Increased Severity of 2009 Pandemic Influenza A Virus Subtype H1N1 Infection in Alveolar Type II Cells From Patients With Pulmonary Fibrosis

To the Editor—Weinheimer et al [1] reported that influenza A viruses preferentially infect alveolar epithelial type II (ATII) cells in lung tissue explants and suggested that the pathogenicity of influenza A viruses is associated with the ability of these viruses to replicate in ATII cells and induce the production of inflammatory cytokines. In contrast, an epidemiological study by Nguyen-Van-Tam et al [2] demonstrated that chronic pulmonary comorbidities such as pulmonary fibrosis were risk factors for poor outcomes for 2009 pandemic influenza A virus subtype H1N1 (A[H1N1]pdm09) infection. Pulmonary fibrosis is a collective and clinicopathological term for a disease characterized by shortness of breath during exercise, abnormal lung physiology, reduced gas transfer, and excessive scar formation in the lung interstitium. Two studies using genome-wide RNA interference screening identified host factors that are required for the replication of influenza virus [3, 4]. These studies suggest that not only viral strains but also host factors associated with existing pulmonary diseases should be studied to gain a better understanding of the mechanisms of influenza A virus pathogenesis.

To our knowledge, there are no studies investigating whether influenza virus infection of ATII cells in diseased lungs is more severe than in normal lungs. On the basis of an epidemiological study [2], we hypothesized that the infection of ATII cells with A(H1N1)pdm09 is more severe for cells from pulmonary fibrotic lungs than for cells from nonfibrotic lungs. To address this question, we infected ATII cells isolated from fibrotic lungs and nonfibrotic lungs with A(H1N1)pdm09 and then compared viral titers in the culture supernatants. This study was approved by the ethics committees of the Tohoku University School of Medicine and the Japanese Red Cross Ishinomaki Hospital. All patients gave their informed consent.

Nonfibrotic lung tissue specimens were obtained from 4 patients who underwent surgery because of lung cancer. The nonfibrotic tissue specimens were resected from portions distal to the cancerous lesions. A histopathological examination confirmed that these nonfibrotic lung tissues did not contain any lesions, including those associated with cancer, fibrosis, emphysema, or inflammatory changes (data not shown). Fibrotic lung tissue specimens were obtained from patients who underwent open lung biopsy or lung resection because of lung cancer. The clinical diagnoses based on pathological examinations of the fibrotic lesions included idiopathic pulmonary fibrosis (in 3 patients), cellular nonspecific interstitial pneumonia (in 1), and chronic hypersensitivity pneumonitis (in 1). We excluded patients in whom other respiratory disorders, such as asthma or chronic obstructive pulmonary disease, were diagnosed that might also be risk factors for poor outcomes for A(H1N1)pdm09 infection [5]. We also excluded patients who had been treated with chemotherapy before surgery.

Figure 1. Increased viral titers in the supernatants of alveolar epithelial type II (ATII) cells from fibrotic lungs relative to the titers for cells from nonfibrotic lungs. We purified and cultured ATII cells from 5 fibrotic lungs and 4 nonfibrotic lungs. The ATII cells were infected with the 2009 pandemic influenza A virus subtype H1N1 for 1 hour. The viral titers in the supernatants were quantified by infecting replicate wells of confluent Madin-Darby canine kidney (MDCK) cells in plastic 96-well dishes with serial 10-fold dilutions of virus-containing supernatant. The viral titers in the supernatant are expressed as median tissue culture infective doses (TCID_{50}). Data are shown as a scatter plot. The bars show the median values. Statistical analysis was conducted using GraphPad Prism version 5.0d (GraphPad Software, La Jolla, CA). The Mann–Whitney U test was performed to evaluate statistical significance. A P value of <.05 was considered statistically significant.
ATII cells were purified using a method that we recently established [6]. The freshly isolated ATII cells were cultured on recombinant human laminin-5 (Oriental Yeast, Tokyo, Japan) in Dulbeccco’s modified Eagle’s medium (Life Technologies, Carlsbad, CA) containing 0.5% bovine serum albumin (Sigma-Aldrich, St. Louis, MO), penicillin, streptomycin, amphotericin B (Life Technologies), and human recombiant keratinocyte growth factor (10 ng/mL; PeproTech, Rocky Hill, NJ). After 24 hours, the ATII cells were infected with 0.5 multiplicity of infection of A(H1N1)pdm09 for 1 hour. We collected culture supernatant fluids 24 hours after addition of the influenza virus and quantified the viral titers in the culture supernatants as previously described [7]. We found that the viral titers for ATII cells isolated from fibrotic lungs were significantly higher than the titers for cells from nonfibrotic lungs (Figure 1).

Our data suggest that the infection of ATII cells is more extensive in fibrotic lungs than in normal lungs and support epidemiological data indicating that pulmonary fibrosis is a risk factor for poor outcomes of A(H1N1)pdm09 infection [2]. Moreover, our study highlights that host factors associated with the severity of influenza virus infection differ between ATII cells subjected to profibrotic conditions and those in normal niches. This result indicates that it is important to identify host factors related to preexisting disease because the identification of such factors might lead to the development of personalized medicine for the treatment of influenza virus infection.

**Notes**

**Acknowledgments.** We thank the Biomedical Research Core of the Tohoku University Graduate School of Medicine for technical support.

**Financial support.** This work was supported by a grant from the Japan Society for the Promotion of Science (22390163 to H. K.).

**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


Received 1 September 2012; accepted 15 October 2012; electronically published 29 November 2012.

**Correspondence.** Hiroshi Kubo, MD, PhD, Department of Advanced Preventive Medicine for Infectious Disease, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai; and 3Department of Thoracic Surgery, Japanese Red Cross Ishinomaki Hospital, Ishinomaki, Japan.

**The Journal of Infectious Diseases 2013;207:692–3**

© The Author 2012. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com.

DOI: 10.1093/infdis/jis739