Case Study

Marked Thrombocytosis in Chronic Eosinophilic Pneumonia and Analysis of Cytokine Mechanism

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A 47-year-old woman with marked thrombocytosis of $1,650 \times 10^9/L$ was diagnosed with chronic eosinophilic pneumonia (CEP) based on imaging of the lung and abundant eosinophils in bronchoalveolar lavage fluid. Known gene abnormalities that cause eosinophilia were not detected in bone marrow cells. Treatment with oral prednisolone at 20 mg/day relieved the CEP and resolved the laboratory abnormalities, including eosinophilia and thrombocytosis. Serum concentrations of interleukin (IL)-5 and IL-6 were elevated to 9.6 and 14.0 pg/mL, respectively. The megakaryocyte-potentiating activity of IL-6 and possibly, that of IL-1$\beta$, which is known to be secreted by activated eosinophils, may have caused the marked thrombocytosis in this patient. (J Clin Exp Hematop 55(2) : 97-102, 2015)

Keywords: thrombocytosis, chronic eosinophilic pneumonia, IL-6, IL-1$\beta$, megakaryocyte-potentiating activity

INTRODUCTION

Reactive thrombocytosis is observed in various diseases and clinical conditions, such as after surgery, during infection, and during malignant tumor development, and the platelet count occasionally exceeds $500 \times 10^9/L$. Reactive thrombocytosis is typically observed in rheumatoid arthritis (RA), and it has been reported that the platelet count is proportional to disease activity. However, reactive thrombocytosis with a platelet count of more than $1,000 \times 10^9/L$ is relatively rare. In addition, reactive thrombocytosis usually disappears with a normalized platelet count along with improvement or cure of the underlying disease.

Chronic eosinophilic pneumonia (CEP) is characterized by dyspnea, wheezing, fever, and eosinophilia in the peripheral blood and bronchoalveolar lavage (BAL) fluid. On chest X-ray, a periphery-predominant infiltrative shadow that is rapidly progressive is typically seen. Although CEP usually responds well to corticosteroid therapy, it often recurs, and, thus, CEP is thought to be a kind of allergic disease with a chronic clinical course. We encountered a rare case of CEP associated with marked thrombocytosis with a platelet count of more than $1,600 \times 10^9/L$, and we measured the serum concentrations of thrombopoietic cytokines in order to investigate the mechanism of thrombocytosis in this patient.

CASE REPORT

A 47-year-old woman was admitted to our hospital because of cough, dyspnea, slight fever, eosinophilia, and marked thrombocytosis in November 2012. She had a medical history of bronchial asthma and allergic rhinitis in childhood, pleuritis at the age of 16, and drug eruption caused by azithromycin. She had no history of smoking. She developed productive cough, wheezing, night sweating, and general fatigue in September 2012. She was treated for bronchial asthma in a clinic without improvement. Laboratory tests in the clinic showed that the white blood cell (WBC) count was $19.2 \times 10^9/L$ with 32% eosinophils, the platelet count was $1,450 \times 10^9/L$, and the serum concentration of C-reactive protein (CRP) was 6.6 mg/dL. On chest X-ray, an extensive infiltrative shadow was seen in the bilateral lung fields. Thus, she was referred to our hospital and admitted.

Physically, she had tachypnea with a ventilatory frequency of 24 times/min and tachycardia with a pulse of 92/min, and was slightly febrile with a body temperature of 37.7°C. The oxygen saturation on pulse-oximetry was decreased to 92% in room air. On auscultation of the chest and back, course crackles but not stenotic sounds were heard with left-sided dominance. Skin rash, edema, superficial lymphadenopathy, and hepatosplenomegaly were not noted. Neurological examination showed no sign of multiple mono-
neuritis. Chest X-ray showed an infiltrative shadow in the bilateral upper lung fields with left-sided dominance (Fig. 1). Computed tomography scan of the chest showed infiltration with upper lobe and peripheral dominance, suggesting CEP (Fig. 2). A cytospin preparation of BAL fluid showed that eosinophils comprised 77% of BAL cells (Fig. 3). Flow cytometric analysis of the fluid revealed that CD4+ and CD8+ cells comprised 50.3% and 33.6% of small-sized mononuclear cells, respectively. Culture of the BAL fluid yielded no growth of bacteria or fungi.

The results of laboratory examination on admission are shown in Table 1. Hematologic examination revealed a WBC count of 19.5 × 10^9/L with 0.3% promyelocytes, 0.3% promyelocytes, 50.8% neutrophils, 35.0% eosinophils, 0.3% basophils, 4.7% monocytes, 8.3% lymphocytes, and 0.3% atypical lymphocytes, a hemoglobin concentration of 12.2 g/dL, and a platelet count of 1,310 × 10^9/L. A serological test showed that CRP was elevated to 4.8 mg/dL (normally below 0.3 mg/dL), while the serum concentration of IgE was within normal limits. Serum concentrations of myeloperoxidase-anti-neutrophil cytoplasmic antibody and proteinase 3-anti-neutrophil cytoplasmic antibody were below 1.0 U/mL and 7.2 U/mL, respectively. Serum biochemical examination yielded unremarkable results. A bone marrow aspirate showed that the nucleated cell count (NCC) was increased to 264 × 10^9/L with 26.8% eosinophils, the majority of which were of the mature form. Megakaryocytes were increased in number in proportion to the NCC, being mostly mature and slightly large without dysplastic features. Neither an increased number of macrophages nor hemophagocytosis was observed. Because of the absence of excess blasts and dysplastic features of marrow cells including eosinophils, we considered that the eosinophilia in the present patient was reactive. Indeed, cytogenetic or genetic analysis of the marrow cells showed a normal 46, XX karyotype and the absence of the bcr-abl fusion gene, 4q interstitial deletion, and JAK2 V717F mutation. From these findings, a diagnosis of CEP associated with marked reactive thrombocytosis was made. Churg-Strauss syndrome appeared to be unlikely because of

![Fig. 1. Chest X-ray on admission shows left-dominant consolidations in bilateral upper lung.](image1)

![Fig. 2. Chest computed tomography on admission shows peripheral-dominant consolidations around the upper lobe.](image2)
the lack of bronchial asthma, skin eruption, and multiple mononeuritis, and low serum titers of myeloperoxidase- and proteinase 3-anti-neutrophil cytoplasmic antibodies.

To investigate the cause of the thrombocytosis in the present patient, we measured serum cytokine concentrations before corticosteroid treatment. As shown in Table 2, the concentrations of cytokines with megakaryocyte colony-stimulating factor (Meg-CSF) activity, such as interleukin-3 (IL-3), thrombopoietin (TPO), and granulocyte macrophage colony-stimulating factor (GM-CSF), were within normal limits, while that of IL-6, which has megakaryocyte-potentiating (Meg-POT) activity,7,8 was elevated to 14 pg/mL. However, the serum concentration of IL-1β, which is another cytokine that has Meg-POT activity,8-10 was not elevated. As for other cytokines, serum concentrations of IL-5, tumor necrosis factor-α (TNF-α), and granulocyte colony-stimulating factor (G-CSF) were elevated to 9.6, 10.7, and 6.7 pg/mL, in accordance with eosinophilia, fever, and neutrophilia, respectively.

As shown in Fig. 4, the peak platelet count before treatment was 1,730 × 10^9/L, and the patient was treated for CEP with oral prednisolone (PSL) (20 mg/day), with the immediate resolution of respiratory symptoms such as productive cough, dyspnea, and wheezing. The abnormal imaging of the lung had almost disappeared. The abnormal laboratory findings such as eosinophilia, thrombocytosis, neutrophilia, and elevated CRP also promptly improved, returning to their respective normal levels. Regarding the changes of blood cytokine levels, re-examination was performed in December 2012, when CEP symptoms and abnormal laboratory findings had disappeared with PSL treatment. As shown in Table 2, concentrations of IL-5 and IL-6 returned to normal levels. However, in January to February 2013, mild eosinophilia and thrombocytosis recurred along with the tapering of PSL, and the platelet count occasionally exceeded 400 × 10^9/L, but did not thereafter (Fig. 4). PSL was tapered and discontinued at the end of March 2013. The patient was free from CEP-related symptoms and the respective laboratory data were within normal limits; however, she again developed CEP with recurrent thrombocytosis of 900 × 10^9/L, eosinophilia, and high CRP in September 2013. She was again treated with oral PSL at 20 mg/day, with the prompt resolution of CEP and the improvement of abnormal laboratory findings, including the platelet count.
Fig. 3. A bronchoalveolar lavage cytospin preparation shows 77% eosinophils and many macrophages. Neut, neutrophil; Lym, lymphocyte; Eos, eosinophil

<table>
<thead>
<tr>
<th>Cells</th>
<th>9.9 × 10^5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neut</td>
<td>9%</td>
</tr>
<tr>
<td>Lym</td>
<td>8%</td>
</tr>
<tr>
<td>Eos</td>
<td>77%</td>
</tr>
<tr>
<td>Macrophages</td>
<td>6%</td>
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</tbody>
</table>

Fig. 4. Clinical course. PSL, prednisolone; Plt, platelet; WBC, white blood cell; Eos, eosinophil; CRP, C-reactive protein
DISCUSSION

Marked thrombocytosis in CEP may be rare; to the best of our knowledge, only two cases have been reported, in which respective platelet counts were 900 × 10^9/L and 1,078 × 10^9/L. Fever may be common because a fever over 38°C was described in these 2 cases, and the present patient had a peak fever of 37.7°C. The causes of thrombocytosis and fever, however, were not investigated in these 2 cited case reports.

In the present patient, we measured the serum concentrations of cytokines that cause thrombocytosis. Serum levels of cytokines exhibiting Meg-CSF activity were all within their normal ranges; therefore, IL-6, which has Meg-POT activity, appeared to be one of the factors causing the marked thrombocytosis in the present patient. Meg-POT activity itself does not promote the growth of megakaryocytes. IL-6 promotes megakaryocytic maturation through its receptors expressed on megakaryocytes and, as a result, causes thrombocytosis. IL-6, therefore, plays a central role in reactive thrombocytosis as in RA.

In the present patient, the serum concentration of IL-6 before prednisolone treatment was increased to 14 pg/dL, and the serum level of CRP of 4.8 mg/dL was consistent with that of IL-6. However, the serum level of IL-6 appeared to be insufficient to cause the marked thrombocytosis. Therefore, we have to consider the presence of some other factors that enhanced the Meg-POT activity of IL-6 in the present patient. IL-1 is another cytokine with Meg-POT activity, and megakaryocytes express the IL-1 receptor and the IL-1 genes although the serum concentration of IL-1β was not elevated in the present patient (Table 2). In fact, high IL-1 levels in the blood, pleural effusion, or BAL fluid have not been reported, while high concentrations of IL-5, IL-6, or G-CSF in the blood or pleural effusion, or those of IL-5, IL-6, or IL-10 in BAL fluid have been documented in patients with CEP. However, it was reported that human eosinophils produce IL-1β; therefore, it is likely that IL-1β secreted by bone marrow eosinophils exhibited Meg-POT activity and enhanced the platelet production of megakaryocytes, which were located close to eosinophils in the present patient. In this fashion, high serum IL-6 and in situ-acting IL-1β may have additively exhibited Meg-POT activity on megakaryocytes.

Thrombocytosis, however, could occur in all patients with CEP if this hypothesis is correct. To test this hypothesis, we collected laboratory data from CEP patients admitted to our hospital. As shown in Table 3, mild to moderate thrombocytosis was observed in all CEP patients before corticosteroid treatment showing the peak platelet count of the present patient. Therefore, the hypothesis of the additive effect of IL-6 and in situ-acting IL-1β on platelet production of megakaryocytes may be partially supported. However, marked thrombocytosis as seen in the present patient may not be completely explained by this hypothesis. As another possibility, we hypothesized the additive effect of IL-5 on Meg-POT activity by IL-6 or IL-1; however, we could find neither such studies nor information regarding the expression of IL-5 receptor on megakaryocytic cells in the literature.

Regarding the high blood TNF-α level on admission of the present patient, Th17 immunity might have acted in addition to activated Th2 status in CEP because Th17 cells play an important role in CEP-related disease, bronchial asthma. IL-17 stimulates macrophages to produce TNF-α and IL-1β. In the present patient, macrophages residing in the lung, as seen in Fig. 3, might have been the source of TNF-α in response to IL-17. These macrophages may also have been recruited to the pulmonary inflammatory site in response to monocyte chemotactic protein-1 (MCP-1) produced by themselves. In addition, the elevated TNF-α appears to be attributable to low-grade fever observed upon admission of the patient.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age</th>
<th>Plt (10^9/L)</th>
<th>WBC (10^9/L)</th>
<th>% of EOS</th>
<th>Absolute EOS count (× 10^9/L)</th>
<th>CRP (mg/dL)</th>
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<tr>
<td>1</td>
<td>M</td>
<td>80</td>
<td>287</td>
<td>7.3</td>
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<td>2</td>
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<td>M</td>
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<td>243</td>
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<td>4</td>
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<td>327</td>
<td>11.7</td>
<td>27.1</td>
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<tr>
<td>5</td>
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<td>223</td>
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<td>5.7</td>
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<td>0.07</td>
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<tr>
<td>13</td>
<td>F</td>
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<td>424</td>
<td>16.3</td>
<td>23</td>
<td>3.7</td>
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<td>Present patient</td>
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<td>47</td>
<td>1,310</td>
<td>19.5</td>
<td>35</td>
<td>6.8</td>
<td>4.75</td>
</tr>
</tbody>
</table>

Table 3. Laboratory data in patients with chronic eosinophilic pneumonia on admission to our hospital

M, male; F, female; Plt, platelet; WBC, white blood cell; EOS, eosinophil; CRP, C-reactive protein
present patient. IL-1β and MCP-1, which were slightly elevated in the serum, might also have been produced by Th17-stimulated macrophages; however, it is unknown why the elevation occurred at a time after steroid treatment but not before it. Thus, further investigation is needed in similar cases or in the present patient with recurrent CEP.

DISCLOSURE
The authors declare that there are no conflicts of interest with any individuals or companies.

REFERENCES