Elevated Serum Levels of Lysozyme in Desquamative Interstitial Pneumonia

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Abstract

We present a case of desquamative interstitial pneumonia (DIP) with elevated serum levels of angiotensin-converting enzyme and lysozyme, which are often found in sarcoidosis. After steroid therapy, improvements in the lung opacity and the serum lysozyme level were observed. Retrospective evaluation of four additional DIP cases showed that four of the five cases studied had an elevated serum lysozyme level. In an immunohistochemical analysis of the lung specimens, increased expression of lysozyme were found in the neutrophils and the alveolar macrophages. Elevated levels of serum lysozyme can occur in diseases such as DIP in which the neutrophil and macrophage activity may affect a patient’s pathological condition.

Key words: desquamative interstitial pneumonia, lysozyme, neutrophil, alveolar macrophage

Introduction

Desquamative interstitial pneumonia (DIP) is an interstitial lung disease of unknown etiology that was first reported by Liebow et al in 1965; its pathological characteristics include widespread cuboidal cell metaplasia of the alveolar epithelium and a large number of macrophages accumulated in the airspaces (1). DIP is a subtype of idiopathic interstitial pneumonia that responds well to steroid therapy and generally has a good prognosis (2). However, compared to idiopathic pulmonary fibrosis (IPF), the frequency of its occurrence is very low, and although there are occasional reports regarding its histological characteristics and related bronchoalveolar lavage fluid (BALF) findings (3), the details of its pathological conditions are still unknown.

Case Report

A 64-year-old man who had been suffering from exertional breathlessness for approximately 10 years was admitted to the hospital because of abnormal chest opacities on a health checkup. He had a smoking habit of 20 cigarettes per day for 37 years, but he had quit smoking 7 years earlier. His occupation was farming and fishing and he had no apparent history of dust inhalation. Minimal fine crackles were heard in both lung fields. A serological examination revealed abnormal values that were often seen in cases of sarcoidosis, including an angiotensin-converting enzyme (ACE) level of 23.5 IU/L (normal value < 21.4 IU/L), a lysozyme level of 12.7 μg/mL (< 10.2 μg/mL), and a soluble interleukin-2 receptor level of 676 U/mL (< 519 U/mL). There were increases in the patient’s Ig levels, including an IgG level of 1,870 mg/dL (< 1,700 IU/mL), an IgA level of 724 mg/dL (< 410 IU/mL), and an IgE level of 2,341 IU/mL (< 173 IU/mL), and there were also increases in his serum markers for interstitial pneumonia, including a KL-6 level of 1,150 U/mL (< 500 U/mL), a surfactant protein (SP)-D level of 153 ng/mL (< 110 ng/mL), and a SP-A level of 131 ng/mL (< 43.8 ng/mL). In addition, while the patient tested positive for antinuclear antibodies (× 80; speckled type), the results for all other autoantibodies were negative. An arterial blood gas analysis revealed hypoxemia (partial pressure of arterial oxygen; 69.2 Torr, partial pressure of arterial carbon dioxide; 33.7 Torr in room atmosphere), and the pulmonary function test demonstrated an obstructive respiratory dys-
Figure 1. A chest roentgenogram shows bilateral reticular opacities in the middle and lower lung fields. A chest computed tomographic scan reveals a diffuse emphysematous change, a ground-glass attenuation, and mild mediastinal lymphadenopathy.

function (forced expiratory volume in 1s as percent of forced vital capacity; 62.9%) and a reduced percent diffusion capacity for carbon monoxide of 39.7%. A chest roentgenogram showed bilateral reticular opacities in the middle and lower lung fields and a chest computed tomographic scan (Fig. 1) revealed a diffuse emphysematous change, a ground-glass attenuation, and mild mediastinal lymphadenopathy. Gallium scintigraphy showed no uptake in the mediastinum or hilum of the lung, but mild accumulations were observed in the lower lungs. While there was no lymphocytosis in the BALF, there were increases in the levels of neutrophils (25.2%) and eosinophils (20.7%). Through a surgical lung biopsy, we were able to pathologically rule out the possibility of sarcoidosis and diagnose the patient to have DIP (Fig. 2). Based on the findings of careful investigations using both BALF and biopsied lung specimens, we were unable to find any pathological microorganisms. We subsequently administered steroid therapy due to deterioration in the patient’s condition, and thereafter improvements of the patient’s lung opacity in the image and decreased serum levels of ACE and lysozyme were observed.

Immunochemistry of lung tissues

An immunohistochemical analysis was performed for lysozyme using paraffin-embedded lung sections obtained from the patient. Briefly, after deparaffinization and rehydration, the tissue sections (4-μm thick) were soaked in 0.3% hydrogen peroxide with absolute methanol for 20 min to inactivate endogenous peroxidases. The lung sections were incubated for 60 min at 4°C with a primary antibody of lysozyme (Novocastra Laboratories Ltd., Newcastle upon Tyne, UK) in a moist chamber. After washing in phosphate-buffered saline, the sections were incubated for 30 min with EnVision+™ (Peroxidase, Mouse, DakoCytomation, Glostrup, Denmark), and then developed with 3,3′-diaminobenzidine. The primary antibody was replaced by an irrelevant IgG1 as a negative control. As a result, lysozyme was expressed in a small but significant minority of neutrophils in the airspace and interstitium (Fig. 2c), and in the alveolar macrophages that had aggregated in the alveoli (Fig. 2d), in comparison to the negative control.

Retrospective analysis using other DIP patients’ samples

We measured the serum levels of ACE and lysozyme, and also performed an immunohistochemical analysis in four other patients with DIP from our departments (2002-2008), using their lung specimens and frozen serum which had been stored before the surgical lung biopsy and the start of steroid therapy. All these patients listed in Table 1 [including the present case and two previously-reported cases (4, 5)] were diagnosed to have pathologically-defined DIP and thus were successfully treated with corticosteroids. Interestingly, including the present case, we found that four of our five cases of DIP showed elevated serum levels of lysozyme. As in our previous report (5), all patients showed positive findings for antinuclear antibodies. In the lung specimens of all patients, immunohistochemical staining for lysozyme demonstrated high levels of immunoreactivity in the alveolar macrophages and the partly-scattered neutrophils. In addition, the levels of lysozyme in BALF were also measured in our five cases with DIP and in five untreated cases with IPF as a control disease, in which no BALF neutrophilia were observed. The BALF levels of lysozyme in the DIP patients were relatively high (Table 1) in comparison with those in IPF patients [median 1.6 (range 0.8-5.6) μg/mL, statistically not significant]. Furthermore, a retrospective analysis of fifteen patients in total with other idiopathic interstitial pneumonias (IPF and pathologically-diagnosed nonspecific interstitial pneumonia) showed no elevation of lysozyme in the serum [4.6 (0.4-9.2) μg/mL].
Figure 2. (a, b) A surgically biopsied lung specimen (Hematoxylin and Eosin staining), showing a homogeneous distribution, a moderate number of interstitially infiltrating cells with mild fibrosis, and hyperplastic alveolar pneumocytes. Macrophages are densely packed in the alveolar lumina (a; original magnification ×40, b; ×400). (c, d) Immunohistochemical staining for lysozyme revealed the hyperimmunoreactivity in neutrophils (arrowheads) and alveolar macrophages (c; ×1,000, d; ×400).

Discussion

DIP is an idiopathic interstitial pneumonia that was first reported by Liebow et al in 1965 (1), and it has been indicated that smoking and exposure to dust are involved in its pathogenesis (2, 6). The main pathological diagnostic criteria were a homogeneous distribution, a large number of macrophages accumulated in the airspaces, a small to moderate number of interstitially infiltrating cells with mild to moderate fibrosis and widespread cuboidal cell metaplasia (7, 8). In the present case, we were able to provide a definite pathological diagnosis of DIP. In addition, the patient presented with abnormal serum ACE and lysozyme levels. The serum ACE levels are known to reach high values in patients with sarcoidosis, and it is thought that ACE levels are higher in sarcoid lymph nodes than in lung tissues and likely indicate macrophage activity. Immunohistological investigations of lysozyme have shown that although lysozyme is expressed in macrophages and giant cells of sarcoidosis and granuloma of Crohn’s disease, it is scarcely expressed in foreign body granulomas and is therefore correlated with the activity of these diseases (9, 10). Lysozyme is also known to be one of the most abundant antimicrobial factors present in human airways and the lysozyme activity has been reported to correlate positively with the BALF neutrophil counts, but not with the bacterial colony counts in patients with cystic fibrosis (11). These findings suggest that some types of cell-mediated immunity affect the lysozyme activity.

In this study, an immunohistochemical analysis of lysozyme using the DIP patient’s lung tissue showed a lysozyme expression in the neutrophils and alveolar macrophages simultaneously with the elevation of serum and BALF lysozyme levels, thus suggesting that, the increased lysozyme had leaked into the circulation. Moreover, steroid therapy improved the patient’s lung opacity and the serum level of lysozyme, and in our five DIP cases, we did not find any clinical factors, except for the BALF neutrophilia, which could distinguish the four DIP patients with elevation of serum lysozyme from the other patient. Taken together, it was suggested that the increased lysozyme level might be due to the increased production from the activated macrophages or neutrophils in DIP, and that these patients’ conditions had been caused by the DIP itself rather than as a complication related to sarcoidosis.

Common BAL fluid findings in DIP include increases in the levels of neutrophils and particularly eosinophils (3-5), and similar findings were made in our cases including the present case (Table 1). However, in the pathological findings of all the cases including the present case, only small amounts of eosinophils were observed in the alveolar walls.
Table 1. Patient Characteristics of Pathologically-defined DIP from 2002 to 2008

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Duration of smoking (months)</th>
<th>BALF Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>M</td>
<td>50</td>
<td>Neutrophils 12.0, Eosinophils 2.0</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>F</td>
<td>60</td>
<td>Neutrophils 35.0, Eosinophils 9.5</td>
</tr>
<tr>
<td>3</td>
<td>260</td>
<td>M</td>
<td>60</td>
<td>Neutrophils 2.9, Eosinophils 19.3</td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
<td>F</td>
<td>7</td>
<td>Neutrophils 37.4, Eosinophils 10.0</td>
</tr>
<tr>
<td>Present</td>
<td>740</td>
<td>M</td>
<td>7</td>
<td>Neutrophils 25.2, Eosinophils 20.7</td>
</tr>
</tbody>
</table>

BALF, bronchoalveolar lavage fluid; ANA, antinuclear antibodies; ACE, angiotensin converting enzyme.

or alveoli, and the cause for this discrepancy is unknown (3-5). In this case, the serum IgE levels also reached high values that later decreased after treatment, but the patient tested negative for any specific allergy diseases. Therefore, we concur with Kawabata et al (3) and speculate that immunological mechanisms comprising a type I allergic reaction to exogenous dust such as cigarette smoke can affect the pathogenesis of DIP.

While there are occasional reports that describe the hilar and mediastinal lymph nodes in cases of DIP (7,12), the histopathological manifestation of the lymph nodes included only hyperplasia with no granuloma formation. In the present case, though we observed mild mediastinal lymphadenopathy, gallium scintigraphy showed no signs of uptake and so a biopsy was not performed. In addition, among cases of idiopathic interstitial pneumonia, it has been reported that while IPF frequently accompanies mediastinal lymphadenopathy, its histopathological manifestation only includes reactive hyperplasia (13,14). Bergin and Castellino (14) speculated that mediastinal lymphadenopathy in cases of IPF may cause inflammatory changes in reaction to cytokine induced by activated alveolar macrophages. The causal mechanisms of mediastinal lymphadenopathy in cases of DIP remain unknown, but because it is characterized by significant aggregations of alveolar macrophages in the alveolar space, hyperplasia may also be caused by reaction to cytokines induced by alveolar macrophages.

This case with DIP was relatively typical in terms of the BAL findings and histopathological manifestations, but it was an interesting case with elevated serum levels of ACE and lysozyme. Although elevated serum levels of lysozyme are common in cases of sarcoidosis, our study suggests that these findings can also occur in diseases such as DIP in which the macrophage and neutrophil activity may be involved in the patient’s pathological condition. However, as the pathogenesis of DIP has not yet been elucidated and may also include heterogeneous subtypes, further studies are required not only to prove a link between an increased production of lysozyme and the pathogenesis of DIP, but also to prospectively elucidate the potential of serum lysozyme levels as a diagnostic tool for DIP.

Acknowledgement

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