Osteopontin Exacerbates Pulmonary Damage in Influenza-Induced Lung Injury

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SUMMARY: The level of osteopontin (OPN) increases during bacterial lung infection. However, the OPN level in virus-induced lung injury is unclear, and the relationship between the hyper-production of OPN and lung injury remains to be thoroughly understood. Therefore, we sought to determine whether a relationship exists between OPN and pulmonary damage. Particularly, pulmonary edema and the destruction of pulmonary tissue. In this study, we found that the OPN level was significantly elevated in patients with pulmonary damage, and there was a positive correlation between the OPN serum level and disease severity in influenza lung injury. The epithelial sodium channel (ENaC) is the main mechanism of clearance of pulmonary edema fluid, and matrix metalloproteinase 7 (MMP7) can degrade the extracellular matrix. In lung epithelial cells, OPN markedly decreased the mRNA expression of the α-subunit of ENaC through integrin β3 and CD44 (OPN receptors); however, the expression of MMP7 was promoted by OPN interaction with integrin β1 and CD44. In addition, OPN increased the levels of tumor necrosis factor-α and interleukin-6. These findings suggested that OPN might increase influenza virus-induced lung injury by augmenting lung epithelial cell apoptosis and impairing ENaC and extracellular matrix destruction.

INTRODUCTION

Influenza virus infection can lead to highly contagious respiratory diseases with clinical manifestations of fever, cough, dyspnea, and severe pneumonia. At present, direct transmission of avian influenza viruses from domestic poultry to humans has been observed for the following virus subtypes H5N1, H7N2, H7N3, H7N7, and H9N2 (1,2). A novel influenza A virus (IAV), H7N9, was identified in China in March 2013, and, until June 2014, some sporadic cases had been reported (3). Most patients infected with the H7N9 subtype are hospitalized with severe symptoms, particularly, acute respiratory distress syndrome (ARDS), mediastinal emphysema, multi-organ dysfunction, disturbance of consciousness, and some even die (4). Chest radiography of patients infected with the H7N9 subtype revealed multiple ground-glass opacities and pulmonary consolidation in both lungs, which indicated severe lung injury.

Influenza virus infections can lead to significant lung tissue damage with massive pulmonary edema and extensive destruction of the respiratory epithelium (5,6). Human respiratory disease caused by infection by the H5N1 subtype of the virus is characterized by pulmonary edema (5). Recent research showed that most patients infected with the H7N9 subtype who present severe and fatal respiratory diseases develop ARDS, and their extravascular lung water index is increased (7). Pulmonary edema rapidly leads to a severe impairment of gaseous exchange and even respiratory failure; however, augmented alveolar fluid clearance enhances the recovery from lung injury and decreases the mortality rate. Sodium absorption by the epithelial sodium channel (ENaC) is the main mechanism of clearance of pulmonary edema fluid; therefore, ENaC expression and activity is very important in the prevention of lung injury.

The alveolar epithelium comprises alveolar type I (ATI) and type II (ATII) cells, forming a tight epithelial barrier, which play an important role in maintaining pulmonary function. ATI cells are thought to provide only passive barrier function while ATII cells are responsible for the active secretion of surfactant. Respiratory epithelial cells are the major target of viral replication (8). In vitro, the H5N1 subtype of the influenza virus causes substantial death of mammalian airway epithelial cells due to the induction of apoptosis (9). The apoptosis or death of the alveolar epithelium, especially, ATII cells, is an underlying mechanism of alveolar damage in murine and human influenza virus infections (10).

Osteopontin (OPN) plays the role of an inflammatory cytokine in diverse pulmonary diseases, such as Klebsiella pneumonia (11) and tuberculosis (12). OPN levels are also increased in idiopathic pulmonary fibrosis (IPF), which induces the pathogenesis of airway remodeling and contributes to fibroblast activation (13).
Notably, increased OPN expression levels in mice infected with IAV are correlated with pathogenicity (14). However, the function of OPN in virus-induced lung injury is poorly understood. In this study, we showed that OPN levels were significantly elevated in patients with pulmonary damage. Furthermore, a positive correlation was identified between serum OPN levels and disease severity in influenza virus-induced lung injury. To further investigate the role of OPN in pulmonary damage, we analyzed the influence of OPN on lung epithelial cell destruction and pulmonary edema.

MATERIALS AND METHODS

Patients and associated procedures: Our study was conducted in 10 patients with confirmed influenza A H1N1 infection and 12 hospitalized patients with laboratory-confirmed H7N9 virus subtype infection recruited at the First Affiliated Hospital College of Medicine, Zhejiang University, Hangzhou, China. Patients infected with the H7N9 subtype presented with new-onset respiratory symptoms and severe lung injury confirmed by chest radiography (15). For comparison, we studied 10 healthy volunteers recruited during the same period. This study was approved by the Institutional Review Board of the First Affiliated Hospital College of Medicine, Zhejiang University, Hangzhou, China (reference number 2013–166). Clinical data and physical examination details were collected and analyzed for all enrolled patients (Table 1). The acute physiology and chronic health evaluation II (APACHE-II) score (16) of each enrolled subject was calculated as a measurement of disease severity. The day of clinical symptom onset was designated day 1. Sera were collected 3–5 d after clinical symptom onset.

Evaluation of patient viral load: The viral load in patients’ respiratory secretions was determined immediately upon admittance, as previously described (15). Briefly, H1N1 and H7N9 virus subtypes were detected with RT-PCR, and the cycles threshold values of these genes in endotracheal aspirate samples were determined immediately upon admittance, as previously described (15).

<table>
<thead>
<tr>
<th>Characteristic of patient</th>
<th>Patient with H1N1</th>
<th>Patient with H7N9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>54 ± 7.90 (43–66)</td>
<td>63.50 ± 16.50 (36–84)</td>
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<tr>
<td><strong>Sex (M/F)</strong></td>
<td>4/6 (66.7)</td>
<td>6/6 (50)</td>
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<tr>
<td><strong>Symptoms</strong></td>
<td></td>
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<tr>
<td>Fever</td>
<td>38.46 ± 0.42</td>
<td>39.19 ± 0.88</td>
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<tr>
<td>Sore throat</td>
<td>7/10 (70)</td>
<td>0/12 (0)</td>
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<tr>
<td>Cough</td>
<td>7/10 (70)</td>
<td>10/12 (83.3)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>0/10 (0)</td>
<td>7/12 (58.3)</td>
</tr>
<tr>
<td>Underlying medical disorders</td>
<td>5/10 (50)</td>
<td>9/12 (75)</td>
</tr>
<tr>
<td>Initial CT scan</td>
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<td></td>
</tr>
<tr>
<td>Extensive bilateral multilobar infiltrates</td>
<td>2/10 (20)</td>
<td>11/12 (91.7)</td>
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<tr>
<td><strong>Detection of virus</strong></td>
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<tr>
<td>FLU(A/C)</td>
<td>30.03 ± 3.90</td>
<td>29.63 ± 5.45</td>
</tr>
<tr>
<td>H1/H7(CI)</td>
<td>30.22 ± 2.55</td>
<td>29.38 ± 5.58</td>
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<td>N1/N9(Ct)</td>
<td>31.19 ± 3.11</td>
<td>30.76 ± 4.87</td>
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<td><strong>Complication</strong></td>
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<td>ARDS</td>
<td>0/10 (0)</td>
<td>6/12 (50)</td>
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<td>Respiratory failure</td>
<td>0/10 (0)</td>
<td>8/12 (66.7)</td>
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<td>Shock</td>
<td>0/10 (0)</td>
<td>2/12 (16.7)</td>
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<td>Length of hospital stay  (d)</td>
<td>5 ± 1.90 (3–9)</td>
<td>15.80 ± 5.30 (11–29)</td>
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<td><strong>Treatment</strong></td>
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<td>Oselatimivir</td>
<td>6/10 (60)</td>
<td>12/12 (100)</td>
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<td>Glucocorticoid given</td>
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<td>7/12 (58.3)</td>
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<td>Advanced respiratory</td>
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<td>Support</td>
<td>0/10 (0)</td>
<td>7/12 (58.3)</td>
</tr>
<tr>
<td>In-hospital death</td>
<td>0/10 (0)</td>
<td>1/12 (8.3)</td>
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<tr>
<td>APACHE-II</td>
<td>2.00 ± 0.82 (1–3)</td>
<td>22.08 ± 11.34 (7–39)</td>
</tr>
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</table>

1): Advanced respiratory support included non-invasive ventilation by continuous positive airway pressure, mechanical ventilation, and extracorporeal membrane oxygenation.
2): APACHE-II = Acute physiology and chronic health evaluation II. Categorical variable data are given as percentage (%). Continuous variable data are given as mean ± SD (range).

Cell culture: Murine lung epithelial cells (MLE-12) and human lung epithelial cells (A549) were propagated at 37°C with 5% CO2 in Dulbecco’s modified Eagle’s medium/F-12 (DMEM/F-12) (HyClone, Logan, UT, USA) and in DMEM (Gibco, Gaithberg, MD, USA) supplemented with 100 μg/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum (FBS) (Gibco). Cells were seeded onto 48-well tissue culture plates at a density of 2.0 × 105 cells/ml. Following adherence of 60% to 70% of cells, 100 ng/ml recombinant OPN (rOPN; R&D, Minneapolis, MN, USA) was added (13). After incubation for 24 h, cell culture supernatants were harvested, and MMP7 levels were assayed by ELISA. In parallel experiments, cells were pretreated for 2 h with anti-β1 integrin (10 μg/ml), anti-β3 integrin (10 μg/ml), and anti-CD44 (10 μg/ml), and recombinant murine OPN was added (100 ng/ml). Alveolar macrophages were purified from female C57BL/6 (B6) (aged 6 w) mice as described previously (17). All mice were maintained under specific pathogen-free conditions, and the Institutional Animal Care and Use Committee preapproved all procedures. Alveolar macrophages were cultured at 37°C with 5% CO2 in RPMI 1640 medium (Gibco) supplemented with 100 μg/ml penicillin, 100 μg/ml streptomycin, and 10% FBS. Approximately 24 h before the study, cells were seeded into 96-well tissue culture plates at a density of 2.0 × 103 cells/ml with 2 μg/ml murine rOPN (R&D). Cell culture supernatants were harvested, and tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels were assayed by ELISA.

ELISAs: ELISA kits (OPN [from R&D], MMP7, TNF-α, and IL-6 [all from eBioscience, San Diego, CA, USA]) were used to determine the concentrations of OPN, MMP7, TNF-α, and IL-6 according to the manufacturers’ instructions. The absorbance was measured at 450 nm by an enzyme-linked immunosorbent assay (ELISA) microplate reader.

Analysis of mRNA expression: Total RNA was isolated using TRIzol (TaKaRa, Shiga, Japan). First strand cDNA was synthesized by reverse transcription with random primers (Invitrogen, Carlsbad, CA, USA). The specific primers used were as follows: glyceraldehyde-3-phosphate dehydrogenase (G3PDH), 5′-ACCACAGTCATGCACATGCAC-3′ (sense), 5′-TCCACCCACCCCT
GTGTGCTGTA-3′ (antisense); αENaC: 5′-GCTCAACC TTGACCTAGACCT-3′ (sense); βENaC: 5′-GGGCCAG GCTACACCTACA-3′ (sense); γENaC: 5′-GGCACCGTAA GCAGGAACC-3′ (antisense); ENaC: 5′-GCTCAACC TTGACCTAGACCT-3′ (antisense); αENaC: 5′-GGGCCAG GCTACACCTACA-3′ (sense); γENaC: 5′-GGCACCGTAA GCAGGAACC-3′ (antisense). Quantitative real-time PCR analysis of mRNA expression was carried out using Light Cycler Fast Start DNA Master SYBR Green I systems (TaKaRa). Data were normalized to G3PDH.

Apoptosis assays: MLE-12 cells were seeded onto 48-well tissue culture plates at a density of 4 × 10⁵ cells/ml. Following adherence of 60% to 70% of cells, 100 ng/ml mouse rOPN (R&D), 60 μg/ml polyinosinic polycytidylic acid (polyI:C) (Sigma-Aldrich, St. Louis, MO, USA), and 5 ng/ml TNF-α (R&D) were added. Apoptosis was measured using an Annexin V-FITC Apoptosis Detection Kit (BD, Franklin Lakes, NJ, USA), as specified by the manufacturers. The apoptosis rate was determined by flow cytometry (Beckman Coulter FC500 MPL) with CXP software. Annexin +/−PI + cells were defined as late apoptosis, while annexin +/−PI − cells were defined as early apoptotic.

Statistical analysis: Data are presented as mean ± SD and are representative of at least 2 independent in vitro experiments. The significance of differences between 2 groups was determined using the Student’s t-test. *P < 0.05, **P < 0.01, ***P < 0.001.

RESULTS

Positive correlation between serum OPN levels and disease severity in influenza-pneumonia patients: OPN is an inflammatory cytokine that has been implicated in inflammation and fibrosis/tissue repair in lung diseases. We compared the serum OPN levels between patients infected with the H1N1 and H7N9 subtypes of IAV and healthy controls. Compared with the H1N1 subtype and healthy control groups, the serum OPN levels were significantly elevated in the H7N9 subtype group (Fig. 1A), which had severe lung injury; there was no significant difference between the serum OPN levels of the H1N1 subtype group and the healthy control group. In order to elucidate the association between OPN expression and the severity of lung damage, we determined the APACHE-II score of patients infected with the H7N9 and H1N1 subtypes and found a positive correlation between serum OPN levels and APACHE-II score (P < 0.0001) (Fig. 1B). MMP7 (18), TNF-α, and IL-6 (19) are related to lung injury. Analysis of the serum MMP7 levels in patients infected with the H7N9 and H1N1 subtypes, compared to healthy controls, revealed that MMP7 levels in patients infected with the H7N9 subtype were significantly higher than in patients infected with the H1N1 subtype and healthy controls (Fig. 1C). However, there were no obvious differences in the MMP7 levels detected in patients infected with the H1N1 subtype and healthy controls. TNF-α and IL-6 function as important acute-phase cytokines during inflammatory processes and enhance susceptibility to infectious diseases (20). We examined the production of TNF-α and IL-6 in the serum of patients infected with the H1N1 and H7N9 subtypes of IAV. Results showed that, compared to the control groups, patients infected with the H7N9 and H1N1 subtypes produced significantly higher levels of TNF-α and IL-6 (Fig. 1D, 1E). Interestingly, compared to patients infected with the H1N1 subtype, patients with the H7N9 subtype had significantly higher serum levels of TNF-α and IL-6 (Fig. 1D, 1E).

![Fig. 1. OPN, MMP7, and cytokine levels in influenza virus-infected patients. (A) OPN levels were measured by ELISA in the serum of patients infected with the H1N1 and H7N9 subtypes. Serum specimens of healthy subjects (HC) were included as controls. (B) A scatter plot shows the relationship between OPN serum levels and disease severity. The line represents the regression line (R = 0.829, P < 0.0001). (C) MMP7 concentrations in the serum of patients infected with the H1N1 and H7N9 subtypes were detected by ELISA. The levels of TNF-α (D) and IL-6 (E) were elevated in the serum of patients infected with the H1N1 and H7N9 subtypes when compared to controls. Data represent the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.]
OPN increased the apoptosis of alveolar epithelial cells and inhibited αENaC expression: The role of OPN in lung damage remains unclear. The death or apoptosis of alveolar epithelium leads to lung dysfunction. To elucidate the role of OPN in this process, we analyzed the effect of OPN on the apoptosis of murine alveolar epithelial cells and found the effect of OPN alone on cell apoptosis was very weak. However, the apoptosis of murine alveolar epithelial cells was significantly increased following stimulation with both polyI:C and OPN compared to stimulation only with polyI:C (Fig. 2A, 2B). In other words, OPN increased polyI:C-induced apoptosis in the alveolar epithelium.

It is well-known that pulmonary edema leads to the alveolar epithelial damage, while sodium absorption by ENaC is the main mechanism of liquid clearance from the lungs. In order to investigate whether OPN can modulate the expression of ENaC, we treated murine alveolar epithelial cells with OPN for 24 h. Results indicated that OPN significantly decreased the mRNA expression of the α-subunit of ENaC (Fig. 2C); however, there was no impact on the expression of the β- and γ-subunits (data not shown). OPN interacts with a variety of cell surface receptors, including αvβ1, αvβ3, αvβ6, αvβ1, and αvβ1 integrins, as well as CD44 (20). In order to analyze which receptors play an important role in the effect of OPN on αENaC production, murine alveolar epithelial cells were pre-treated with antibodies specific to different receptors. The inhibition of αENaC expression was abolished by treatment with anti-β3 and anti-CD44 antibodies (Fig. 2A).

OPN increased the production of MMP7: MMP7 degrades proteoglycans, fibronectin, type IV collagen, and other components of the lung matrix. OPN increases MMP7 expression in pulmonary fibrosis (13); however, the effect of OPN on the expression of MMP7 in lung damage is not clear. In our study, stimulation of human alveolar epithelial cells with OPN for 24 h revealed that MMP7 production was significantly augmented by OPN treatment (Fig. 3A). Moreover, OPN increased MMP7 production in murine alveolar epithelial cells (Fig. 3B). In order to understand which receptors interact with OPN in MMP7 production, murine alveolar epithelial cells were pre-treated with antibodies specific for different receptors. The induction of MMP7 expression was partially blocked by anti-β1 integrin and anti-CD44 antibodies, while it was not affected by the anti-β3 integrin antibody (Fig. 3B).

OPN enhanced inflammatory cytokine production in alveolar macrophages: Given that OPN, TNF-α, and IL-6 were significantly higher in patients infected with the H7N9 subtype than in healthy subjects, we examined whether OPN affected the production of TNF-α and IL-6 in alveolar macrophages. Alveolar macrophages from wild-type mice were treated with murine rOPN for 24 h. OPN induced a significant increase in the levels of TNF-α and IL-6 (Fig. 4A, 4B). Moreover, TNF-α induced apoptosis of murine alveolar epithelial

![Graph](image-url)

**Fig. 2.** OPN affected the apoptosis of lung epithelial cells and the expression of ENaC (A and B). MLE-12 cells were incubated with polyI:C (60 μg/ml) alone or with OPN (100 ng/ml) for 24 h. The percentage of apoptotic cells was detected by flow cytometric analysis of annexin V/PI staining. Apoptosis was assessed by counting the percentage of early apoptotic and late apoptotic cells. (C) MLE-12 cells were stimulated with 100 ng/ml rOPN for 24 h. ENaC subunits were detected by real-time PCR. In parallel, osteopontin-stimulated cells were treated with anti-β1, anti-β3, and anti-CD44 antibodies. Data represent the mean ± SD. ###P < 0.001 vs. control, *P < 0.05 vs. OPN group, **P < 0.01.
Fig. 3. Effect of OPN on the expression of MMP7 following incubation with rOPN (100 ng/ml) for 24 h. MMP7 levels were detected by ELISA in culture supernatants of (A) A549 cells and (B) MLE-12 cells. In parallel experiments, OPN-stimulated cells were treated with anti-β1, anti-β3, and anti-CD44. Data represent the mean ± SD. ###P < 0.001 vs. control, *P < 0.05 vs. OPN group.

Fig. 4. OPN enhanced TNF-α and IL-6 production in alveolar macrophages. TNF-α (A) and IL-6 (B) were measured by ELISA in the culture supernatants of murine alveolar macrophages 24 h after stimulation with rOPN (2 μg/ml). MLE-12 (C and D) cells and A549 (E and F) cells were incubated with murine TNF-α (5 ng/ml) and human TNF-α (20 ng/ml), respectively for 24 h. The percentage of apoptotic cells was detected by flow cytometric analysis of annexin V/PI staining. Apoptosis was assessed by counting the percentage of early apoptotic and late apoptotic cells. Data represent the mean ± SD. *P < 0.05, **P < 0.01.

cells (Fig. 4C, 4D). Similar results were observed when human alveolar epithelial cells were incubated with TNF-α (Fig. 4E, 4F). Therefore, OPN may also further increase the apoptosis of alveolar epithelial cells by inducing TNF-α production.

DISCUSSION

OPN is implicated in subacute and chronic pulmonary inflammation. Although research has shown that increased OPN expression during IPF (13), tuberculo-
sis, and silicosis (12), studies of the role of OPN in influenza virus pneumonia are very limited. Moreover, patients infected with the H7N9 subtype present with severe lung injury and even die (4). In this study, we showed that OPN levels were significantly elevated in patients infected with the H7N9 subtype who had pulmonary damage, and we identified a positive correlation between serum OPN levels and disease severity in influenza-induced lung injury. OPN induced lung injury by influencing the apoptosis of alveolar epithelial cells, inhibiting ENaC $\alpha$-subunit expression, and increasing MMP7 production.

The alveolar epithelium is very important in maintaining pulmonary function, with alveolar epithelial cell apoptosis or death leading to lung dysfunction. In influenza virus pneumonia, alveolar epithelial cell apoptosis is induced by macrophages through the release of tumor necrosis factor-related apoptosis-inducing ligand (6). Avian influenza A virus H5N1 induces apoptosis in alveolar epithelial cells via the caspase-dependent pathway (9). PolyI:C, an analog of dsRNA, can induce a marked inflammatory response in the lungs and lead to impairment of pulmonary function. In our study, OPN increased the rate of apoptosis of alveolar epithelial cells induced by polyI:C. TNF-$\alpha$ also induced apoptosis of lung epithelial cells, and we found that OPN induced the production of TNF-$\alpha$, indicating that OPN directly and indirectly induces alveolar epithelial cell apoptosis and may play an important role in pulmonary damage. During viral lung infection, the expression of many inflammatory cytokines, including TNF-$\alpha$ and IL-6, is increased, leading to inflammatory responses and further recruitment of infiltrating lymphocytes (21). TNF-$\alpha$ and IL-6, which are produced by macrophages exposed to respiratory viruses, influence the susceptibility to and severity of infection as well as recovery from respiratory virus infections (19). In the process of lethal IAV infections, TNF-$\alpha$ and IL-6 are thought to correlate with the magnitude of viral replication, multiple organ disorders, and activation of caspase 1 (22). During pneumococcal pneumonia induced in OPN knockout mice by infection with S. pneumoniae, the concentrations of TNF-$\alpha$ and IL-6 in the serum and spleen tissue were decreased compared to WT mice (23). In our study, high levels of TNF-$\alpha$ and IL-6 were detected in patients with influenza A H1N1 and H7N9 viral subtypes. Moreover, OPN induced the production of TNF-$\alpha$ and IL-6 in alveolar macrophages. In addition, TNF-$\alpha$ induced alveolar epithelial cell apoptosis and led to lung tissue damage, while OPN was also shown to be indirectly involved in the process of lung damage by inducing the production of inflammatory cytokines.

MMP7 deficiency, which confers protection against severe lung injury, is accompanied by decreased neutrophilic inflammation and chemokines in the alveolar fluid (24). Although, increased MMP7 expression is detected in lung epithelial cells in Gram-negative bacterial infections (25), the level of MMP7 in influenza virus infections is unclear. In this study, we found that MMP7 production in lung injury patients was significantly increased. OPN regulates the expression of some matrix metalloproteinases. Upon further investigation, we found that the production of MMP7 in alveolar epithelium cells was significantly augmented by OPN treatment and that the effect was mediated by $\beta_1$-integrin and CD44 receptors, consistent with previous reports (20).

Influenza virus infection leads to significant lung tissue damage with extensive destruction of the respiratory epithelium and massive pulmonary edema (5,6). Pulmonary edema, which is common in diverse lung diseases, is triggered by a multitude of inflammatory cytokines and leads to excessive leakage of fluid into the interstitial and alveolar spaces. Sodium absorption by ENaC, which is composed of 3 related subunits ($\alpha$, $\beta$, and $\gamma$), is the main mechanism by which fluids are cleared from the lungs. The $\alpha$-subunit is sufficient to activate the sodium channel, while the $\beta$-subunit or $\gamma$-subunit alone are unable to induce amiloride-sensitive currents, which is required for maximal expression of the active sodium channel (26). Moreover, mice that are genetically deficient in $\alpha$ENaC are unable to clear fluid from their airways and die from respiratory distress (27). However, lung fluid clearance is not affected in $\beta$ENaC knockout mice (28). The influenza virus has been shown to reduce ENaC activity in murine ATII cells, with concomitantly impaired clearance of sodium and fluid from the airways (29). Pseudomonas infections hamper the clearance of fluid from the lungs and accelerate edema formation by reducing ENaC expression (30). In our study, we found that OPN mediated a significant decrease in $\alpha$ENaC expression through the $\beta_3$-integrin and CD44 receptors. It can be speculated that this effect, at least in part, is the mechanism by which OPN increases pulmonary edema and lung damage.

In conclusion, OPN expression correlates positively with the severity of lung injury. This effect is mediated by OPN-induced apoptosis of lung epithelial cells and destruction of the extracellular matrix. Furthermore, OPN impairs clearance of sodium and fluid from the airways by reducing the expression of the ENA-C $\alpha$-subunit.

In summary, the present study revealed the role of OPN in lung damage caused by influenza-induced pneumonia and implicates serum OPN levels as a marker of lung injury.

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Conflict of interest None to declare.

REFERENCES

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