Eosinophilic Granulomatosis with Polyangiitis Demonstrating IgE-immune Complexes and the Possible Involvement of IgE Autoantibodies

Yasushi Ota¹, Mutsunori Fujiwara², Yoji Hirabayashi³, Toshio Kumasaka⁴, Tamiko Takemura⁴, Rumi Ota⁴, Mitsuya Suzuki¹ and Shigeko Inokuma⁶

Abstract

We herein report a case of female eosinophilic granulomatosis with polyangiitis (EGPA) in which polyethylene glycol (PEG) precipitation was used to evaluate the patient’s levels of IgE-immune complexes (IC). Her serum IgE (7,110 IU/mL) and IgE-IC (1,880 IU/mL) levels were observed with an IgE PEG precipitation index of 26.4%. We speculate that the circulating IgE-IC were formed by anti-neutrophil IgE autoantibodies. Therefore, the large amount of IgE autoantibodies in the patient’s serum appears to have induced a constant allergic pathology. This pathology may have resulted in a marked infiltration of eosinophils into the tissues, as well as intensified the EGPA pathology.

Key words: MPO-ANCA, eosinophil, Churg-Strauss syndrome

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Introduction

Eosinophilic granulomatosis with polyangiitis (EGPA), also known as Churg-Strauss syndrome, is an intractable disease that first presents with allergy symptoms, such as bronchial asthma and allergic rhinitis, followed by symptoms of vasculitis, including an elevated number of circulating eosinophils, purpura, polyarthritis, myalgia, muscle weakness, and mononeuritis multiplex. EGPA frequently results in high levels of serum IgE, in addition to the presence of myeloperoxidase (MPO) anti-neutrophil cytoplasmic antibodies (ANCA), a type of anti-neutrophil autoantibody (1). Interestingly, the anti-IgE monoclonal antibody omalizumab has been reported to be effective in a number of recent cases, thus indicating the involvement of IgE in EGPA pathology (2-4).

We herein report a case of EGPA in which gel filtration and polyethylene glycol (PEG) precipitation were used to measure the levels of immune complexes of IgE (IgE-IC), IgG (IgG-IC), and IgA (IgA-IC) in the patient’s serum. Additionally, we also discuss the role of IgE in EGPA.

Case Report

In August 1998, a 66-year-old woman experienced a cold which thereafter developed into asthma. In October 1998, she began treatment with inhaled corticosteroids (fluticasone) and β2 agonists (salbutamol). She subsequently began treatment with anti-leukotriene agents (montelukast), which were able to control her asthma attacks.

In July 2006, an elevated number of eosinophils (3,700/μL; 37%) was detected in the patient’s blood, resulting in symptoms that included pain, numbness, imperception in both legs, and a painful erythema nodosum in the right forearm and left femur. EGPA was suspected, and the patient was admitted to the Department of Allergology and Rheumatology at the Japanese Red Cross Medical Center. Nerve...
and skin biopsies revealed eosinophil infiltration, and the patient was diagnosed with EGPA according to the asthma, the high level of circulating eosinophils, onset of neuritis, and histology findings. Following steroid pulse therapy, she was treated with an initial dose of 40 mg of prednisolone, which ameliorated her symptoms. The steroid dosage was gradually tapered; she exhibited a favorable progression which ameliorated her symptoms.

The laboratory results were as follows: leukocytes: 5,900/μL, neutrophils: 2,020/μL (32.0%), lymphocytes: 1,390/μL (22.5%), monocytes: 220/μL (4.0%), basophils: 50/μL (1.5%), eosinophils: 2,260/μL (33.5%), platelets: 308,000/μL, C-reactive protein: 0.20 mg/dL, aspartate transaminase: 21 IU/L, alanine transaminase: 19 IU/L, lactate dehydrogenase: 212 IU/L, total protein: 6.8 g/dL, albumin: 4.0 g/dL, blood urea nitrogen: 13 mg/dL, creatinine: 0.49 mg/dL, IgG: 1,166 mg/dL, IgA: 223 mg/dL, IgM: 102 mg/dL, and IgE: 7,517 IU/mL. Therefore, we proceeded to follow-up care on an outpatient basis.

In July 2010, the patient complained of symptoms indicative of upper airway inflammation. The chest radiographic findings indicated pneumonia, and she was once again admitted to the Department of Allergology and Rheumatology. Computed tomography (CT) of the paranasal sinus revealed mild sinusitis (Fig. 1), and a biopsy of the paranasal sinus mucosa was performed. The biopsy findings revealed inflammatory cell infiltration consisting primarily of eosinophils, similar to the patient’s previous skin and nerve biopsies. The patient was diagnosed with chronic bronchiectasis and EGPA, with a comorbid bacterial infection that was subsequently improved after the administration of antibacterial agents (cefepime). At this time, the anti-leukotriene medication was discontinued, and the patient was started on suplatast tosilate and procaterol hydrochloride. She continued taking the 1 mg/day of prednisolone.

In August 2013, the patient presented with coughing, expectoration, nasal congestion, rhinorrhea, and mild respiratory distress. Chest radiography and CT scans revealed infiltrative shadows in the lungs (Fig. 2), and a marked increase in the number of circulating eosinophils was observed. The patient's expectoration and coughing symptoms were spontaneously resolved, and her eosinophil count and her level of serous IgE mildly decreased (eosinophils: 1,320/μL, 22.5%; IgE: 5,697 IU/mL). Therefore, we proceeded to follow up with the patient only at the treatment appointments and did not prescribe any additional treatments (e.g., increased steroid dose). The patient was discharged in September 2013 and is currently undergoing follow-up care on an outpatient basis.

**Histopathological findings**

Figs. 3 and 4 demonstrate the histopathological images

Figure 1. A CT image of the paranasal sinus reveals mild sinusitis. Mild mucosal thickness in the ethmoid and sphenoid sinuses is indicated (white arrowheads).

Figure 2. A chest CT image reveals infiltrative shadows in the lungs (white arrowheads).
from the nerve and muscle biopsies taken in 2006, while Figs. 5 and 6 show the histopathological image from the paranasal sinus mucosa biopsy taken in 2010. Immunohistochemical staining was performed using a rabbit anti-human IgE secondary antibody (PA1-29206, Thermo Scientific, Waltham, USA), followed by Hematoxylin and Eosin staining. The nerve and muscle tissues were infiltrated with eosinophils and IgE-positive cells, albeit in small numbers, while the paranasal sinus mucosa tissue was infiltrated by large numbers of eosinophils and IgE-positive cells. Fig. 6 shows the vasculitis without fibrinoid necrosis in the paranasal sinus mucosa.

**Measurement of the immune complexes in the patient’s serum**

Using blood collected from the patient upon her 2013 admission, we used 4% PEG precipitation to measure the levels of circulating IgE-IC, IgG-IC, and IgA-IC.

Each serum sample was centrifuged at 1,000x g at 4°C for 15 minutes. Synthetic PEG (Wako Pure Chemical Industries, Ltd., Osaka, Japan) with a mean molecular mass of 7,500 g/mol was dissolved in 0.01 M phosphate-buffered saline (pH 7.4) at a concentration of 4% (weight/weight). From this stock, 0.1 mL of dissolved PEG was mixed with the serum samples, and this mixture was centrifuged at 1,000x g at 4°C for 1 hour. The precipitate was dissolved in 0.1 mL phosphate buffered saline, and the IgE levels in the precipitate and supernatant (serum) were measured using a paper radioimmunosorbent test. The percentage of the IgE in the precipitate vs. in the serum was calculated and defined as the PEG precipitated index (the PP index). Similar measurements were also performed for IgG and IgA.

The patient’s level of IgE in the untreated serum was 7,110 IU/mL, while the level of IgE-IC in the PEG-treated serum was 1,880 IU/mL. The IgE values were markedly elevated (normal range <170 IU/mL). The patient’s IgE PP index was 26.4%, which was also higher than the normal value (<20%). The levels of IgG in the untreated and PEG-treated sera were 1,172 mg/dL and 140 mg/dL, respectively, and the IgG PP index was 11.9%. However, the IgG values were all within the normal level (<20%). The level of IgA in the untreated serum was 233 mg/dL, the level of IgA-IC in the PEG-treated serum was 22 mg/dL, and the IgA PP index was 9.4%. These values were also within the normal range (<20%). The details are shown in the Table.
PEG precipitation has been used to measure the serum levels of IgE in infants and children with bronchial asthma and atopic dermatitis, and IgE was found to exist as an immune complex in the blood of infants and children with bronchial asthma (5). It was therefore concluded that this complex plays an immunological role in the presentation of asthma attacks (5).

In the present study, we used the same technique to measure the immune complexes in the serum of a woman with EGPA. Her serum level of IgE was higher than the normal value, as was her IgE PP index (normal PP index <20%). However, her serum levels of IgG and IgA were within the normal ranges. The respective immune complex levels and PP indices were also within the normal value. Thus, only serum IgE and serum IgE-IC were markedly elevated in the present case. Additionally, as there was no elevation in the IgG levels, the IgE-IC appears to be comprised IgE bound to an antigen, rather than IgE complexed with IgG.

Interestingly, the same technique has also been used to measure IgE-IC not only in EGPA, but also in cases of allergies and rheumatoid arthritis. Although minimal IgE-IC was found in cases of allergy or rheumatoid arthritis, high levels of IgE-IC were observed in cases of EGPA (6). In these cases, if the IgE-IC comprised IgE complexed with IgG, it would be expected that the IgE-IC levels would be high in those patients with allergy and rheumatoid arthritis displaying high levels of IgE. Therefore, these results support the idea that the elevated levels of IgE-IC observed in cases of EGPA comprises IgE bound to an antigen.

In our patient’s serum, the positive RAST results were all extremely low (Candida: 67 IU/mL; Aspergillus: 11.8 IU/mL; Alternaria: 48.1 IU/mL, house dust 1.1 IU/mL; mites: 0.76 IU/mL). Thus, no obvious antigen for IgE was detected in the serum. However, in cases of EGPA, ANCA targeting proteinase-3 and MPO are produced. As with these IgG autoantibodies, it is feasible to consider that high levels of anti-neutrophil IgE autoantibodies are also present in the serum. It is also plausible that IgM antibodies undergo a class switch in the serum, thereby becoming IgE antibodies.

Moreover, these anti-neutrophil IgE antibodies may form IgE-IC in the serum by binding to large amounts of circulating neutrophils (or parts of neutrophils), thereby explaining the large amount of IgE-IC that was present in the patient’s serum. In addition, as these IgE autoantibodies would be anti-neutrophil, they would not react to existing RAST, which may explain why no obvious antigen was detected. Interestingly, the pathology of EGPA has been reported to be caused by the deposition of IgE-IC on the vascular wall (6). By this mechanism, the deposition results in histological damage, and the large amounts of IgE autoantibodies (which are continuously produced over an extended period) induce the chronic release of histamines and other allergic compounds from mast cells, thereby promoting the constant allergic pathology. This mechanism is consistent with our observations in the present case, given the IgE and eosinophil infiltration into the tissues, as well as the efficacy of the recent treatments with anti-IgE biologicals.

In contrast to the elevated levels of IgE-IC, no elevation in the IgG-IC and IgA-IC levels was observed. In addition, the patient’s PP indices for IgA and IgG were nearly identical to the normal values. Elevated levels of IgG-IC are typically associated with the presence of ANCAs, although the results of proteinase-3 ANCA and MPO-ANCA tests were both negative. It thus appears that the pathology related to the presence of IgE autoantibodies was more important in the present case, compared to the pathology related to the presence of IgG autoantibodies. According to this interpretation, the large amount of circulating IgE autoantibodies resulted in a constant allergic pathology, which may have become one cause of the marked infiltration of eosinophils into the tissue, as well as the intensified EGPA pathology.

IgE autoantibodies for atopic dermatitis and bullous pemphigoid has been previously demonstrated (7, 8). These IgE autoantibodies play important roles in each disease. In a similar manner, anti-neutrophil IgE antibodies may play important roles in EGPA.

The nerve and muscle tissues were infiltrated with eosinophils and IgE-positive cells in the present case. The paranasal sinus mucosa tissue was infiltrated by large numbers of eosinophils and IgE-positive cells. These IgE-positive cells were distinguishable from eosinophils. According to the shape of these cells, they were mostly likely mast cells or plasma cells. Additionally, a relationship between the IgE-positive cells and the eosinophils may exist, and the IgE-positive cells may thus cause the eosinophil infiltration.

We herein described a case of EGPA in which PEG precipitation was used to evaluate the patient’s levels of IgE-IC. In this case, a marked elevation of serum IgE (7,110 IU/mL) and IgE-IC (1,880 IU/mL) levels were observed, with an IgE PP index of 26.4%, which was higher than the normal range. However, IgG-IC, IgA-IC, and their respective PP indices were within the normal ranges. According to the presence of ANCA IgG in EGPA, we speculate that the cir-

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Discussion

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

References