Inhibitory effect of pulmonary surfactant on Sendai virus infection in rat lungs

Brief Report

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Summary. Intranasal infection of rats with active (infectious) Sendai virus enhances secretion of tryptase Clara, a Sendai virus-activating protease, into the bronchial lumen by Clara cells of the bronchial epitheliums, and inversely suppresses secretion of pulmonary surfactant, an inhibitor of the protease, into the lumen [Kido H et al. (1993) FEBS Lett 322: 115–119]. A trypsin-resistant mutant, TR-2, showed similar effects, although its replication was restricted to a single cycle in the lungs. In contrast, neither nonactive (noninfectious) wild-type virus possessing receptor-binding activity and lacking envelope fusion activity nor UV-inactivated virus retaining receptor binding and envelope fusion activities altered the mode of secretions. These results indicate that viral replication is required for producing a condition in the bronchial lumen for proteolytic activation of progeny virus, thereby infection is extended to a fatal pneumonia. On the other hand, intranasal administration of infected rats with pulmonary surfactant suppressed activation of progeny virus and pathological changes in the lungs, suggesting a therapeutic use of pulmonary surfactant for influenza pneumonia.

Proteolytic activation of the fusion (F) glycoprotein by host proteases is necessary for Sendai virus, a murine parainfluenza virus 1 of the paramyxovirus genus, to acquire membrane fusion activity and infectivity [4, 11]. Cleavage site of the F protein consists of a single arginine residue, at position 116, that is cleavable by specific proteases such as trypsin in vitro [4, 11], clotting factor Xa in the chorioallantoic fluid of chick embryos [3, 7] and tryptase Clara in rat lungs [6, 16]. Sendai virus infections in rodents are restricted to bronchial epithelial
cells [13, 16]. We have shown that tryptase Clara, a trypsin-like protease secreted by the Clara cells of rat bronchial epithelia, is a principal protease which activates Sendai virus in the lungs [15, 16]. Progeny virus is activated inside the bronchial lumen by the protease and therefore undergoes multiple cycles of replication along the respiratory tract resulting in fatal pneumonia.

We have also shown that pulmonary surfactant, a phospholipoprotein complex also secreted by the Clara cells as well as type II alveolar cells, is a potent inhibitor specific for tryptase Clara [5]. Surfactant inhibits in vitro proteolytic activation of Sendai virus by tryptase Clara. In normal rat lungs, secretion of the protease remains at a low level, while surfactant is secreted to a relatively large extent. However, within 12 to 24 h after intranasal infection with Sendai virus, secretion of tryptase Clara is enhanced up to three times, whereas that of surfactant is reduced, thereby producing a condition favorable for proteolytic activation of progeny virus in the bronchial lumen. This is a likely mechanism involved in the pulmonary pathogenesis of Sendai virus infection [5, 8, 9].

On the other hand, we previously showed that when nonactive Sendai virus with a precursor F protein, F₀, was inoculated intranasally to mice, viral replication did not occur in the lungs, suggesting the absence of virus activating protease inside the airway lumen [13]. This appears inconsistent with the results that viral activation occurs inside the lumen by secreted tryptase Clara [15, 16]. To answer the question, we investigated the secretion of tryptase Clara and pulmonary surfactant upon infection with various Sendai virus preparations.

Nonactive wild-type Sendai virus (Z strain) grown in LLC-MK₂ cells was activated in vitro by trypsin treatment [13]. The activated virus was irradiated with a UV-lamp to inactivate infectivity but not receptor binding and membrane fusion activities [13]. A trypsin-resistant mutant TR-2, which is also resistant to tryptase Clara [16], was activated in vitro with chymotrypsin [13]. Receptor binding and envelope fusion activities measured by hemagglutination and hemolysis tests, respectively, are shown in Table 1. Specific-pathogen-free, 5-week-old male rats of the CD (SD) strain were inoculated intranasally with the virus preparations. At various time intervals, bronchoalveolar lavage was collected for measuring the amount of tryptase Clara and surfactant protein A by the quantitative Western immunoblotting and for assaying protease activity as described previously [5, 6].

When rats were infected intranasally with activated wild-type virus, secretion of tryptase Clara was enhanced, whereas that of surfactant was decreased as reported previously [5, 8, 9]. To determine which step of viral infection process is involved in this phenomenon, nonactive virus possessing receptor binding activity but lacking envelope fusion activity and infectivity ([13], also see Table 1) was inoculated intranasally to rats. Nonactive virus caused neither viral replication nor pathological changes in the lungs as long as an observation period of 8 days (data not shown). For these animals, secretion of tryptase Clara and surfactant protein A remained unchanged (Fig. 1). Similarly, intranasal inoculation with UV-irradiated active virus, which had no more infectivity but retained the receptor binding and envelope fusion activities (Table 1), did not