CASE REPORT

Immunohistochemical Findings of an Autopsied Lung Specimen from a Patient with Pandemic Influenza (A/H1N1pdm) Virus Infection

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Abstract

A 24-year-old female presented with fever and dry cough. Influenza A virus infection was suspected and the patient was treated with neuraminidase inhibitors. Five days after diagnosis, the patient developed persistent fever and dyspnea, and was diagnosed with severe pneumonia. Despite intensive treatment, the pneumonia worsened and the patient died 14 days after admission. At autopsy, a diffuse alveolar damage (DAD) pattern was observed. Immunohistochemical evaluation indicated severe epithelial damage, resulting in successive regeneration of alveolar type II cells followed by marked proliferation of smooth muscle cells and an increase of collagen fibers at the tip of alveolar orifices.

Key words: influenza virus, pneumonia, alveolar orifice, type II epithelial cells, smooth muscle cells

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Introduction

The influenza virus is a common cause of lower respiratory tract infections in adults. Infections can occur as a pandemic, an epidemic, or sporadic outbreaks. Clinical pneumonia attributable to the influenza virus infection is uncommon, but when it does occur, secondary bacterial infection (secondary bacterial pneumonia) as well as the influenza A virus itself (purely influenza viral pneumonia) need to be considered as the possible cause. Purely influenza viral pneumonia is usually mild, but it can be overwhelming in some patients and prove fatal within 24 hours after onset.

A pandemic caused by influenza A/H1N1pdm virus occurred during the 2009/2010 influenza seasons. In addition, several reports have described the pathological findings of pneumonia caused by influenza A/H1N1pdm (1-12), however, there are few reports that have evaluated lung specimens, immunohistochemically. The aim of our study was to describe the pathological and immunohistochemical findings of an autopsied case of influenza A/H1N1pdm virus pneumonia. We demonstrate that diffuse damage of epithelial cells at the alveolar orifices played a significant role in the pathogenesis leading to respiratory failure caused by influenza A/H1N1pdm virus infection.

Case Report

On August 26, 2009, a 24-year-old female with mental retardation had symptoms of fever and dry cough. The rapid diagnostic test for influenza using throat swab was performed at the outpatient clinic and a positive result of influenza A antigen was obtained. In addition, the patient’s mother was also diagnosed with influenza A infection.
days before the onset of the patient’s symptoms. Therefore, the patient was diagnosed with influenza A virus infection and was prescribed neuraminidase inhibitors. However, the patient was not compliant and did not use the prescribed neuraminidase inhibitors. Five days later, the patient developed further symptoms of persistent fever and dyspnea, and was diagnosed with severe pneumonia and admitted to the hospital. Chest radiographs and chest computed tomography (CT) scans of the chest upon admission are shown in Fig. 1. RT-PCR for influenza A/H1N1pdm was positive using the throat swab specimen obtained on admission.

After admission, steroid pulse therapy (methylprednisolone 1 g/day for 3 days) was performed and successive oral prednisolone was administered. In addition, oseltamivir (75 mg, twice a day for 5 days), and several antibiotics (meropenem [0.5 g four times a day, for 11 days], vancomycin [1 g twice a day, for 5 days], cefazolin [1 g twice a day, for 4 days]) were administered. Furthermore, sivelestat (4.8 mg/kg for 10 days) and gabexate mesilate (20 mg/kg for 8 days) were administered. For respiratory failure, mechanical ventilation was performed from admission to the time of death (16 days). In addition, extracorporeal membrane oxygenation was performed for 11 days from day 2 to day 12. After this treatment, chest X-rays at day 16 showed slight improvement of consolidation, however, the patient died due to subarachnoid hemorrhage.

The patient was diagnosed with purely influenza viral pneumonia and not secondary bacterial pneumonia based on following evidence: i) the presence of infiltrates in chest radiographs and chest CT scans; ii) influenza virus A infection was confirmed by the rapid diagnosis kit as well as RT-PCR for influenza A/H1N1pdm; and iii) positive staining for influenza antigen was confirmed by immunohistochemistry without significant bacterial infection confirmed by repeated Gram stains.

In addition to hematoxylin-eosin stain, Azan stain for collagen fibers and Elastica van Gieson (EVG) stain for elastic fibers were also performed on tissue sections. Immunohistochemical stains were performed using the avidin-biotin peroxidase complex (ABC) method (LSAB kit; Dako, Carpinteria, CA, USA) using antibodies, as listed in Table 1. After confirming the specificity of these antibodies, the appropriate positive and negative controls were stained in parallel. All staining procedures were performed according to the
manufacturers’ instructions. To analyze the distribution of the influenza virus antigen, immunohistochemistry using a mouse monoclonal antibody against influenza A nucleoprotein was performed.

At autopsy, lung specimens were diagnosed as being consistent with the organizing phase of diffuse alveolar damage (DAD) (Fig. 2A). In the areas of organized DAD, the surfaces of alveolar ducts or spaces were covered by irregular membranous substances, consisting of loosely proliferated plump myofibroblasts, light eosinophilic myxoid matrix, and loose mature collagen fibers. In these parts, epithelial cell linings stained with AE1/AE3 (pancytokeratins) antibody were rarely demonstrated. Instead, discontinuous thin epithelial cell fragments were occasionally detected and myofibroblasts stained for α-smooth muscle actin and mature collagen fibers were frequently demonstrated. In the present case, marked nodular thickening at the location of alveolar orifices was demonstrated (Fig. 2A, B). Immunohistochemical staining for influenza A virus (using mouse monoclonal antibody against influenza A nucleoprotein) demonstrated few viral antigen-positive macrophages in both lungs (Fig. 3). However, influenza A antigen was not detected in epithelial cells. In addition, no significant bacterial respiratory infection was confirmed at autopsy.

A schematic view of the normal structure of alveolar orifices to compare pathological findings is demonstrated in Fig. 4. From the results of Azan and EVG stains, no increase in elastic fibers was observed, but both collagen fibers and smooth muscle cells increased in nodular fashion at the alveolar orifices. Marked proliferation of alveolar type II cells were detected by immunohistochemistry as revealed by AE1/AE3 (Fig. 5A, B), Ck19, CAM 5.2, EMA, KL-6, SP-A, and TTF-1 immunostains around alveolar ducts. A thin layer of alveolar epithelial cells as viewed by AE1/AE3 (Fig. 5B), CK19, CAM5.2, and KL-6, covered the surface of the tip of the alveolar orifices. MIB-1 positive nuclei of alveolar epithelial cells were often detected around alveolar ducts, suggesting mitotic abilities. In addition, an increase in smooth muscle cells at alveolar orifices was revealed by h-caldesmon staining, a marker of mature smooth muscle, and α-smooth muscle actin immunostain (Fig. 6A, B). The proliferation of smooth muscle as well as an increase in collagen fibers might be a major cause of irregular and tortuous alveolar ducts, resulting in marked deformity and dysfunction of peripheral airways in this case. As for lymphocytes, CD3, CD20, CD4 and CD8 were stained, but no specific distribution of lymphocytes was observed. CD3-positive cells were diffusely distributed in the lung tissue, and CD4-positive cells were more prevalent compared with CD8-positive cells. CD20 and CD79a-positive cells were not detected. The true cause of the increase in collagen and smooth muscle cells at alveolar orifices should be investigated in the future to clarify the exact genesis.

Discussion

Previously reported pathological findings in cases of pneumonia caused by H1N1-2009 pandemic influenza viruses are presented in Table 2, where the main pathological
finding was DAD. In some cases, secondary bacterial pneumonia was observed (Table 2).

In the human lung, the dynamic movement of the alveolar wall is very important for gas exchange. To inflate and deflate, extension and contraction of alveolar orifices play a major role in changing the size of the alveoli. Several reports have analyzed the pathology found in lung specimens of patients with influenza A/H1N1pdm virus infection (1-12). In pneumonia caused by influenza A/H1N1pdm virus, DAD or alveolar hemorrhage are the predominant pathological features (1-12). However, immunohistochemical analyses are rarely performed and the precise mechanism of

**Figure 4.** A schematic view of the alveolar wall. Arrows demonstrate the points of the alveolar orifice. This figure was adapted from the textbook, “Gendai No Soshigakku” with the permission of the Kanehara & Co., Ltd.

**Figure 5.** Immunohistochemical staining using anti-cytokeratin antibody. A: low magnification (100×), B: high magnification (400×). Marked proliferation of alveolar type II cells is shown. A thin layer of alveolar epithelia was observed at the alveolar orifices (B).

**Figure 6.** Immunohistochemical staining using anti-α-smooth muscle actin antibody. A: low magnification (100×), B: high magnification (400×). Marked proliferation of myofibroblasts is demonstrated just beneath the alveolar epithelium at the location of the alveolar orifices (B).
respiratory failure has not been demonstrated.

In the present study, immunohistochemical analysis was performed on autopsied lungs which had evidence of DAD caused by influenza A/H1N1 pdm virus infection. The present case demonstrated severe damage of epithelial cells and marked proliferation of smooth muscle cells at the point of the alveolar orifices. In addition, there was an organizing phase of DAD as well as a regeneration of type II alveolar cells in which several epithelial cell markers and MIB-1-positivity were detected. However, regenerated type II cells did not entirely encompass the tip of the alveolar orifices. In addition, a marked proliferation of smooth muscle cells and increased collagen fibers were also demonstrated at the subepithelial tissue at the location of the alveolar orifices.

In normal alveolar structure, capillaries are observed at the tip of the alveolar orifice. Therefore, the disruption of capillary flow was likely due to the marked proliferation of smooth muscle cells at this point and reduced the mobility of the blood supply at this point and reduced the mobility of the alveoli in gas exchange.

In conclusion, severe epithelial damage was determined as the main mechanism of respiratory failure caused by influenza A/H1N1 pdm virus infection. In addition, successive regeneration of alveolar type II cells followed by marked uneven proliferation of smooth muscle cells and collagen fibers at the tip of alveolar orifices of the distorted irregular alveolar ducts might be the cause of dysfunction of peripheral airways. The precise mechanisms surrounding these observations will be investigated in detail in future studies.

The authors state that they have no Conflict of Interest (COI).

**References**

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