Serum Antibody Against Granulocyte/Macrophage Colony-Stimulating Factor and KL-6 in Idiopathic Pulmonary Alveolar Proteinosis

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Here we describe a case of idiopathic pulmonary alveolar proteinosis (I-PAP), in which anti-granulocyte/macrophage colony-stimulating factor (GM-CSF) antibody and high level of KL-6 were found in the serum. Anti-GM-CSF antibody is responsible for I-PAP, and KL-6 is a serum marker for the activity of diffuse interstitial lung disease. A 38-year-old woman, who had no symptoms, was found to have an abnormal shadow on chest radiograph 5 years previously at a health check-up. Chest radiograph showed a patchy shadow in the left lower lung field. Thoracoscopic biopsy was performed because the shadow had gradually expanded during the 5 years. Histological examination revealed proteinous material filling the alveoli and positive staining by the PAS method, suggesting PAP. Anti-GM-CSF antibody and a high level of KL-6 were detected in the serum at the time of diagnosis. Three years later, the shadow disappeared spontaneously. During this period, the level of KL-6 dramatically decreased, although that of GM-CSF antibody remained unchanged. The present case suggests that the serum level of the anti-GM-CSF antibody represents a useful marker for the diagnosis but not for follow-up of the clinical course. On the contrary, KL-6 is an excellent marker for the assessment of the clinical course of I-PAP.

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Pulmonary alveolar proteinosis (PAP) is a rare and enigmatic disorder that is characterized by proteinaceous material accumulation within the alveoli and a variable natural history (Seymour and Presneill 2002; Trapnell et al. 2003). The disease is a heterogeneous group consisting of congenital (C)-PAP and acquired PAP (Seymour and Presneill 2002; Trapnell et al. 2003). More than 90% of the patients with PAP present a primary acquired disorder, that is, idiopathic PAP (I-PAP) (Seymour and Presneill 2002; Trapnell et al. 2003). The primary symptoms in patients with I-PAP are shortness of breath with exercise and/or cough (Wang et al. 1997; Goldstein et al. 1998), and chest radiographs in such patients typically show bilateral air-spaces of a nodular or confluent pattern (Wang et al. 1997). Bronchoalveolar lavage (BAL) and/or lung biopsy (transbronchial [TBLB] or thoracoscopic or open lung biopsy) are needed to confirm the diagnosis (Wang et al. 1997). We describe here the case of a female patient with I-PAP in which 5 years were required to make the diagnosis because of atypical clinical features (e.g., she had no symptoms, her chest radiograph showed a unilateral patchy shadow, routine laboratory data were unremarkable).

Recent investigation has revealed that loss of anti-granulocyte/macrophage colony-stimulating factor (GM-CSF) activity caused by neutralizing anti-GM-CSF autoantibody crippled normal function of alveolar macrophages. This dysfunction that reduces surfactant clearance is responsible of I-PAP (Kitamura et al. 1999; Trapnell et al. 2003). In brief, I-PAP is an autoimmune disease with neutralizing antibody of GM-CSF (Kitamura et al. 1999). In our case, GM-CSF antibody was detected in the serum at the time of diagnosis. And, the level was not changed even after the chest radiograph was improved. On the contrary, the serum level of KL-6 has decreased according to the improvement of the chest radiographic shadow and the diffusing capacity of carbon monoxide (D_{LCO}).

**CASE REPORT**

A 38-year-old non-smoking woman was found to have an abnormal shadow on chest radiograph at a health check up 5 years prior to visiting our clinic. At that time, when she visited her family doctor, she had no symptoms. The chest radiograph showed a patchy shadow in the left lower lung field without abnormal findings in the peripheral blood, serum and sputum analysis. In the TBLB and BAL no significant findings were obtained. The abnormal shadow gradually spread and new patchy shadows emerged also in the right lung field during the 5 years of observation. On August 5, 2002, she was referred to our clinic for close examination. On admission to our hospital, arterial blood gas values under room air were as follows: PaO_{2} 82.0 Torr; PaCO_{2} 40.1 Torr; pH 7.413. Peripheral blood analysis showed normal leukocyte (8.100/μl), red blood cell (4.35 × 10^{6}/μl), platelet (269 × 10^{3}/μl) counts and hemoglobin (12.3 g/dl) level. Also, no laboratory findings suggestive of disorders of the immune system or connective tissue were obtained. C-reactive protein and tumor markers (carcino-embryonic antigen, cytokeratin 19 fragment, pro-gastrin-releasing peptide, and sialyl lewis X antigen) were all negative except for an increase in KL-6 (1,493 U/ml). Her pulmonary function test showed normal function (vital capacity [VC] 2.27L; a prediction of VC 84.4%; forced expiratory volume in one second [FEV_{1}] 1.82L; a prediction of FEV_{1} 83.1%) with a reduction in the diffusing capacity for D_{LCO} (D_{LCO} 13.92 ml/min/mmHg; a prediiction of D_{LCO} 70.5%). Sputum examinations were negative for tuberculous bacteria, fungi or malignant cells. Urinalysis was normal. A chest radiograph showed bilateral diffuse infiltrative shadows (Fig. 1A). The patchy shadows were confluent. Chest computerized tomography (CT) scanning showed opacification with a patchy distribution (Fig. 1B). The opacification had a ground-glass appearance. The distribution was widespread but more prominent at the peripheral portion (Fig. 1B). Lung biopsy under video-assisted thoracic surgery (VATS) was performed. In the thoracoscopic survey the left lung showed patches with a change in hue without any effusion and with a smooth or normal surface in both the visceral and parietal pleura. Histological examination of the biopsy specimens from the...
lingular inferior segment showed eosinophilic dense homogenous material filling in the alveoli (Fig. 2). This precipitate had a fine granular appearance (Fig. 2). The eosinophilic material was periodic acid Schiff reaction (PAS)-positive and diastase resistant. There were also collections of foamy histiocytes engulfing this alveolar material (Fig. 2). The inflammatory reaction was absent to slight in the affected alveoli. Based on these findings, we made the diagnosis of PAP. More than 90% of patients with PAP show a primary acquired disorder of unknown etiology, that is, I-PAP (Seymour and Presnell 2002; Trapnell et al. 2003). I-PAP has been reported as an autoimmune disease with neutralizing antibody of GM-CSF (Kitamura et al. 1999). Kitamura et al. (1999) reported that the GM-CSF-neutralizing antibody was found in sera from all I-PAP patients.
Fig. 3. Radiographic appearance 3 years after the diagnosis (April 25, 2005). Diffuse infiltrative shadows on chest radiogram which were seen on August 5, 2002 had disappeared (A). The opacification with a patchy distribution on chest CT also resolved (B).

Table 1. Time course of serum anti-GM-CSF antibody, KL-6, pulmonary function data and chest images

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<tr>
<td>Serum anti-GM-CSF antibody (μg/ml)</td>
<td>27.66</td>
<td>26.84</td>
</tr>
<tr>
<td>KL-6 (U/ml)</td>
<td>1,493</td>
<td>680</td>
</tr>
<tr>
<td>VC (L)</td>
<td>2.27</td>
<td>2.46</td>
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<tr>
<td>FEV₁ (L)</td>
<td>1.82</td>
<td>2.13</td>
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<tr>
<td>D_LCO (ml/min/mmHg)</td>
<td>13.92</td>
<td>15.34</td>
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<tr>
<td>Prediction of D_LCO (%)</td>
<td>70.5</td>
<td>84.3</td>
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<tr>
<td>Chest images</td>
<td>Fig. 1</td>
<td>Fig. 3*</td>
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</tbody>
</table>

* Chest images in Fig. 3 are taken on April 25, 2005.

Fig. 4. Time course of serum KL-6 level (●) and anti-GM-CSF antibody (Ab) level (○). The level of KL-6 dramatically decreased, although the level of GM-CSF Ab did not decline.
examined but not in sera from secondary PAP (S-PAP) patients or normal subjects. In this case, there were no underlying causes for the S-PAP, such as malignancies, hematopoietic disorders, immunodeficiency disorders, or inhalational syndromes. We measured the serum antibody as described (Seymour et al. 2003). The anti-GM-CSF antibody was detected, and the concentration of the antibody was 27.66 μg/ml. Therefore, the diagnosis of I-PAP was confirmed. The shadow on chest radiograph and CT disappeared spontaneously 3 years after the diagnosis (Fig. 3). D\textsubscript{LCO} also improved (D\textsubscript{LCO} 13.92 ml/min/mmHg, a prediction of D\textsubscript{LCO} 70.5% on August 5, 2002 and D\textsubscript{LCO} 20.10 ml/min/mmHg, a prediction of D\textsubscript{LCO} 112.3% on July 14, 2005) (Table 1). During this period, the level of KL-6 dramatically decreased (from 1,493 U/ml to 156 U/ml), although the level of GM-CSF antibody had not declined (from 27.66 μg/ml to 26.84 μg/ml) (Fig. 4, Table 1).

**DISCUSSION**

PAP is a rare lung disorder characterized by the accumulation of proteinous material in the alveoli (Seymour and Presneill 2002; Trapnell et al. 2003). The common presenting symptoms are dyspnea on exertion and cough (Wang et al. 1997; Goldstein et al. 1998). Chest radiographs in patients with PAP usually show bilateral and patchy consolidation, although there are no specific features suggesting PAP (Goldstein et al. 1998; Shah et al. 2000). In this disease, the serum levels of lactate dehydrogenase, tumor markers and KL-6 have been reported to increase (Shah et al. 2000), and the predominant abnormality in pulmonary function tests is a restrictive ventilatory defect with a reduction in diffusing capacity (Goldstein et al. 1998; Shah et al. 2000). Our patient had no symptoms during the 5 years of observation. Her chest radiograph showed a unilateral patchy shadow at the time of visiting her family doctor. Her laboratory data were unremarkable except for an increase in KL-6 and a slight reduction in the diffusing capacity. These atypical clinical features made it difficult to reach a diagnosis.

The anti-GM-CSF antibody was detected in the present case. I-PAP has been reported as an autoimmune disease with neutralizing antibody of GM-CSF (Kitamura et al. 1999). Neutralization of GM-CSF bioactivity by the antibody causes a dysfunction of alveolar macrophages, which results in reduced surfactant clearance (Kitamura et al. 1999). In addition, recently, administration of aerosolized GM-CSF has reported to improve respiratory function of patients with I-PAP (Arai et al. 2004; Tazawa et al. 2005). Interestingly, Kitamura et al. (1999) reported that the antibody was found in sera from all I-PAP patients but not in sera from S-PAP patients or normal subjects. In our case, the concentration of the antibody did not change, although the shadow on chest radiograph disappeared and D\textsubscript{LCO} improved. Arai et al. (2004) reported a case with PAP successfully treated with inhaled GM-CSF. In their case as well the concentration of anti-GM-CSF antibody was not correlated with the disease severity. They described that the binding capacity of the antibody with GM-CSF was different in each case, although the neutralizing capacity of GM-CSF showed a correlation with the disease activity (Arai et al. 2004). It therefore appears that the absolute quantity of the antibody does not determine the disease severity. In our case, the binding capacity of the antibody may have become weaker during the 3 years since the diagnosis, although the quantity of the antibody has not changed. KL-6 is a mucin-like high molecular weight glycoprotein and a novel serum marker for the activity of diffuse interstitial lung disease (Kohno et al. 1989). However, there are reports that the serum KL-6 level was also high in patients with PAP and that this correlated well with the clinical activity (Nakajima et al. 1998; Takahashi et al. 1998). Takahashi et al. (1998) described that the serum KL-6 levels in patients with PAP were significantly higher than those in patients with other diffuse interstitial lung diseases. In the present case as well the KL-6 level was high, although other laboratory data were unremarkable. In addition, the serum level of KL-6 dramatically decreased with the remission by the chest radiograph appearance and D\textsubscript{LCO}, suggesting that KL-6 is a useful marker for the clinical course of I-PAP.
In conclusion, our case suggests that there are distinct two markers for I-PAP. The serum level of the antibody represents a useful marker for the diagnosis but not for follow-up of the clinical course, but KL-6 is an excellent for the assessment of the clinical course of I-PAP.

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References


