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Sendai virus infection changes the subcellular localization of tryptase Clara in rat bronchiolar epithelial cells

K. Sakai*, T. Kohri*, M. Tashiro+, Y. Kishino*, H. Kido**

Sendai virus infection changes the subcellular localization of tryptase Clara in rat bronchiolar epithelial cells. K. Sakai, T. Kohri, M. Tashiro, Y. Kishino, H. Kido. ©ERS Journals Ltd 1994.

ABSTRACT: Tryptase Clara activates the infectivity of Sendai and influenza viruses proteolytically. In this study, we investigated changes in the subcellular localization of tryptase Clara in rat bronchioles with progression of Sendai virus infection. Tryptase Clara and Sendai virus F2 antigen were localized by light and electron immunohistochemical studies.

In the uninfected rat lung, tryptase Clara was specifically localized in the secretory granules of respiratory bronchiolar epithelial nonciliated cells, but not in bronchiolar ciliated, or alveolar cells. In the initial stage of Sendai virus infection with slight pathological changes, however, anti-tryptase Clara was highly reactive in luminal peripheral membranes of both nonciliated and ciliated epithelial cells of the bronchioles together with some Sendai virus envelope glycoprotein, F2 antigen. In the progressed stage, tryptase Clara was hard to detect, with heavy accumulation of F2 antigen in the epithelial cells.

These immunohistochemical results support our previous findings that in the bronchial lavage fluid tryptase Clara is significantly increased both in amount and activity after viral infection. These results suggest that Sendai virus stimulates the secretion of tryptase Clara from nonciliated bronchiolar epithelial cells to the airway lumen. Accumulation of tryptase Clara on the luminal surface of the bronchiolar epithelial cells and/or in the airway lumen may produce favourable conditions for proteolytic viral activation and multiplication.

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*Dept of Nutrition, School of Medicine, and **Division of Enzyme Chemistry, Institute for Enzyme Research, The University of Tokushima, Tokushima, Japan. +Dept of Virology, Jichi Medical School, Minami-Kawachi, Tochigi, Japan.

Correspondence: K. Sakai
Division of Rheumatology, Allergy, and Immunology
Medical College of Virginia
Virginia Commonwealth University
MCV Station, Box 263
Richmond
Virginia 23298
USA

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The pathogenicity of Sendai and influenza viruses is considered to be determined primarily by a host-cell protease(s) that activates viral infectivity by proteolytic cleavage of the envelope glycoproteins, fusion glycoprotein (F) and haemagglutinin (HA) [1–7]. These viruses infect nasal, tracheal, bronchial and bronchiolar epithelial cells and some alveolar epithelial cells [8, 9], suggesting the presence of a specific trypsin-like protease(s) in the respiratory tract, that converts the envelope glycoprotein precursors to mature forms with membrane fusion activity.

Recently, we isolated a novel trypsin-type serine protease, named tryptase Clara, from the rat lung [10]. This protease is localized in the secretory granules of nonciliated secretory Clara cells in the bronchi and bronchioles of rats [11], and some of the enzyme in the granules is normally secreted into the airway lumen [10]. Purified tryptase Clara converts F0 of Sendai virus to subunits F1 and F2, and HA of influenza A/Aichi/2/68 (H3N2) virus to subunits HA1 and HA2. It also activates the infectivities of these viruses dose-dependently *in vitro* and in organ cultures of rat lung [10, 12]. Intranasal instillation of antibody against tryptase Clara inhibits Sendai virus activation and multiple cycles of viral replication in the lungs of infected rats [12]. These findings suggest that tryptase Clara is a primary host factor involved in the pneumopathogenicity of Sendai virus and that proteolytic viral activation occurs extracellularly in the respiratory lumen [12]. However, the precise mechanism of pneumotropic virus activation by tryptase Clara *in vivo* has not been clarified. To elucidate the mechanism of Sendai virus activation by tryptase Clara *in vivo* and the pathophysiological role of tryptase Clara in the respiratory tract after infection of pneumotropic viruses, we examined the subcellular localization of tryptase Clara in respiratory epithelial cells from rats infected with Sendai virus by light and electron immunohistochemical studies.

Here we report changes in the subcellular localization of tryptase Clara with progress of Sendai virus infection in the rat lung. We also discuss the role of tryptase Clara in the mechanism of Sendai virus activation *in vivo*.

Materials and methods

Antibodies

Antibody against tryptase Clara from rat lung was prepared as described in detail previously [10]. Antibody

against the Sendai virus F2 subunit, designated F2C12, was raised against a synthetic peptide composed of 13 amino acids, NDTTQNAGAPQSC, that correspond to the carboxyl terminus of the F2 subunit (residues 104 to 116), except that the carboxyl terminal arginine was replaced by cysteine. This allowed formation of a peptide-Keyhole Limpet haemocyanin conjugate using sulphosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate [13]. The conjugate (1 mg) in 1 ml of saline was emulsified with an equal volume of Freund's complete adjuvant and injected subcutaneously into New Zealand White rabbits (Japan SLC; Shizuoka, Japan). Monospecific antibody against the peptide was purified by affinity chromatography on a column containing antigen peptide-coupled Sepharose 4B (Pharmacia LKB Biotechnology; Uppsala, Sweden).

Virus and cells

Active (infectious) Sendai virus (Z strain) was propagated, purified, and assayed for infectivity as described previously [1, 14]. Inactive (noninfectious) virus was prepared in LLC-MK cells, an established line of monkey kidney cells, in the absence of protease in culture medium as described previously [1, 15, 16].

Electrophoresis and Western immunoblotting

For Western blot analysis, inactive and activated Sendai viruses were subjected to electrophoresis in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (10–20% gradient) under reducing conditions by the method of LAEMMLI [17], and transferred electrophoretically to an Immobilon transfer membrane (Millipore, Tokyo, Japan). The membrane was soaked in 1% bovine serum albumin (BSA) to block nonspecific binding, and then incubated for 15 h at room temperature with 1:200 dilution of an affinity purified F2C12 antibody (0.8 $mg \cdot ml^{-1}$). Bound antibodies were detected by the anti-rabbit alkaline phosphatase method (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions. Prestained SDS-PAGE low range standards (Bio-Rad, Richmond, CA, USA) were used as molecular weight markers.

Protein measurement

The protein concentration was measured with bicinchoninic acid (Pierce Chemical Co., Rockford, IL, USA) by the method of SMITH *et al*. [18].

Infection of rats with Sendai virus

Specific-pathogen-free male Wistar strain rats (n=6) (Japan SLC) weighing 60–80 g were infected intranasally with 2×10^4 plaque forming units of activated virus [12]. The rats were sacrificed 24 h after infection and their lungs were stained immunohistochemically. Control rats (n=6) were inoculated with saline and their lungs were examined in the same manner.

Immunohistochemical staining for light microscopy

Rats were anaesthetized with an intraperitoneal injection of sodium pentobarbital and 200 units of heparin solution in 0.2 ml of saline was injected into the inferior vena cava. A cannula was inserted through the inferior vena cava to the right atrium of the heart. The lungs were perfused at 20 cmH_2O pressure with Ca^{2+}- and Mg^{2+}-free Dulbecco's phosphate buffered saline (Gibco; Grand Island, NY, USA) *via* the cannula, as described previously [10, 11, 19]. The lungs were then fixed by tracheal instillation and lung perfusion at 20 cmH_2O pressure with 10% buffered formalin pH 7.2, excised, fixed in the same fixative for 24 h at 4°C and embedded in paraffin.

Three serial lung sections of 4 µm thickness were placed on glass slides coated with 1% aqueous gelatin. One of them was stained with haematoxylin and eosin (HE) and the others were stained immunohistochemically by the avidin-biotin-peroxidase complex method [20, 21]. For immunohistochemical staining, sections were deparaffinized and soaked in 0.3% hydrogen peroxide in absolute methanol for 30 min at room temperature. After hydration and rinsing in phosphate buffered saline (PBS; 0.01 M phosphate buffer, pH 7.2, containing 0.85% NaCl), the sections were incubated with normal goat serum at 1:60 dilution for 20 min at room temperature to reduce nonspecific staining, then at 4°C overnight with 6 $\mu g \cdot ml^{-1}$ of anti-tryptase Clara or 5 $\mu g \cdot ml^{-1}$ of F2C12 in PBS containing 0.1% BSA in a moist chamber. The sections were then rinsed with PBS and incubated for 50 min at room temperature with a 1:200 dilution of biotinylated goat anti-rabbit immuno-globulin G (IgG) (Vectastain ABC Kit, Vector Labora-tories, Burlingame, CA, USA). After rinsing in PBS, the sections were incubated for 60 min at room temperature with the avidin-biotin complex. They were then rinsed in PBS, and stained for 5 min with 50 mM Tris-HCl, pH 7.6, containing 0.1% 3,3'-diaminobenzidine tetrahydrochloride, 0.02% hydrogen peroxide and 0.65 $mg \cdot ml^{-1}$ of sodium azide. The sections were washed with PBS, counterstained for 10 min with 1% methyl green, dehydrated and mounted. Controls were prepared in the same way, except that nonimmunized rabbit IgG was used instead of the primary antibody.

Immunoelectron microscopy

Rat lungs were perfused as described above and fixed by instillation into the trachea and perfusion of the lungs at 20 cmH_2O pressure with freshly prepared periodate-lysine-paraformaldehyde solution for 10 min. The lungs were then excised and fixed in the same fixative overnight at 4°C. The fixed lungs were cut into 3 mm slices and washed sequentially with 0.1 M phosphate buffer (PB),

pH 7.4, containing 10, 15 and 20% sucrose (PB-sucrose). The lung slices were rapidly frozen in liquid nitrogen, sectioned at 10 µm thickness with a cryostat, thawed, and dried on glass slides coated with 1% aqueous gelatin. The sections were then incubated with 1.14 mg·ml^{-1} of sodium periodate in PB for 10 min at room temperature, washed with PB-sucrose and incubated with 20% normal goat serum in PB for 30 min at room temperature to block nonspecific binding. F(ab')$_2$ fragments of antigen-affinity purified anti-tryptase Clara (60 µg·ml^{-1}) were incubated with the sections overnight at 4°C as the primary antibody. The sections were then washed with PB-sucrose and incubated with the peroxidase labelled Fab fragment of goat anti-rabbit IgG (Organon Teknika Corp.; Durham, NC, USA) at 4°C for 4 h. The sections were washed with PB-sucrose and fixed in 1% glutaraldehyde in PB for 10 min at 4°C. They were then washed with PB, and reaction products were located by incubation for 10 min in 50 mM Tris-HCl, pH 7.6, containing 0.1% 3,3'-diaminobenzidine tetrahydrochloride, 0.02% hydrogen peroxide, 0.65 mg·ml^{-1} of sodium azide, and 1% dimethyl sulphoxide. The stained sections were post-fixed with 1% OsO_4 in PB for 60 min at room temperature, dehydrated in an ethanol series and embedded in Epon. Ultrathin sections were examined with a Hitachi HU-12 electron microscope at 75 kV. Controls were prepared in the same manner, except that nonimmunized rabbit F(ab')$_2$ fragments of IgG (60 µg·ml^{-1}) were used instead of the primary antibody.

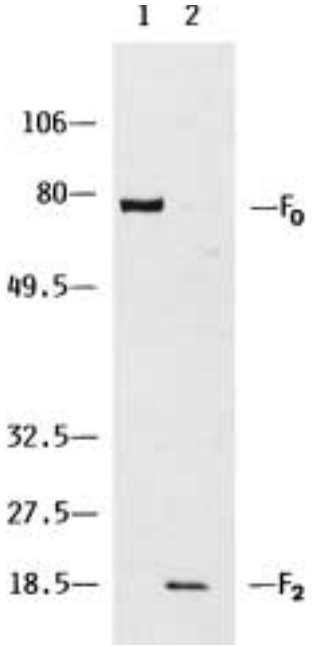

Fig. 1. – Immunochemical analysis of the specificity of antibody against Sendai virus F2 glycoprotein, F2C12. Western blots of nonactive Sendai virus with (lane 2) or without (lane 1) digestion by tryptase Clara (50 µg·ml^{-1}). The viral proteins (1.3 µg) were separated by SDS-PAGE (10–20% gradient) under reducing conditions and transferred electrophoretically to an Immobilon transfer membrane. The membrane was developed with a 1:200 dilution of F2C12 (0.8 mg·ml^{-1}) and stained by the anti-rabbit alkaline phosphatase method as described under "Materials and methods". The molecular mass was determined by comparison with prestained Bio-Rad low range standards: ovalbumin (49.5 kDa), carbonic anhydrase (32.5 kDa), soybean trypsin inhibitor (27.5 kDa) and lysozyme (18.5 kDa).

Results

Immunochemical analysis of specificity of an antibody against the F2 subunit of Sendai virus envelope glycoprotein

The specificity of an antibody raised against the carboxyl terminal peptide, NDTTQNAGAPQS, of the F2 subunit was evaluated by Western blotting. The antibody (F2C12) reacted specifically with F2 and its precursor F0 under reducing (fig. 1; lanes 1 and 2, respectively) and nonreducing conditions (data not shown). These results indicate that the carboxyl terminus regions of F2 is an antigenic epitope both in the uncleaved and cleaved forms of the F protein. The antibody did not react with other viral proteins, such as haemagglutinin, neuraminidase and the F1 subunit. For immunohistochemical studies, we also tested the other antibodies against Sendai virus, such as a monoclonal antibody against Sendai virus F protein and polyclonal antibodies against whole particles of Sendai virus. Among the antibodies tested, F2C12 was the most sensitive for immunostaining of Sendai virus and also gave the lowest background (data not shown).

Histology and immunostaining of serial lung sections of the respiratory tract of uninfected rats

In serial sections of the lungs of control rats inoculated intranasally with saline, the respiratory bronchioles were stained with HE (fig. 2A), and immunohistochemically with antibody against F2C12 (fig. 2B) or tryptase Clara (fig. 2C and D). HE staining revealed

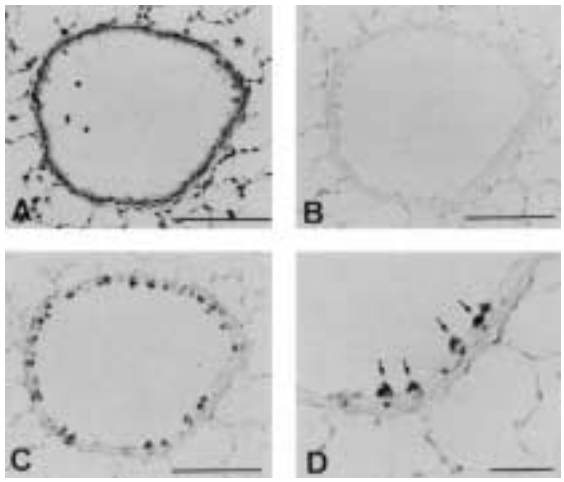

Fig. 2. – Histology and immunostaining of serial lung sections of a respiratory bronchiole of a rat 24 h after intranasal inoculation with saline. A) HE staining shows an intact bronchiole with neither a disorder of the epithelial lining cells, nor infiltration by inflammatory cells. (Bar=100 µm). B) No immunoreactivity with antibody F2C12 is detectable. (Bar=100 µm). C) Immunoreactivity against tryptase Clara is present in bronchiolar nonciliated epithelial Clara cells. (Bar=100 µm). D) High magnification of (C). Heavy deposits of immunoreactive products with anti-tryptase Clara are seen in Clara cells (arrows), but not in ciliated cells. (Bar=40 µm).

intact epithelial nonciliated Clara cells and ciliated cells lining the bronchioles, with no infiltration of inflammatory cells. No immunoreactive deposits were detected with F2C12 antibody. On the other hand, heavy immunoreactive deposits of antibody against tryptase Clara were seen specifically in bronchiolar nonciliated Clara cells, and not in bronchiolar ciliated cells or alveolar cells. No reaction products were seen in control sections, in which the primary antibody was nonimmunized rabbit IgG (data not shown).

Histology and immunostaining of serial lung sections of rats infected with Sendai virus

Serial sections of the lungs of rats obtained 24 h after infection with Sendai virus showed various degrees of pathological change in each lobe of the lung, probably due to differences in the amount of viral particles that had reached individual lobes after intranasal instillation. Figure 3 shows slight pathological changes in the section of a bronchiole in the initial stage of virus infection, as judged by the mild infiltration of mononuclear cells, slight swelling of the epithelium (figs. 3A and B) and slight immunoreactive deposits of Sendai virus F2 antigen in some epithelial cells (fig. 3C). At higher magnification, the F2 antigen was thinly scattered on both the apical surface of Clara cells and the cilia of ciliated cells (fig. 3D), indicating that the progeny virus buds from the luminal surface of these bronchiolar epithelial cells. In serial sections, however, reaction products with antitryptase Clara were heavily deposited in the apical domains of almost all bronchiolar epithelial lining cells (figs. 3E and F), in contrast to its specific localization in

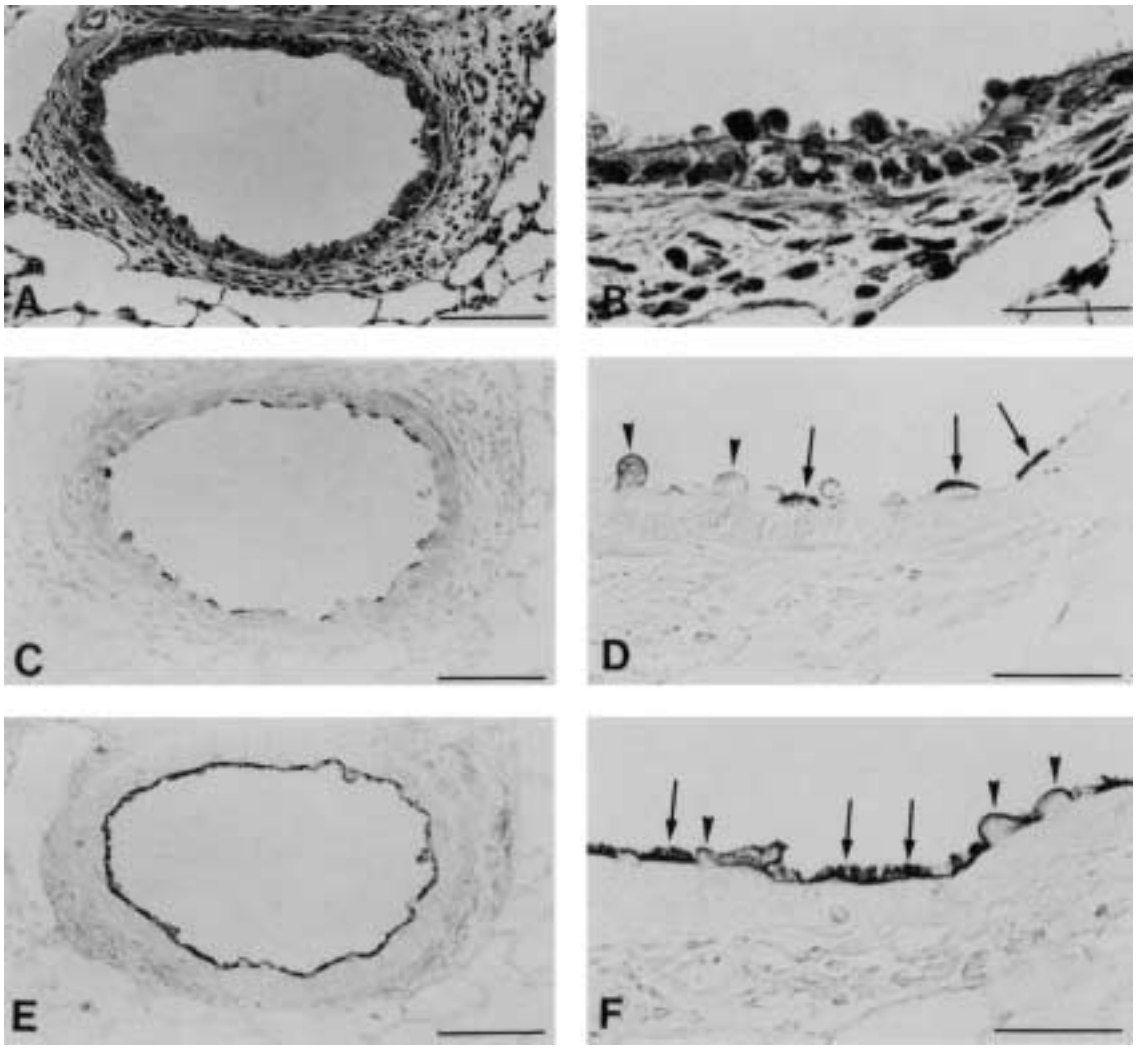

Fig. 3. – Histology and immunostaining of serial sections of a bronchiole of rat lung in a locus with slight pathological changes 24 h after inoculation with Sendai virus. A) HE staining shows slight infiltration of mononuclear cells into the peribronchiolar epithelium. (Bar=100 μm). B) Higher magnification of (A) shows a slight disorder of epithelial lining cells. (Bar=40 μm). C) Sendai virus F2 antigens are seen in some epithelial cells. (Bar=100 μm). D) Higher magnification of (C). Immuno-positive reactions with F2C12 antibody are seen in the apical regions of several Clara cells (arrow heads) and the cilia of several ciliated cells (arrows). (Bar=40 μm). E) Intense immunoreactions of tryptase Clara are present in the apical surface of almost all epithelial lining cells. (Bar=100 μm). F) Higher magnification of (E). The cilia of ciliated cells (arrows) and luminal cell surface of Clara cells (arrow heads) are major immunoreactive sites. The cytoplasm of these cells shows little immunostaining. (Bar=40 μm).

the cytoplasmic space of Clara cells in the uninfected lung (fig. 2C). In this locus, no significant pathological changes were evident in respiratory subepithelial tissues, alveolar lining epithelial cells or alveolar macrophages.

Figure 4 shows severe pathological changes in bronchiolar sections at an advanced stage of Sendai virus infection. HE staining revealed peribronchiolar infiltration of numerous mononuclear cells and marked swelling of the epithelium (figs. 4A and B). Heavy immunoreactive deposits of Sendai virus F2 antigen were widespread on the luminal surface of almost all epithelial cells (figs. 4C and D). At this stage, scarcely any tryptase Clara immunoreactivity was evident in the epithelial cells (figs. 4E and F), but some mononuclear cells infiltrating the bronchiolar airway space were, in part, weakly positive. No reaction products were seen in control sections prepared using nonimmunized rabbit IgG as the primary antibody (data not shown).

Electron immunohistochemical appearance of tryptase Clara in rat lungs infected with Sendai virus

The subcellular localization of tryptase Clara in the section with slight pathological changes in the initial stage of Sendai virus infection was examined by electron immunomicroscopy. In sections of uninfected rats, tryptase Clara was detected only in the cytoplasmic secretory granules of nonciliated Clara cells (fig. 5A), not in bronchiolar ciliated (fig. 5C), or alveolar cells (data not shown). These results are consistent with our previous findings on electron immunocytochemical staining by the protein A-gold technique [11]. At the initial stage of infection, immunoreactive deposits of tryptase Clara were mainly present in the peripheral membranes of both bronchiolar Clara (fig. 5B) and ciliated cells (fig. 5D), with little detection in the secretory granules of infected Clara cells. No immuno-positive organelles,

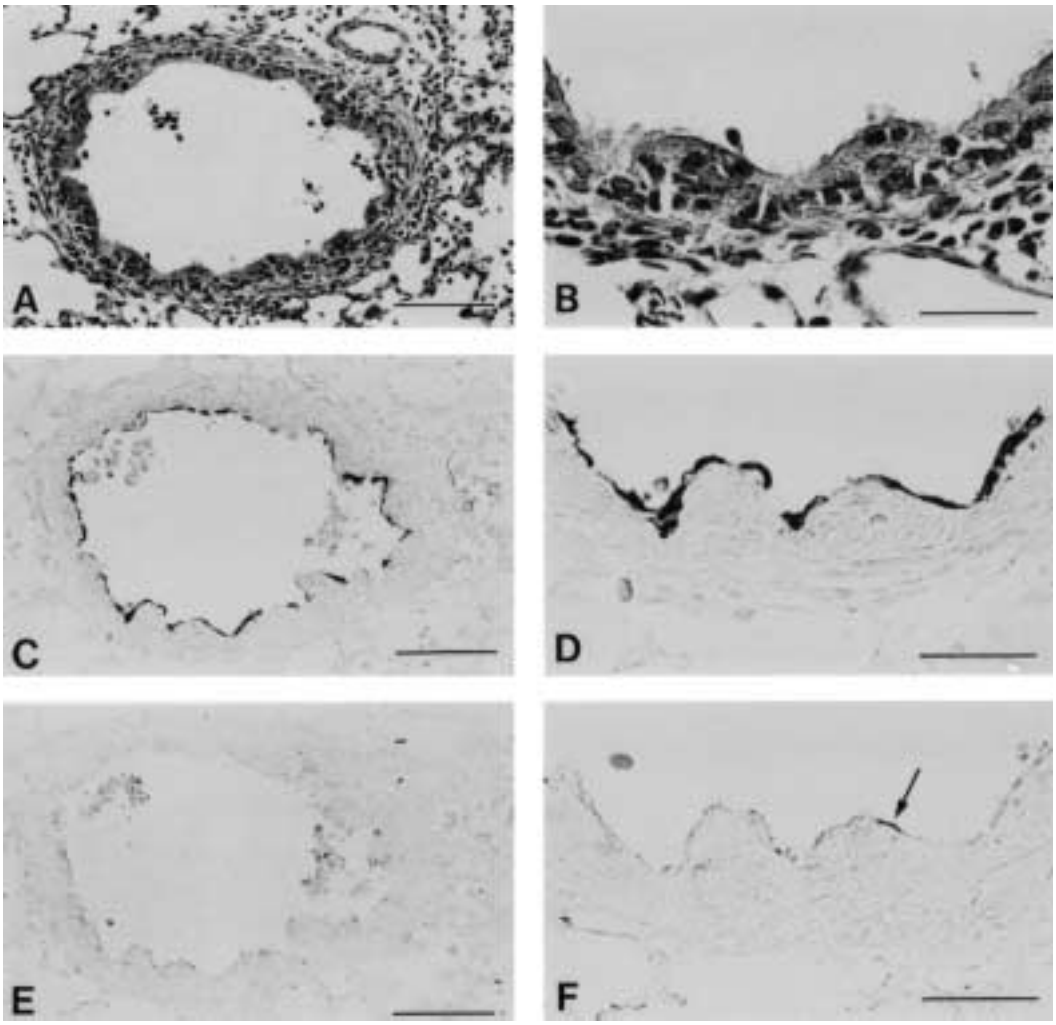

Fig. 4. – Histology and immunostaining of serial sections of a bronchiole of rat lung in a locus of severe pathological changes 24 h after Sendai virus infection. A) HE staining shows infiltration of numerous mononuclear cells into the peribronchiolar epithelium and into the respiratory and adjacent alveolar spaces. (Bar=100 μm). B) Higher magnification of (A) shows marked swelling of the bronchiolar epithelium. (Bar=40 μm). C) Positive staining for Sendai virus F2 antigen is seen in the luminal surface of almost all epithelial cells. (Bar=100 μm). D) Higher magnification of (C). Heavy deposits of immunoreactive products with F2C12 antibody are seen in the luminal surface of the swollen epithelium. (Bar=40 μm). E) Little or no staining for tryptase Clara is present in the epithelial lining cells, but some mononuclear cells infiltrating into the respiratory airway space are weakly positive. (Bar=100 μm). F) Higher magnification of (E). There is little immunoreactivity for the protease in the apical cell surfaces of epithelial cells (arrow). (Bar=40 μm).

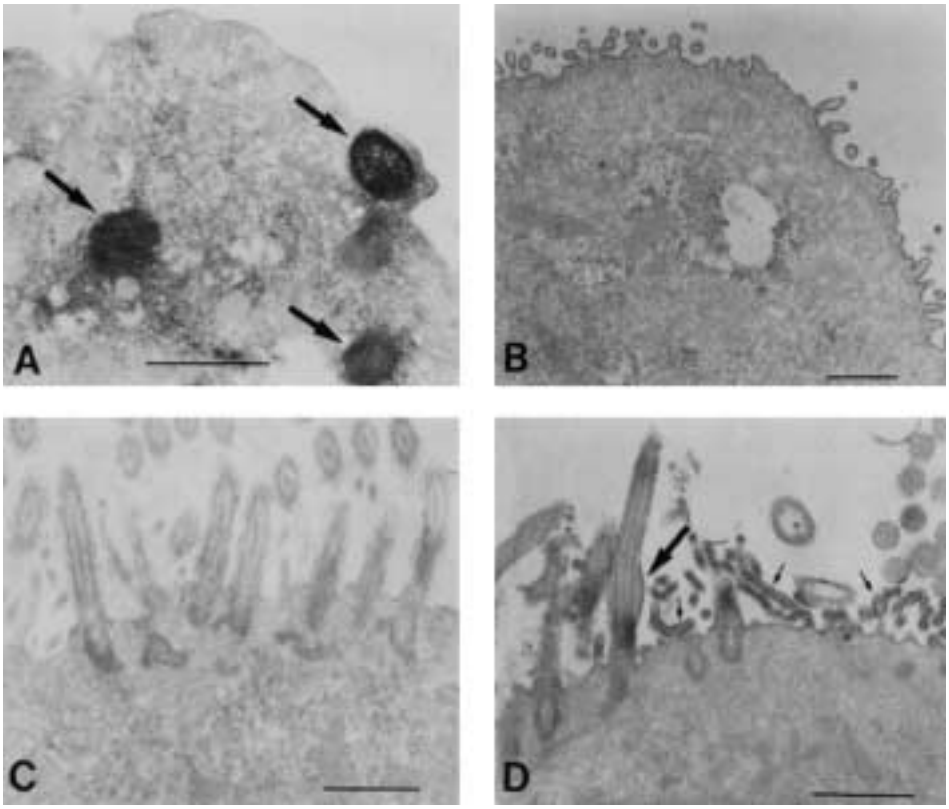

Fig. 5. – Immunoelectron micrographs of weakly infected respiratory epithelial Clara cells and ciliated cells of rats 24 h after inoculation with Sendai virus. A) Heavy deposits of immunoreactive products of tryptase Clara (arrows) are mainly located in the secretory granules of Clara cells of control rats inoculated with saline instead of Sendai virus. (Bar=1 μm). B) The peripheral luminal membrane of Clara cells of rats infected with the virus is intensely labelled with deposits of immunoreactive products of tryptase Clara. Little immunoreactivity is noted in secretory granules. (Bar=1 μm). C) There is no immunoreactivity for tryptase Clara in ciliated cells of control rats. (Bar=1 μm). D) Ciliated cells of rats infected with Sendai virus show immunoreactivity for tryptase Clara in the peripheral membranes of cilia (large arrow) and some microvilli (small arrows). No organelles are labelled. Note diminished cilia and disorder of the cell surface. (Bar=1 μm).

such as the Golgi apparatus and endoplasmic reticulum, were seen in infected ciliated or Clara cells. There were no immunoreactive deposits of tryptase Clara in the peripheral membranes of the epithelial cells at the advanced stage of infection (data not shown). There were no specific deposits in control sections using nonimmune rabbit $F(ab')_2$ fragments instead of the first antibody (data not shown).

Discussion

We demonstrated by histological and immunohistochemical studies, that the subcellular localization of tryptase Clara in rat bronchioles changed with the progression of pathological changes induced by Sendai virus infection.

Various pathological changes at different stages occurred in rat bronchioles 24 h after an intranasal inoculation with Sendai virus. At the initial stage of infection with slight pathological changes, Sendai virus F2 antigen was present in the apical domains of some epithelial lining cells. At this stage, there was a dramatic change in the subcellular localization of tryptase Clara. Tryptase Clara antigenicity was found in the peripheral membranes of almost all bronchiolar epithelial cells on the luminal side, but scarcely in the secretory granules, Golgi apparatus or endoplasmic reticulum of the epithelial cells. This was in contrast to its specific localization in the secretory granules of Clara cells in the uninfected lung. At an advanced stage of infection, heavy deposits of Sendai virus F2 antigen were observed in almost all epithelial cells with marked peribronchiolar infiltration of mononuclear cells and large swelling of the epithelium. Although tryptase Clara antigens almost disappeared from the apical domains of epithelial cells, we previously reported that two- to threefold increases in the amount and activity of the protease in bronchial lavage fluid from rat 12–24 h after intranasal infection with Sendai virus [22]. The precise mechanisms of the altered localization of tryptase Clara in bronchiolar epithelial cells during Sendai virus infection are unclear. However, our results suggest that secretion of tryptase Clara from the secretory granules of Clara cells into the airway lumen is stimulated by infection with Sendai virus and that increased levels of tryptase Clara in the luminal surface of the epithelial cells produce favourable conditions for viral activation and multiplication.

We recently found that intranasal administration of antibody against tryptase Clara [12] and F2C12 antibody

[23] significantly inhibited Sendai virus activation and suppressed the multiple cycles of viral replication and pathological changes in the lungs of infected rats. These findings indicate that proteolytic activation of Sendai virus mainly occurs extracellularly in the lumen of the respiratory tract, and that tryptase Clara is a primary host factor involved in the pneumopathogenicity of the virus. In addition, the progeny of Sendai virus in rat lungs underwent proteolytic activation by tryptase Clara in the airway lumen for 5–6 days after infection [12]. These findings were supported by the present demonstration of the changes in the localization of tryptase Clara in bronchioles after Sendai virus infection. Although the protease was scarcely detectable in the lung sections at the advanced stage of infection, amount of tryptase Clara in the bronchial lavage fluid was increased and was held constantly at high level after inoculation of Sendai virus for 12–24 h [22]. The occasional staining with anti-tryptase Clara in mononuclear cells that infiltrated the bronchiolar airway space at the advanced stages of infection may be due to phagocytosis of the protease in the bronchiolar airway fluid. Taken together, these results present the correlation between change in localization of tryptase Clara in rat bronchiolar epithelial cells and its proteolytic activation of Sendai virus in the respiratory lumen.

From the present findings and our previous observations, we conclude that activated virus altered the subcellular localization of tryptase Clara together with accumulation of the protease in the airway fluid from bronchiolar and bronchial Clara cells, and that the protease in the airway fluid and/or the peripheral surface of epithelial cells, where progeny virus buds, induces activation and multiplication of inactive progeny viruses extracellularly.

References

1. Tashiro M, Homma M. Pneumotropism of Sendai virus in relation to protease-mediated activation in mouse lungs. *Infect Immun* 1983; 39: 879–888.
2. Scheid A, Choppin PW. 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* 1974; 57: 475–490.
3. Nagai Y, Klenk H-D, Rott R. Proteolytic cleavage of the viral glycoproteins and its significance for the virulence of Newcastle disease virus. *Virology* 1976; 72: 494–508.
4. Gorman JJ, Nestorowicz A, Mitchell SJ, Corino GL, Selleck PW. Characterization of the sites of proteolytic activation of Newcastle disease virus membrane glycoprotein precursors. *J Biol Chem* 1988; 263: 12522–12531.
5. Klenk H-D, Rott R. The molecular biology of influenza virus pathogenicity. *Adv Virus Res* 1988; 34: 247–281.
6. Lazarowitz SG, Choppin PW. Enhancement of the infectivity of influenza A and B viruses by proteolytic cleavage of the hemagglutinin polypeptide. *Virology* 1975; 68: 440–454.
7. Garten W, Bosch FX, Linder D, Rott R, Klenk H-D. Proteolytic activation of the influenza virus hemagglutinin: the structure of the cleavage site and the enzymes involved in cleavage. *Virology* 1981; 115: 361–374.
8. Giddens WE Jr, van Hoosier GL Jr, Garlinghouse LE Jr. Experimental Sendai virus infection in laboratory rats. II. Pathology and immunohistochemistry. *Lab Anim Sci* 1987; 37: 442–448.
9. Castleman WL. Respiratory tract lesions in weanling outbred rats infected with Sendai virus. *Am J Vet Res* 1983; 44: 1024–1031.
10. Kido H, Yokogoshi Y, Sakai K, *et al*. Isolation and characterization of a novel trypsin-like protease found in rat bronchiolar epithelial Clara cells. A possible activator of the viral fusion glycoprotein. *J Biol Chem* 1992; 267: 13573–13579.
11. Sakai K, Kawaguhi Y, Kishino Y, Kido H. Electron immunohistochemical localization in rat bronchiolar epithelial cells of tryptase Clara, which determines the pneumotropism and pathogenicity of Sendai virus and influenza virus. *J Histochem Cytochem* 1992; 41; 89–93.
12. Tashiro M, Yokogoshi K, Tobita K, Seto JT, Rott R, Kido H. Tryptase Clara, an activating protease for Sendai virus, is involved in pneumopathogenicity. *J Virol* 1992; 66: 7211–7216.
13. Ishikawa E, Imagawa M, Hashida S, Yoshitake S, Hamaguchi Y, Ueno T. Enzyme-labeling of antibodies and their fragments for enzyme immunoassay and immunohistochemical staining. *J Immunoassay* 1983; 4: 209–327.
14. Tashiro M, Pritzer E, Khoshnan MA, *et al*. Characterization of a pantropic variant of Sendai virus derived from a host range mutant. *Virology* 1988; 165: 577–583.
15. Tashio M, Yamakawa M, Tobita K, Klenk H-D, Rott R, Seto JT. Organ tropism of Sendai virus in mice: proteolytic activation of the fusion glycoprotein in mouse organs and budding site at the bronchial epithelium. *J Virol* 1990; 64: 3627–3634.
16. Tashiro M, James I, Karri S, *et al*. Pneumotropic revertants derived from a pantropic mutant, F1-R, of Sendai virus. *Virology* 1991; 184: 227–234.
17. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227: 680–685.
18. Smith PK, Krohn RI, Hermanson GT, *et al*. Measurement of protein using bicinchoninic acid. *Anal Biochem* 1985; 150: 76–85.
19. Sakai K, Kweon MN, Kohri T, Kishino Y. Effects of pulmonary surfactant and surfactant protein A on phagocytosis of fractionated alveolar macrophages: Relationship to starvation. *Cell Mol Biol* 1992; 38: 123–130.
20. Hsu S-M, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 1981; 29: 577–580.
21. Hsu S-M, Raine L, Fanger H. A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am J Clin Pathol* 1981; 75: 734–738.
22. Kido H, Sakai K, Kishino Y, Tashiro M. Pulmonary surfactant is a potential endogenous inhibitor of proteolytic activation of Sendai virus and influenza virus. *FEBS Lett* 1993; 322: 115–119.
23. Tashiro M, Takeda M, Tanaka S, Nishimura N, Takenaka M, Kido H. Antibody against the carboxyl terminus of the F2 subunit of Sendai virus fusion glycoprotein inhibits proteolytic activation. *Virol* 1993; 194: 882–885.