysis assay. To 0.5 ml of virus suspension which had been frozen and thawed three times was added 2 ml of 2% chicken erythrocytes in phosphate-buffered saline (PBS), pH 7.2. After adsorption for 10 min at 0°C, the erythrocytes were washed with cold PBS and suspended in 2.5 ml of the same buffer. The suspension was incubated at 37°C for 60 min, and the released hemoglobin was measured by the optical density at 575 nm (15).

Infection of animals. Specific-pathogen-free, 3-week-old male mice of the ICR/CRJ(CD-1) strain weighing 9 to 10 g were purchased from Charles River Japan Inc. They were kept under germfree conditions at 25°C and 55% humidity throughout the experiments. The normal animal sera did not contain hemagglutination inhibition antibody to Sendai virus, which was measured by the standard microtitration method (10), using egg-grown wild-type seed virus as antigen.

Each mouse was inoculated intranasally with 25 μl of virus samples under anesthetization with ether. The animals were investigated daily for clinical signs, body weight, lung consolidation, virus recovery from the lung, and serum hemagglutination inhibition antibody to Sendai virus. The lung consolidations were graded from 1 to 4 according to the extent of lung lesion, and one point was added when the mice died (12). To recover the virus from the lung, 10% homogenates in MEM were made with the aid of a mortar and pestle at 0°C. They were then clarified by centrifugation at $3,000 \times g$ for 15 min, and the supernatants were measured for infectivity either by the plaque method or by the immunofluorescent cell-counting method.

Examination of lung section. The paraffin sections of the lungs were stained with hematoxylin and eosin for histological examinations. For detection of the viral antigens, an immunoperoxidase method described by Taylor (45) was followed; an anti-Sendai virus rabbit serum (2,048 hemagglutination inhibiting units per ml) was diluted 1:400 as the primary antibody. An anti-rabbit immunoglobulin G (IgG) swine serum was used as the secondary antibody after dilution to 1:20. The PAP (horse radish peroxidase-antiperoxidase soluble complexes) was reacted after dilution to 1:100, and the counterstaining of the nuclei was made by hematoxylin. Specificity for the viral antigens was ensured by control studies for each step.

Polyacrylamide gel electrophoresis. Samples were solubilized in 1% sodium dodecyl sulfate and 1% 2-mercaptoethanol by boiling at 100°C for 2 min, and 10% polyacrylamide gels were run by the method of Laemmli (26) followed by staining with Coomassie brilliant blue.

TABLE 1. In vitro activation of Sendai virus grown in LLC-MK_2 cells

Virus	Treatment	HAU/ml	Hemolytic activity (optical density at 575 nm [×10])	Infectivity[a] (CIU/HAU)
Wild-type seed virus	None	1,024	0.01	6.8×10^2
	Trypsin[b]	1,024	1.24	5.7×10^6
	Chymotrypsin[c]	512	0.09	8.2×10^4
TR-2	None	1,024	0.00	1.7×10^2
	Trypsin[b]	1,024	0.00	1.3×10^2
	Chymotrypsin[c]	512	1.04	3.8×10^6

[a] Assayed by the immunofluorescent cell-counting method (25). CIU, Cell-infecting units.
[b] Treated with 4 μg of trypsin per ml for 10 min at 37°C, pH 7.2
[c] Treated with 25 μg of chymotrypsin per ml for 10 min at 37°C, pH 7.2.

Chemicals and immunoperoxidase reagents. Lyophilized trypsin (thrice crystallized) was obtained from Worthington Diagnostics, Freehold, N.J. α-Chymotrypsin (type II) and soybean trypsin inhibitor were obtained from Sigma Chemical Co., St. Louis, Mo. Components for the polyacrylamide gel were obtained from Nakarai Chemical Ltd., Osaka, Japan. Anti-rabbit IgG swine serum and PAP were obtained from DAKO-immunoglobulin Ltd., Denmark.

RESULTS

Loss of body weight by Sendai virus infection. Serial 10-fold dilutions of both the inactive and the active wild-type seed viruses were inoculated into mice, and the animals were weighed daily thereafter (Fig. 2). The mice which received the active virus at less than 1 HAU/ml gained regularly in weight, whereas those receiving the virus at more than 10 HAU/ml lowered the rate of the increase of body weight on the next day after infection and began to lose the weight on the 3rd day. The latter mice then developed signs of respiratory infection, such as nasal discharge, breathing crepitation, cyanosis, and dyspnea. Most of the diseased mice died within 1 week, although some escaped death. On the contrary, when the inactive virus was administered, neither the loss of body weight nor the clinical signs were evident, except that a slight retardation of the increase in body weight was observed with the mice inoculated with the virus at 1,000 HAU/ml. This might be due to the active virus present in the inactive virus preparation at a ratio of about 1:10,000 (Table 1). It is worth mentioning that, although the reason is not known, the retardation of the increase in body weight in the infected mice was reproducible and parallel with the development of the clinical signs of the respiratory disease. It is a good indication of pathogenicity for mice and suitable for quantitative analysis of morbidity.

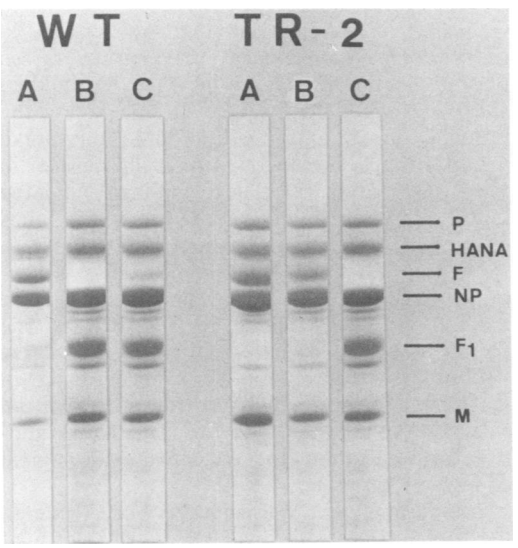

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic patterns of the wild-type seed virus and TR-2 before and after activation by the protease. The inactive viruses of the wild type and TR-2 were prepared in LLC-MK_2 cells (lanes A). They were treated with either trypsin at 4 μg/ml (lanes B) or chymotrypsin at 25 μg/ml (lanes C) at 37°C for 10 min in phosphate-buffered saline, pH 7.2. A sodium dodecyl sulfate-polyacrylamide slab gel was run under a Tris-glycine buffer system (26). Nomenclature for the viral polypeptides followed a proposal by Homma et al. (20). F_2 is not seen in this figure.

Comparison of the pathogenicity of the inactive and the active Sendai viruses in mouse lung. As will be discussed below, the intranasal infection of mice with Sendai virus exclusively caused pathological changes in respiratory organs. To see the difference between the inactive and the active viruses in the pathogenicity to the lungs of mice in detail, each virus at 10 HAU/ml was inoculated intranasally, and the viral replication and lung lesions were investigated daily. All of the mice inoculated with the active virus developed the disease. During 2 to 3 days after inoculation, the lung became swollen and congestive, and then focal consolidation, visible on days 4 to 5, became manifest on day 8. When the mice survived, the lung lesion improved thereafter. Serum hemagglutination inhibition antibody

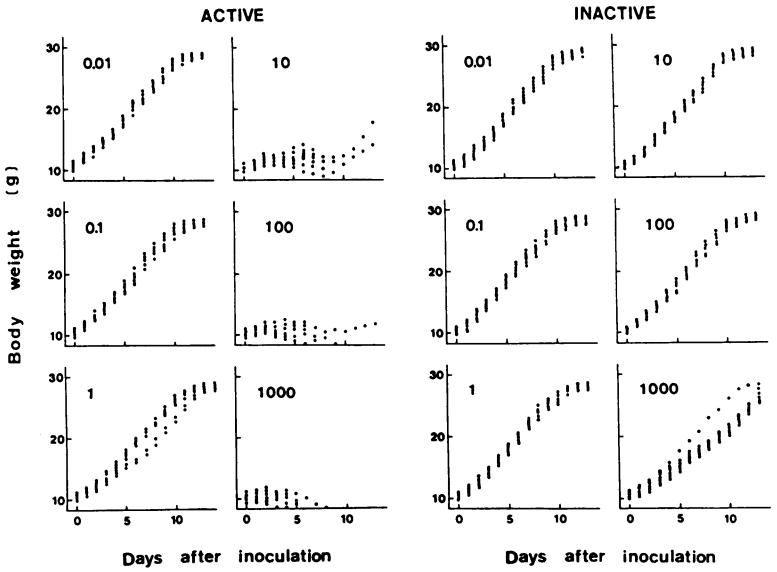

FIG. 2. Time course of the body weight of mice after inoculation with Sendai virus. Wild-type seed virus grown in LLC-MK_2 cells was used before (inactive) and after (active) activation with 4 μg of trypsin per ml at 37°C (pH 7.2) for 10 min. Each virus was diluted 10-fold serially to contain various amounts of the virus from 1,000 to 0.01 HAU/ml. A 25-μl amount of each dilution was inoculated intranasally into a 3-week-old mouse of strain ICR/CRJ(CD-1), and the animal was weighed every day. For each dilution, five to six mice were used.

could be detected on day 5, and the titer increased thereafter up to 256 hemagglutination inhibiting units per ml. The virus growth in the lung occurred soon after the inoculation and reached a maximum on day 4. The virus titer began to decrease at the time when the antibody started to be detected (Fig. 3A). In contrast to the active virus, clinical signs of the respiratory disease, replication of the virus, and the consolidation in lungs were not observed with the mice inoculated with the inactive virus. The serum hemagglutination inhibition antibody titer rose considerably, but the response was slower than the case of the active virus (Fig. 3B).

Histology and virological examinations on the lung section. Histological changes were readily detectable on the next day after inoculation with the active virus, where a slight degree of peribronchial infiltration with large mononuclear cells and swelling of the bronchial epithelium were seen (Fig. 4A and E). On day 3, the peribronchial infiltration was pronounced, and bleeding into the interstitium and degenerative changes of the bronchial epithelium were apparent (Fig. 4B and F). On days 5 to 7, the cell infiltration and the bleeding were increased, and the venous congestion and the destruction of the bronchial epithelium were dominantly seen (Fig. 4C and G).

Immunoperoxidase studies revealed that the viral antigens were detectable only in the epithelial cells of the trachea, bronchus, and bronchiolus but not in those of the alveolus (Fig. 4E to G). In the bronchial epithelium, the viral antigens were condensed at the free surface of the plasma membrane, suggesting that the budding site was limited to the cell surface facing the bronchial lumen and infection could not spread inside through submucosa, as in the case of in vitro observation (7). These findings indicated that the target cells of Sendai virus in the lungs of mice are confined to bronchial epithelium.

Neither histological change nor development of the viral antigens was demonstrable when the inactive virus was inoculated (Fig. 4D and H); the fact suggested that the incoming inactive wild-type seed virus could not be activated in the lumen of the respiratory tract and that the activation of the inoculum virus was a prerequisite for replication and to cause pathological changes in the lung.

Pathogenicity of UV-irradiated virus. Although the foregoing results revealed the necessity of the activation of the virus before infection to cause the lung lesion, they do not exclude the possibility that noninfectious physical particles of the active form of Sendai virus are enough to cause the pathological changes. To explore the problem, the wild-type seed virus, previously activated by trypsin, was UV irradiated for various periods, and the virus at 1,000 HAU/ml was inoculated into mice. Partially inactivated (2-min UV-irradiated) virus caused a certain degree of virus growth and pathological changes

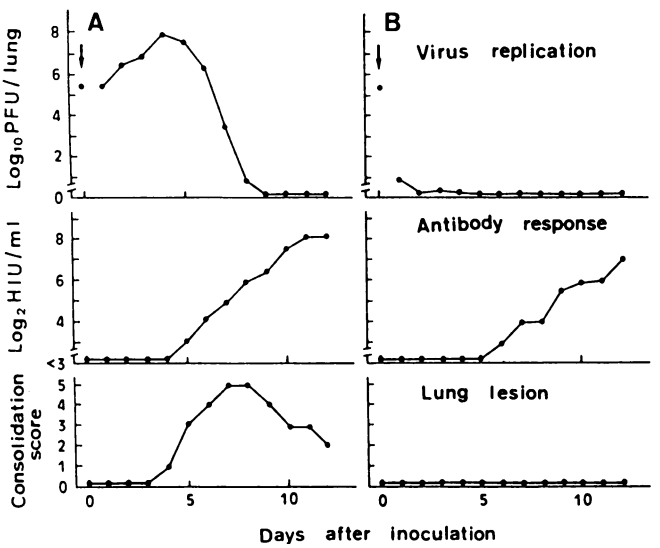

FIG. 3. Time course of viral replication in the lung, antibody response, and lung lesion in mice after inoculation with Sendai virus. The preparation of the viruses was the same as described in the legend to Fig. 2. Each mouse was inoculated intranasally with 25 μl of the active (A) and the inactive (B) wild-type seed virus at 10 HAU/ml. Part of the mice were sacrificed every day, and 10% lung homogenates were made for infectivity assays by the plaque method in the presence of 5 μg of trypsin per ml in the agar overlay medium. The arrow indicates the amount of the virus inoculated per mouse. Measurements of the serum hemagglutination inhibition antibody titer and the lung lesion were described in the text. Each plot is the mean of four to five mice.

in the lung, whereas totally inactivated (30-min UV-irradiated) virus did not (data not shown). The results indicated that the replication of the virus is necessary to cause lung lesion in mice.

Replication of the wild-type seed virus in the lungs of mice. To examine whether the progeny virus produced in the lungs of mice was inactive or already activated, the infectivity was measured either by the immunofluorescent cell-counting method or by the plaque method as described above. Figure 5 shows the time course of the progeny viruses produced in the lungs of mice inoculated with the active wild-type seed virus at 100 or 0.01 HAU/ml. The infectivity of the progeny virus could be measured equally by the two methods at any given time, indicating that the progeny virus of the wild-type seed virus was produced as an activated form. It was also apparent that multiple-cycle replication occurred in the lung when a low dose of the wild-type seed virus was administered (Fig. 5B). This was also supported by the immunoperoxidase studies on the lung sections; the infected cells were hardly seen in the bronchial epithelium on the next day but were increased in number on days 3 to 7 (data not shown). However, the pathological changes in the lung were limited and developed slowly, and the mice apparently remained healthy. These results suggest that some activating mechanism for the progeny virus exists in the lungs of mice by which viral replication proceeds in multiple steps. A possibility that the activation of the virus might occur during the preparation of the lung homogenates could be denied by the fact that the UV-irradiated lung homogenates made from the mice infected with the active wild-type seed virus at 100 HAU/ml for 3 days could not activate the inactive wild-type seed virus (data not shown).

Infection of mice with TR-2. Because the in vitro activation of the wild-type seed virus was caused by the specific cleavage of glycoprotein F by trypsin (18, 33, 40, 41) or a trypsin-like protease (30), the activating principle for the wild-type seed virus in the lungs of mice, if it exists, might have a character similar to that of trypsin. To verify this view and search into the pneumotropism of Sendai virus, a trypsin-resistant mutant, TR-2, was isolated as described above.

Mice were inoculated with TR-2 which had been activated by chymotrypsin at 100 or 0.01 HAU/ml and examined as in the case of the wild-type seed virus (Fig. 6). The mice did not show any sign of the respiratory disease and gained in weight at a nearly normal rate. In the mice which received large doses of the virus at 100 HAU/ml, considerable amounts of the virus were detected. Since the virus was detectable only by the plaque method in the presence of chymotrypsin but not by the immunofluorescent cell-counting method (Fig. 6A), the virus thus recovered was

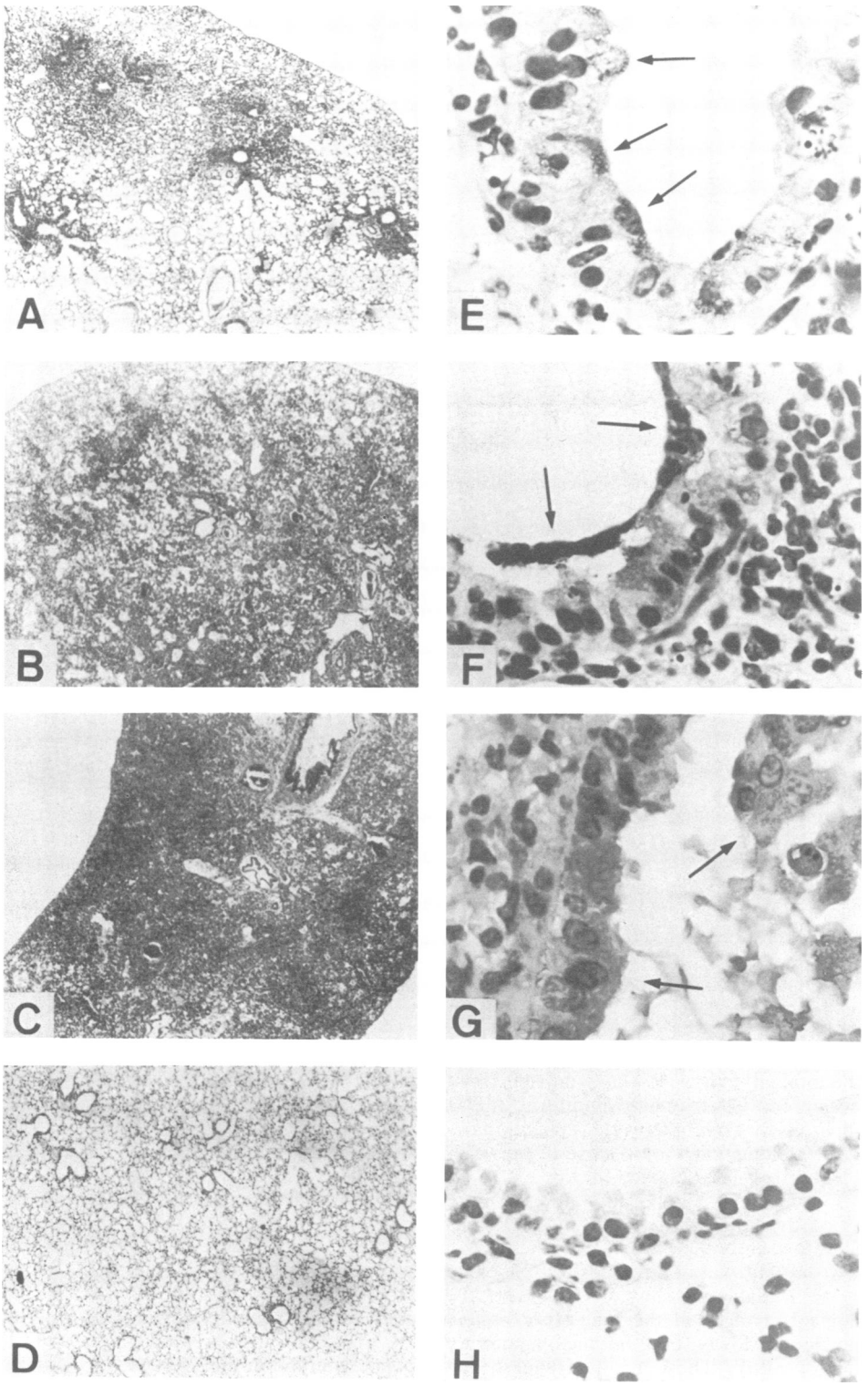
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FIG. 4. Histology and immunoperoxidase staining for Sendai virus antigens on the lung sections of mice inoculated with Sendai virus at 10 HAU/ml. The preparation of the viruses was the same as described in the legend to Fig. 2. (A to D) Staining with hematoxylin and eosin, ×20. (E to H) PAP-immunoperoxidase staining, × 1,000. (A) At day 1 after inoculation with the active wild-type seed virus. Peribronchial cellular infiltration is apparent. (B) At day 3 after inoculation with the active wild-type virus. Cellular infiltrations and bleeding into the interstitium are seen. (C) At day 7 after inoculation with the active wild-type seed virus. The cellular infiltrations, bleeding, and venous congestions are distinct. (D) At day 7 after inoculation with the inactive wild-type seed virus. No pathological changes are seen. (E) The same section as (A). Bronchial epitheliums and the nuclei are swollen, and the viral antigens are seen only in the epitheliums (arrows). (F) The same section as (B). The viral antigens are accumulated at the free surface of the bronchial epitheliums (arrows), in which degenerative changes are remarkable. (G) The same section as (C). Degenerative epitheliums with the viral antigens are sloughed off into the bronchial lumen (arrows). Viral antigens are not detectable either in the subepithelial cells or in the infiltrating mononuclear cells. (H) The same section as (D). Neither pathological changes nor the viral antigens are seen.

an inactive form of the TR-2 progeny but not the residual fraction of the inoculum. The results also imply that there is no activating mechanism of the progeny virus of TR-2 in the lung and the incoming activated TR-2 can replicate only in a single cycle in the lung. This is consistent with the fact that the virus growth was very limited when the small dose of the active TR-2 was applied (Fig. 6B). These growth patterns of TR-2 were in good contrast to those of the wild-type seed virus (Fig. 5). In parallel with the results of virus growth, a certain but limited number of the pathological changes were found when the large dose of TR-2 was given. The immunoperoxidase staining could also detect a limited number of the viral antigen-positive cells in the bronchial epithelium as early as 24 h after infection, but further increase of the antigen-positive cells was not evident (data not shown).

DISCUSSION

Sendai virus grown in LLC-MK_2 cells neither grew nor caused pathological changes in the lungs of mice, indicating that the virus was inactive in vitro and in vivo. Trypsin treatment, which activated the virus in vitro system by cleaving precursor F into subunits F_1 and F_2 (14, 18, 33, 39), also activated the virus in vivo system of the lungs of mice. This has revealed an analogy of the growth characteristics of Sendai virus in vitro with those in vivo as far as the lungs of mice are concerned. However, when mice were infected with small doses of the trypsin-activated wild-type seed virus at 0.01 HAU/ml, virus replication could occur in multiple cycles (Fig. 5B), suggesting that some activating principle (activator) for the progeny virus exists in the lung. The question may then arise as to why the activator could not activate the incoming inactive wild-type seed virus so that it could initiate the infection. A plausible explanation is that the activation does not occur in the bronchial lumen, but the activation of the progeny virus occurs either on the surface or inside the bronchial epithelium, which is the target of Sendai virus in the lungs of mice (Fig. 4). Similar observations have been made in vitro; some primary tissue culture cells could support multiple-cycle replication of the virus without adding trypsin to the culture media (9, 32, 41–43). It

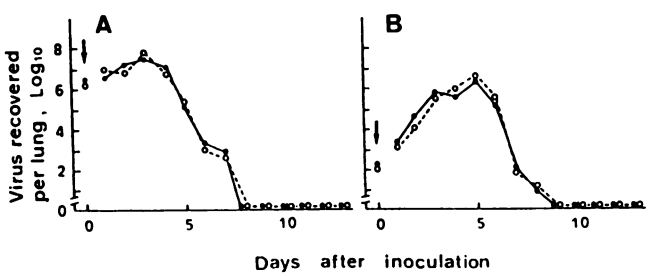

FIG. 5. Time course of the viral replication in the lungs of mice after inoculation with Sendai virus. The preparation of the active wild-type seed virus was described in the legend to Fig. 2. Each mouse was inoculated with 25 μl of the activated wild-type seed virus at 100 HAU/ml (A) or 0.01 HAU/ml (B). Part of the mice were sacrificed every day, and the lung homogenates were prepared as described in the legend to Fig. 3. The infectivity in the lung homogenates was measured by the plaque method as described in the legend to Fig. 3 (●) and by the immunofluorescent-cell-counting method as described in the text (○). The arrows indicate the amount of the virus in the inoculum. Each plot is the mean of four to five mice.

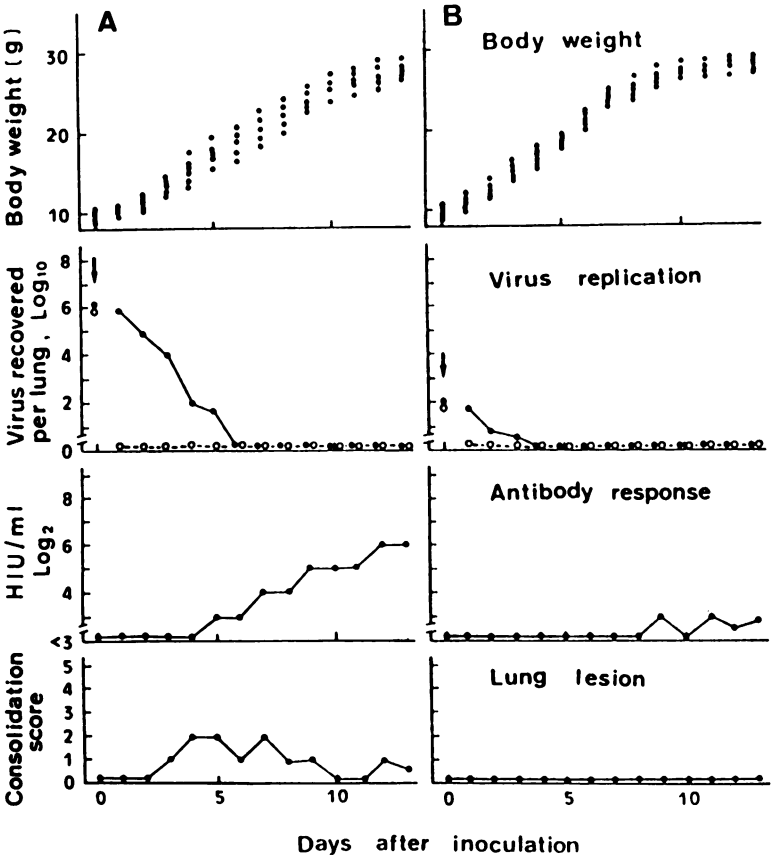

FIG. 6. Time course of the body weight, virus replication in the lung, hemagglutination inhibition antibody response, and lung lesion in mice after inoculation with the active TR-2. The preparation and activation of TR-2 were done as described in the text. Each mouse was inoculated with 25 μl of the active TR-2 at 100 HAU/ml (A) or 0.01 HAU/ml (B) and examined daily for body weight. Part of the mice were sacrificed every day and were measured for the infectivity in the lung, serum hemagglutination inhibition antibody, and lung lesion as described in the legend to Fig. 3, except that the infectivity was assayed by the plaque method in the presence of 5 μg of chymotrypsin per ml instead of trypsin (●) and by the immunofluorescent-cell-counting method (○). Arrows indicate the amount of the virus in the inoculum. Each plot is the mean of four to five mice.

could not be generated, however, by the inactive virus (43). On the basis of some previous observations (30, 40), a trypsin-like protease was predicted as a most likely candidate for the activator. A trypsin-resistant mutant, TR-2, could replicate in the lungs of mice only in a single cycle when activated by chymotrypsin before infection (Fig. 6), suggesting that an activator(s) for the TR-2 progeny does not exist in the lung. The result implies that the activator has a character similar to that of trypsin rather than that of chymotrypsin. Although attempts to detect the activating enzyme directly from the lung homogenates have failed, we could find the proteolytic activation of the virus in a culture system using mouse lung blocks devised specifically for this purpose (manuscript in preparation).

In the establishment of the lung lesion in mice due to Sendai virus infection, many factors, e.g., immunological responses (1, 4–6, 36), difference of mouse strain (34), aging (36, 37), etc., should be considered. Nevertheless, the present study revealed that the pathological changes which have been characterized principally as peribronchitis, similarly to those reported previously (3, 5, 6, 8, 28, 36), were induced subsequent to the replication of the virus in the bronchial cells. The extent of the lung lesion was directly influenced by the number of the bronchial cells infected with the virus. The infection with the 2-min-UV-irradiated virus or with a low dose of the wild-type seed virus (Fig. 5B) caused a considerable delay and reduction in virus growth, which resulted in a limited number of the infected bronchial cells. Similarly, the num-

ber of the infected cells was limited when mice were infected even with large doses of the active TR-2 which was allowed to replicate in the lung only in a single cycle (Fig. 6). These observations suggest that the infected bronchial cells should attain a certain number during the short period after infection to cause marked pathological changes which can be manifested as clinical signs and reduction in body weight. To induce such changes, the inoculum should contain a large number of the virus particles, the virus should be activated before infection, and the progeny virus should also be activated by the activator present in the lungs of mice.

The receptors for Sendai virus were shown to be distributed in various organs of mice, e.g., gastrointestinal tract, kidney, spleen, heart, brain, and lymphnodes (Y. Ito et al., Abstr. 28th General Meeting of the Society of Japanese Virologists, Kurume, 1980, p. 2027). In our preliminary experiments with mice which received either intranasal or intraperitoneal inoculation with Sendai virus, neither replication of the virus nor pathological change in organs, such as kidney and spleen (other than lung), was detectable although viremia was evident (unpublished data). These observations may suggest that the pneumotropism of Sendai virus is not determined merely by the presence of the receptors or by the route of the virus administration. The present results rather suggest that the presence of the specific activator(s) in lungs which was responsible for the multiple-step replication determines the pneumotropism of Sendai virus in mice.

ACKNOWLEDGMENTS

We gratefully acknowledge T. Kasajima of the Department of Pathology, Yamagata University School of Medicine, for his technical aid and valuable discussions on histology and immunoperoxidase studies. Our thanks are also due to K. Ohwada of the Animal Center, Yamagata University School of Medicine, for the animal care.

This research was supported, in part, by a Grant-in-Aid for Scientific Research and a Grant-in-Aid for the Encouragement of a Young Scientist from the Ministry of Education, Science and Culture, Japan, and the Naitoh Foundation Research Grant for 1980.

LITERATURE CITED

1. **Anderson, M. J., D. R. Bainbridge, J. R. Pattison, and R. B. Heath.** 1977. Cell-mediated immunity of Sendai virus infection in mice. Infect. Immun. **15**:239–244.
2. **Anderson, M. J., J. R. Pattison, R. J. R. Cureton, S. Argent, and R. B. Heath.** 1980. The role of host responses in the recovery of mice from Sendai virus infection. J. Gen. Virol. **46**:373–379.
3. **Appell, L. H., R. M. Kovatch, J. M. Reddecliff, and P. J. Gerone.** 1971. Pathogenesis of Sendai virus infection in mice. Am. J. Vet. Res. **32**:1835–1841.
4. **Blandford, G.** 1975. Studies on the immune response and pathogenesis of Sendai virus infection of mice. III. The effect of cyclophosphamide. Immunology **28**:871–883.
5. **Blandford, G., R. J. R. Cureton, and R. B. Heath.** 1971. Studies of the immune response in Sendai virus infection in mice. J. Med. Microbiol. **4**:351–355.
6. **Blandford, G., and R. B. Heath.** 1972. Studies on the immune response and pathogenesis of Sendai virus infection of mice. I. The fate of viral antigens. Immunology **22**:637–649.
7. **Boulan, E. R., and D. D. Sabatini.** 1978. Asymmetric budding of viruses in epithelial monolayer: a model system for study of epithelial polarity. Proc. Natl. Acad. Sci. U.S.A. **75**:5071–5075.
8. **Carthew, P., and S. Sparrow.** 1980. A comparison in germfree mice of the pathogenesis of Sendai virus and mouse pneumonia virus infections. J. Pathol. **130**:153–158.
9. **Darlington, R. W., A. Portner, and D. W. Kingsbury.** 1970. Sendai virus replication: an ultrastructural comparison of productive and abortive infections in avian cells. J. Gen. Virol. **9**:169–177.
10. **Dowdle, W. A., A. P. Kendal, and G. R. Noble.** 1979. Influenza virus, p. 585–609. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral, rickettsial and chlamydial infections, 5th ed. American Public Health Association, Washington, D.C.
11. **Garten, W., W. Berk, Y. Nagai, R. Rott, and H.-D. Klenk.** 1980. Mutational changes of the protease susceptibility of glycoprotein F of Newcastle disease virus: effect on pathogenicity. J. Gen. Virol. **50**:135–147.
12. **Ginsberg, H. S., and F. L. Horsfall.** 1952. Quantitative aspects of the multiplication of influenza A in the mouse lung. J. Exp. Med. **95**:135–145.
13. **Holland, J. J., and B. H. Hoyer.** 1962. Early stage of enterovirus infection. Cold Spring Harbor Symp. Quant. Biol. **27**:101–111.
14. **Homma, M.** 1971. Trypsin action on the growth of Sendai virus in tissue culture cells. I. Restoration of the infectivity for L cells by direct action of trypsin on L cell-borne Sendai virus. J. Virol. **8**:619–629.
15. **Homma, M.** 1972. Trypsin action on the growth of Sendai virus in tissue culture cells. II. Restoration of the hemolytic activity of L cell-borne Sendai virus by trypsin. J. Virol. **9**:829–835.
16. **Homma, M.** 1972. Host-controled modification in HVJ (Sendai virus), p. 23–34. *In* Working conference on newer approaches to investigation and diagnosis of viral diseases. The Japan-United States cooperative medical science program. Sapporo, Japan.
17. **Homma, M.** 1975. Host-induced modification of hemagglutinating virus of Japan (HVJ, Sendai virus). Virus (Tokyo) **25**:7–18.
18. **Homma, M., and M. Ohuchi.** 1973. Trypsin action on the growth of Sendai virus in tissue culture cells. III. Structural differences of Sendai viruses grown in eggs and tissue culture cells. J. Virol. **12**:1457–1463.
19. **Homma, M., and S. Tamagawa.** 1973. Restoration of fusion activity of L cell-borne Sendai virus by trypsin. J. Gen. Virol. **19**:423–426.
20. **Homma, M., H. Tozawa, K. Shimizu, and N. Ishida.** 1975. A proposal for designation of Sendai virus proteins. Jpn. J. Microbiol. **19**:467–470.
21. **Howe, C., and C. Morgan.** 1969. Interaction between Sendai virus and human erythrocytes. J. Virol. **3**:70–81.
22. **Ishida, N., and M. Homma.** 1960. A variant Sendai virus, infectious to egg embryos but not to L cells. Tohoku J. Exp. Med. **73**:56–69.
23. **Ishida, N., and M. Homma.** 1961. Host-controlled variation observed with Sendai virus grown in mouse fibroblast (L) cells. Virology **14**:486–488.
24. **Ishida, N., and M. Homma.** 1978. Sendai virus. Adv. Virus Res. **23**:349–382.
25. **Kashiwazaki, H., M. Homma, and N. Ishida.** 1965. Assays of Sendai virus by immunofluorescence and hemadsorbed cell-counting procedures. Proc. Soc. Exp. Biol. Med. **120**:134–138.
26. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
27. **Matsumoto, T., and K. Maeno.** 1962. A host-induced

modification of hemagglutinating virus of Japan (HVJ, Sendai virus) in its hemolysis and cytopathic activity. Virology **17**:563–570.

28. **Mims, C. A., and F. A. Murphy.** 1973. Parainfluenza virus Sendai infection in macrophages, ependyma, choroid plexus, vascular endothelium and respiratory tract of mice. Am. J. Pathol. **70**:315–328.
29. **Morgan, C., and C. Howe.** 1968. Structure and development of viruses as observed in the electron microscope. IX. Entry of parainfluenza I (Sendai) virus. J. Virol. **2**:1122–1132.
30. **Muramatsu, M., and M. Homma.** 1980. Trypsin action on the growth of Sendai virus in tissue culture cells. V. An activating enzyme for Sendai virus in the chorioallantoic fluid of the embryonated chicken egg. Microbiol. Immunol. **24**:113–122.
31. **Nagai, Y., K. Shimokata, T. Yoshida, M. Hamaguchi, M. Iinuma, K. Maeno, T. Matsumoto, H.-D. Klenk, and R. Rott.** 1979. The spread of a pathogenic and an apathogenic strain of Newcastle disease virus in the chick embryo as depending on the protease sensitivity of the virus glycoproteins. J. Gen. Virol. **45**:263–272.
32. **Nagata, I., K. Maeno, S. Yoshii, and T. Matsumoto.** 1965. Plaque formation by HVJ in calf kidney cells. Arch. Gesamte Virusforsch. **15**:257–259.
33. **Ohuchi, M., and M. Homma.** 1976. Trypsin action on the growth of Sendai virus in tissue culture cells. IV. Evidence for activation of Sendai virus by cleavage of a glycoprotein. J. Virol. **18**:1147–1150.
34. **Parker, J. C., M. D. Whiteman, and C. B. Richter.** 1978. Susceptibility of inbred and outbred mouse strains to Sendai virus and prevalence of infection in laboratory rodents. Infect. Immun. **19**:123–130.
35. **Robinson, T. W. E., R. J. R. Cureton, and R. B. Heath.** 1968. The pathogenesis of Sendai virus infection in the mouse lung. J. Med. Microbiol. **1**:89–95.
36. **Robinson, T. W. E., R. J. R. Cureton, and R. B. Heath.** 1969. The effect of cyclophosphamide on Sendai virus infection of mice. J. Med. Microbiol. **2**:137–145.
37. **Sawicki, L.** 1961. Influence of age of mice on the recovery from experimental Sendai virus infection. Nature (London) **192**:1258–1259.
38. **Sawicki, L.** 1962. Studies on experimental Sendai virus infection in laboratory mice. Acta Virol. **6**:347–351.
39. **Scheid, A., and P. W. Choppin.** 1974. Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis, and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. Virology **57**:475–490.
40. **Scheid, A., and P. W. Choppin.** 1976. Protease activation mutants of Sendai virus. Activation of biological properties by specific proteases. Virology **69**:265–277.
41. **Shibuta, H., M. Akami, and M. Matsumoto.** 1971. Plaque formation by Sendai virus of parainfluenza virus group, type I on monkey, calf kidney and chick embryo cell monolayers. Jpn. J. Microbiol. **15**:175–183.
42. **Shigeta, S.** 1964. Plaque formation and growth characteristics of Sendai virus in chick kidney cell cultures. Tohoku J. Exp. Med. **83**:114–120.
43. **Silver, S. M., A. Scheid, and P. W. Choppin.** 1978. Loss on serial passage of rhesus monkey kidney cells of proteolytic activity required for Sendai virus activation. Infect. Immun. **20**:235–241.
44. **Sugita, K., M. Maru, and K. Sato.** 1974. A sensitive plaque assay for Sendai virus in an established line of monkey kidney cells. Jpn. J. Microbiol. **18**:262–264.
45. **Taylor, C. R.** 1974. The nature of Reed-Sternberg cells and other malignant "Reticulum" cells. Lancet **ii**:802–806.
46. **van Nunen, M. C. J., and J. van der Veen.** 1967. Experimental infection with Sendai virus in mice. Arch. Gesamte Virusforsch. **22**:388-397.