

LETTER TO THE EDITOR

A reduced expression of surfactant protein D in the lungs of fatal influenza H1N1 cases in 2009CH. BOONARKART¹, O. SUPTAWIWAT¹, M. UIPRASERTKUL², A. KONGCHANAGUL¹, P. RODPOTHONG¹,
CH. BUNTHI³, R. CHAMPUNOT⁴, P. AUEWARAKUL^{1,5*}

¹Department of Microbiology, ²Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand; ³International Emerging Infections Program (IEIP), Thailand MOPH-US CDC Collaboration (TUC), Ministry of Public Health, Nonthaburi, Thailand; ⁴Department of Medicine, Buddhachinaraj Hospital, Phitsanulok, Thailand; ⁵Center of Emerging and Neglected Infectious Diseases, Mahidol University, Bangkok, Thailand

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Pulmonary surfactant reduces alveolar surface tension and prevents the collapse of alveolar walls. It is also an important first line defense of the innate immune system. Human lung surfactant is composed of approximately 90% lipids and 10% proteins (1). Among these proteins, there are four types of surfactant proteins: hydrophobic surfactant protein B (SPB) and surfactant protein C (SPC); and hydrophilic water-soluble surfactant protein A (SPA) and surfactant protein D (SPD). SPB and SPC facilitate adsorption and spread of the pulmonary surfactant, while SPA and SPD are involved in the innate defense against various pathogens (2). SPD is a pulmonary collectin, which plays multiple roles in the pulmonary innate immune system by mediating pathogen clearance and regulating pulmonary inflammatory response (3). Pulmonary SPD is synthesized and secreted mainly by type II alveolar pneumocytes (4). SPD binds to influenza A virus (IAV) hemagglutinin and interferes with its receptor binding (5). SPD-deficient mice

showed increased susceptibility to IAV infection (6). The inhibition process involves binding of SPD to high-mannose oligosaccharides associated with the globular head of the hemagglutinin molecule in the proximity of the receptor-binding pocket. The binding of SPD can, therefore, interfere with the hemagglutinin activity and viral attachment to the host cell receptor. SPD can also bind to the neuraminidase protein of IAV and inhibit its enzyme activity (5). IAVs with more glycosylation sites on hemagglutinin tend to be more susceptible to SPD than those with less glycosylation sites. It has been recently shown that H5N1 and the 2009 H1N1 IAVs were less sensitive to SPD than recent seasonal IAVs (7, 8).

SPD appears to have both pro- and anti-inflammatory signaling functions. Collectins are capable of differential binding through either their lectin or their collagen domain to cell membrane receptors and eliciting anti-inflammatory or pro-inflammatory signaling pathways, respectively (9, 10). Using expression microarray, we have recently shown that SPD was among the most down-regulated genes in the lung tissue from fatal H5N1 avian influenza victims (11). The 2009 H1N1 IAV causes severe disease in some patients, and pathological findings in the lungs resembled those observed in H5N1 infected cases (12). We asked whether SPD was also down-regulated in the lungs of severe 2009 H1N1 influenza cases.

*Corresponding author. E-mail: sipaw@mahidol.ac.th; phone: +662-419-7059.

Abbreviations: ARDS = acute respiratory distress syndrome; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; IAV = influenza A virus; SPA = surfactant protein A; SPB = surfactant protein B; SPC = surfactant protein C; SPD = surfactant protein D

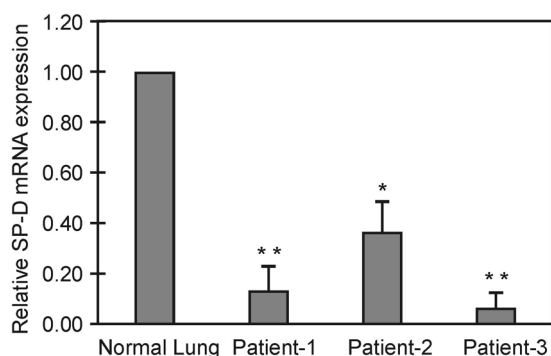


Fig. 1

Relative levels of SP-D mRNA were measured by real time RT-PCR

The assay was performed in triplicates, and the results are shown as mean \pm SD. Statistical significance of the differences compared to normal lung was tested by t-test (* = $p < 0.05$, ** = $p < 0.01$). The SP-D mRNA levels were normalized to GAPDH mRNA as an internal control.

Lung tissues from three fatal cases of the 2009 H1N1 IAV infection were studied. All the patients had severe pneumonia, developed acute respiratory distress syndrome (ARDS) and died of respiratory failure. Autopsies were carried out by standard techniques using precautions to minimize the risk of transmission of the infection. Total RNA was extracted from fresh tissue samples using TRIZOL reagent (Invitrogen, Carlsbad, CA). The levels of SP-D mRNA were measured by real-time RT-PCR, using LightCycler FastStart DNA MasterPLUS SYBR Green I (Roche Diagnostics GmbH, Mannheim, Germany) on a LightCycler 2.0 Instrument (Roche Diagnostics). The following primers were used to detect the expression of specific genes: SP-D forward (13) and reverse (CCAGTTGGCTCAGAACTCGCA); and E14134 and E14135 (14) for the mRNA of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which is a house-keeping gene and was used as an internal control. The amplification reactions contained 1 \times LightCycler Fast Start DNA MasterPLUS SYBR Green I and 0.4 mmol/l of each forward and reverse primer. Melting-curve analyses were performed from 65°C to 95°C. RNA extracted from normal lung tissue was serially diluted and used for standard curve setting of SP-D. SP-D expression levels were normalized with respect to GAPDH expression. Normal lung specimens were obtained at the Department of Forensic Medicine, Faculty of Medicine Siriraj Hospital during routine autopsy examination of cases who died in traffic accidents. All the normal lung specimens showed normal macro- and microscopic morphology.

In accordance to our previously published data in H5N1 avian influenza-infected lungs, the expression of SP-D in all the three 2009 H1N1-infected lungs were significantly lower than that of the normal lung (*t*-test, $p < 0.5$ or $p < 0.01$). The average relative expression of SP-D in the 2009 H1N1-infected lungs was 0.19 ± 0.15 of the level of the normal lung, which

is comparable to what we described for H5N1-infected lungs (0.13 ± 0.06) (11). This indicates that the reduction of SP-D is a common phenomenon in severe influenza pneumonia from both H5N1 and 2009 H1N1 IAVs. Both these viruses were shown to target type II alveolar epithelial cells. Since this cell type is the main source of SP-D, the reduction in SP-D mRNA may be a direct consequence of infection and destruction of these cells.

Our data in H5N1- and 2009 H1N1-infected lungs are in agreement with a recent report showing a reduction of SP-D production from influenza-infected primary human type II alveolar epithelial cells (15). The down-regulation of SP-D may have an important implication for the pathogenesis of influenza pneumonia. Reduced SP-D levels in the lungs might compromise the innate anti-viral mechanism and contribute to the high viral load in the lungs. It has been recently shown that H5N1 and 2009 H1N1 viruses were less sensitive to SP-D than recent seasonal influenza viruses (7, 8). However, since the local concentration of SP-D in alveoli is not known, it is still not clear whether SP-D can inhibit H5N1 and 2009 H1N1 infection in the lung. In addition, the reduction of SP-D may impair innate defense against superinfection by other pathogens (16). Bacterial superinfection has been shown to be a common finding in the lungs of fatal 2009 H1N1 cases (17). Furthermore, since SP-D plays an important role in modulating inflammation (10), a deficiency in SP-D may result in an uncontrolled inflammatory process and contribute to the observed severe inflammation (16). Therapeutic approaches using surfactant components have been proposed and studied in animal models and patients with ARDS (18). A recent report showed a successful attempt to treat a severe 2009 H1N1 influenza case with antiviral and surfactant replacement (19). This together with our data points to a potential benefit of surfactant therapy. This therapeutic approach should be therefore further evaluated clinically in severe influenza cases with viral pneumonia.

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