Reply to Fujino et al

To the Editor—Chronic respiratory conditions, including interstitial lung fibrosis, are well-known risk factors for a severe outcome of influenza virus infection [1–3]. However, the precise underlying mechanisms remain to be established. The report by Fujino et al [4] may open up a new approach to gain a better understanding of this phenomenon in the case of influenza in patients with pulmonary fibrosis. The lungs of patients with pulmonary fibrosis are characterized by areas with deranged lung architecture and emphysema formation, accompanied by alveolar epithelial cell injury, alveolar type II (ATII) hyperplasia, fibrin formation, enhanced extracellular matrix deposition, and myofibroblast activation [5]. By use of healthy explanted human lung tissue specimens, we recently identified ATII pneumocytes as the main target cells in which seasonal and pandemic influenza A viruses replicate in the distal human respiratory tract [6].

Importantly, Fujino et al demonstrated increased propagation of influenza A virus in ATII cells prepared from lungs of patients with pulmonary fibrosis, compared with propagation in ATII cells isolated from healthy controls. We agree with the conclusion that ATII cells from fibrotic lungs may provide an intracellular milieu favoring viral replication and, consequently, disease development, although the host factors involved remain unknown. However, the study by Fujino et al was limited to the analysis of a purified, homogenous ATII cell population. Therefore, it cannot be excluded that altered cellular composition and accumulation of extracellular matrix typically seen in fibrotic lungs may also affect the cellular tropism and/or viral spread in alveolar tissue. To analyze this possibility, we infected lung explants from 3 patients with pulmonary fibrosis with seasonal influenza A virus subtype H3N2 and investigated the occurrence of viral protein synthesis in proliferated ATII and other cells.
Representative images from lung sections for 3 patients with pulmonary fibrosis. A, Hematoxylin–eosin staining 24 hours after mock infection, illustrating the typical morphology of interstitial pulmonary fibrosis of alveolar septae (asterisk), as well as type II pneumocyte proliferation (arrow heads). B, Type II pneumocyte proliferation was proven by immunostaining of pro-SP-C (red, arrowheads) in fibrotic areas (asterisks), demonstrated by differential interference contrast (DIC, grey). Nuclei were stained with DAPI (blue). C, Human fibrotic lung tissue was infected with influenza A virus for 24 hours. Slices were incubated with anti-pro-SP-C antibody (red channel) to detect sites of type II pneumocyte proliferation and fluorescently labeled anti-influenza A virus antibody (green channel) to detect virus-infected cells (white arrowheads). A strong colocalization of H3N2 infection was found in proliferated type II cells (yellow). Interstitial pulmonary fibrosis was confirmed by DIC (asterisk), and nuclei were stained with DAPI (blue). The staining was visualized by confocal microscopy, and tissue autofluorescence was separated from specific fluorescence by spectral unmixing. A–C, Representative images from lung sections for 3 patients with pulmonary fibrosis. Scale bars indicate 50 µm (A) and 20 µm (B and C).

Fresh explants of fibrotic lung tissue were obtained from patients undergoing lung resection at local thoracic surgeries. Written informed consent was obtained from all patients, and the study was approved by the ethics committee of the Charité clinic. The detailed experimental procedure has been previously described [6]. Interstitial pulmonary fibrosis was confirmed through histological diagnosis by an experienced pathologist, revealing strong extracellular matrix deposition, fibrotic thickening of alveolar septae, and ATII cell apoptosis and proliferation (Figure 1A). Proliferating ATII cells were further identified by pro-SP-C staining in fibrotic areas of mock-infected lungs (Figure 1B), and influenza virus–infected and mock-infected fibrotic tissues were, in addition, fluorescently labeled for viral gene expression (Figure 1C and data not shown). This double-staining analysis identified strong tropism of influenza virus for proliferated ATII cells from fibrotic lungs.

As with the data from Fujino et al, our results indicate that not only distinct host factors in ATII cells, but also their increased number, may augment influenza virus propagation in fibrotic lungs. More work is needed to identify the specific molecular factors in fibrotic alveolar tissue contributing to enhanced virus replication, but published data may give some hints. First, expression of ELMO domain containing 2 (ELMOD2) gene, a candidate susceptibility factor for the development of idiopathic pulmonary fibrosis, was required for full activation of antiviral type I and type III interferon responses in human lung cells [7]. Since influenza virus infection decreased ELMOD2 expression, it is possible that virus-infected cells in fibrotic lungs have a generally lower capacity to restrict virus replication, because of their debilitated antiviral interferon response [8]. Second, it was recently demonstrated that influenza virus polymerase proteins interact with members of the WNT/β-catenin pathway [9]. Significantly, reactivation of the developmentally relevant WNT/β-catenin pathway has recently been demonstrated in ATII cells from mice and humans with pulmonary fibrosis [10, 11]. In particular, the WNT1 signaling protein was increased in hyperplastic ATII cells and was associated with proliferation, as well as with the induction of profibrotic markers. Therefore, further investigations should clarify the potential role of WNT/β-catenin pathway activation and enhanced influenza virus replication in ATII cells from fibrotic lungs.

Notes

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Andreas C. Hocke,1 Johanna Berg,2 Anne Becher,2 Jessica Knepper,1 Frederick Klauschen,3 Mario Tönnies,4 Torsten T. Bauer,4 Paul Schneider,3 Jens Neudecker,6 Jens C. Rückert,6 Achim D. Gruber,7 Norbert Suttorp,2 Stefan Hippensstiel,2 and Thorsten Wolff1

1Division of Influenza/Respiratory Viruses, Robert Koch-Institut; 2Department of Internal Medicine/Infectious Diseases and Pulmonary Medicine, and 3Institute of Pathology, Charité Universitätsmedizin Berlin; 4Department of Pneumology and Thoracic Surgery, HELIOS Clinic Emil von Behring, Chest
Hospital Heckeshorn, Department for General and Thoracic Surgery, DRK Clinics, Department of General, Visceral, Vascular and Thoracic Surgery, Universitätsmedizin Berlin, and Department of Veterinary Pathology, College of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

References


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Correspondence: Thorsten Wolff, Div. of Influenza/Respiratory Viruses, Robert Koch-Institut, Nordufer 20, 13353 Berlin, Germany (wolfft@rki.de).

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