Analysis of Serum IgG Subclasses in Churg-Strauss Syndrome—The Meaning of Elevated Serum Levels of IgG4

Motohisa Yamamoto, Hiroki Takahashi, Chisako Suzuki, Tetsuya Tabeya, Mikiko Ohara, Yasuyoshi Naishiro, Hiroyuki Yamamoto, Kohzoh Imai and Yasuhisa Shinomura

Abstract

Objective Mikulicz’s disease (MD) is characterized by symmetrical and persistent enlargement of the lacrimal and salivary glands. Recently it has been categorized as an ‘Ig (immunoglobulin) G4-related disease.’ It presents with elevated serum levels of IgG4 and abundant infiltration of IgG4-bearing plasmacytes in involved organs. Allergic symptoms are often observed in patients with IgG4-related disease. On the other hand, allergic diseases are often complicated with Churg-Strauss syndrome (CSS). Here we focused on CSS and analyzed the relation of IgG4 in its pathogenesis.

Materials and Methods We analyzed five patients (2 men and 3 women) with CSS and 51 patients (20 men and 31 women) with MD who presented at Sapporo Medical University Hospital since 2001. We measured the serum concentrations of IgG subclasses in the patients with MD and CSS, and evaluated renal specimens from CSS patients, staining them for anti-IgG4 antibody.

Results We surprisingly found elevated serum levels of IgG4 not only in MD but also in CSS patients. The renal specimens in CSS patients revealed the infiltration of IgG4-positive plasmacytes.

Conclusion IgG4-bearing plasmacytes may be involved in the pathogenesis of CSS, and it is possible that an allergic reaction plays an important role in the pathogenesis of IgG4-related disease.

Key words: allergy, autoimmune pancreatitis, Churg-Strauss syndrome, IgG4, Mikulicz’s disease, IgG4-related disease

(Inter Med 49: 1365-1370, 2010)
(DOI: 10.2169/internalmedicine.49.3532)

Introduction

Mikulicz’s disease (MD) is characterized by symmetrical and persistent enlargement of the lacrimal and salivary glands (1). MD has been categorized as primary Sjögren’s syndrome since 1953 (2), but more recently it is considered to be an ‘Ig (immunoglobulin) G4-related disease (3)’ because MD presents with elevated serum levels of IgG4 (4) and abundant infiltration of IgG4-bearing plasmacytes in lacrimal and submandibular glands (5). The origin of MD is unknown, but it has been associated with allergic symptoms, such as bronchial asthma and allergic rhinitis in approximately half of MD patients (6). In contrast, Churg-Strauss syndrome (CSS) is a rare systemic necrotizing vasculitis involving small vessels (arterioles, capillaries and venules). CSS invariably involves the lungs and may, additionally, affect a wide variety of other tissues and organs. In most cases of CSS, patients suffer from bronchial asthma or allergic rhinitis. Both MD and CSS are clinically quite different diseases except for the allergic symptoms. Here, we analyzed the serum levels of IgG subclasses in CSS patients, and considered the meaning of elevated levels of IgG4 in MD.
Table 1. The Clinical Diagnostic Criteria of IgG4-related Mikulicz’s Disease (The Japanese Medical Society for Sjögren’s Syndrome, 2008)

<table>
<thead>
<tr>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Persistent (&gt;3 months), symmetrical swelling of the lacrimal, parotid</td>
</tr>
<tr>
<td>and submandibular glands, involving at least two pairs.</td>
</tr>
<tr>
<td>2. Serologically high levels of immunoglobulin (Ig) G4 (≥1.35 g/L).</td>
</tr>
<tr>
<td>3. Marked IgG4-positive plasmacyte infiltration (≥50% IgG4-positive/IgG-</td>
</tr>
<tr>
<td>positive cells in five high power fields) into lacrimal and salivary gland</td>
</tr>
<tr>
<td>tissues.</td>
</tr>
</tbody>
</table>

In terms of diagnosis, IgG4-related Mikulicz’s disease is defined as satisfying item 1 and either item 2 and/or 3. This form of systemic IgG4-related disease often accompanies multiple organ lesions. Sarcoidosis, Castleman’s disease, Wegener’s granulomatosis and malignant lymphoma need to be considered as differential diagnoses.

Patients and Methods

Study patients and materials

We analyzed five patients (2 men and 3 women) with CSS and 51 patients (20 men and 31 women) with MD who presented at Sapporo Medical University Hospital since 2001. The CSS patients met the criteria of the American College of Rheumatology (ACR) for diagnosing CSS (7), and the MD patients were categorized according to the criteria of the Japanese Medical Society for Sjögren’s syndrome (2008) (Table 1) (8). Sapporo Medical University Ethics Committee approved this clinical study. Written informed consent was obtained from all patients. Serum samples were obtained pre-therapy, and stored at -80°C. Formalin-fixed paraffin-embedded blocks of renal tissue from the patients with CSS were analyzed.

Cases 1 and 2 of CSS were relapsed cases and prescribed with a small quantity of prednisolone. The rest of the CSS cases and MD cases were incipient. The mean age of subjects in the study was 35.6±14.1 years in the CSS patients, and 58.7±13.4 years in MD patients. In the CSS group, there was mononeuritis multiplex in all patients, pulmonary involvement and glomerulonephritis in 3 patients. In the MD group, there was autoimmune pancreatitis in 9 patients, tubulointerstitial nephritis in 8 patients, and pulmonary involvement in 5 patients, and retroperitoneal fibrosis in 10 patients (Table 2).

Clinical data

We analyzed the counts of leukocytes and eosinophils, and the values of IgE, total complement activity (CH50), rheumatoid factor (RF), myeloperoxidase anti-neutrophil cytoplasmic antibodies (MPO-ANCA), and C-reactive protein (CRP), which were examined in the course of medical treatment.

Nephelometry

Pre-therapy serum levels of IgG subclasses from patients were measured with a Behring nephelometer (Dade Behring, Deerfield, IL, USA) using IgG subclasses (BS-NIA IgG1-4; The Binding Site, Birmingham, UK) as antibodies for 0.4 mL serum samples. Diluted samples (N-dilution liquid; Oriental Yeast, Tokyo, Japan, in 1: 20-100) of standard and control sera were introduced into the reaction tubes of the nephelometer. Appropriate anti-IgG subclass reagents and reaction buffer (N-responsive buffer liquid; Oriental Yeast) were added. Dispersion strength according to irradiation from a light-emitting diode was measured at a wavelength of 840 nm, and contrasted to dispersion by available light after 10 seconds and again after 6 minutes. IgG subclass concentrations in test samples were calculated relative to calibration curves, obtained using nephelometric IgG subclass standard sera. A control serum was assayed to confirm the validity of calibration curves and the accuracy of IgG subclass determinations.

Immunohistochemistry

For anti-IgG4 antibody immunostaining, the monoclonal antibodies were reacted for 24 hours at 4°C with steam after endogenous peroxidase activity was stopped in each section. Primary antibodies comprised anti-IgG4 antibodies (Mouse anti-human IgG4; The Binding Site, Birmingham, UK) diluted 1 : 500. Secondary antibodies (Biotinylated anti-mouse IgG (H+L)); Vector, Burlingame, CA, USA) were diluted 1 : 500. Nuclear-staining was performed using Hema-toxylin after indirect peroxidase staining in renal specimens from the patients with CSS.

Results

Leukocytosis was observed in all patients with CSS, but in only two patients (3.9%) with MD. Eosinophilia was also detected in all CSS cases and in 14 cases (27.5%) with MD. The eosinophil count in MD was not as high as that in CSS.
Table 2. The Clinical and Serological Data of Five Patients with Churg-Strauss Syndrome and the Mean Data of IgG4-related Mikulicz’s Disease

<table>
<thead>
<tr>
<th>Case</th>
<th>CSS</th>
<th>Average of MD 51 cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y.o.), Sex (M/F)</td>
<td>19 F 25 M 38 F 37 M 51 F</td>
<td>58±7±13.4, 20.31</td>
</tr>
<tr>
<td>Organ failure</td>
<td>P1  G1B  MM  P  P1</td>
<td>AIP 9 cases</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>HAA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes (×10^3 μL)</td>
<td>47,700 13,700 20,500 16,300 18,800</td>
<td>5,868±1,830</td>
</tr>
<tr>
<td>Eosinophils (×10^3 μL)</td>
<td>36,672 8,220 10,455 6,646 10,077</td>
<td>320± 291</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG (g/L)</td>
<td>20.80 9.74 15.10 20.60 21.90</td>
<td>26.78±16.07</td>
</tr>
<tr>
<td>IgG1 (g/L, %)</td>
<td>11.40 38.11 3.81 47.69 4.93 43.82 11.60 49.33 9.36 42.01</td>
<td>11.94±5.59, 41.13±6.29</td>
</tr>
<tr>
<td>IgG2 (g/L, %)</td>
<td>8.56 28.61 3.24 40.86 4.85 40.44 7.33 31.17 6.49 29.13</td>
<td>8.28±2.47, 30.79±6.06</td>
</tr>
<tr>
<td>IgG3 (g/L, %)</td>
<td>0.20 0.66 0.18 2.40 0.20 1.78 0.42 1.80 0.39 1.75</td>
<td>0.67±0.57, 2.35±1.54</td>
</tr>
<tr>
<td>IgG4 (g/L, %)</td>
<td>9.75 32.62 0.75 9.35 1.57 13.96 4.16 17.69 6.04 27.81</td>
<td>8.91±17.33, 25.76±19.89</td>
</tr>
<tr>
<td>IgE (IU/mL)</td>
<td>75 253 650 1120 3300</td>
<td>304±1321.7</td>
</tr>
<tr>
<td>CH50 (U/mL)</td>
<td>66.0 64.7 54.8 48.7 52.3</td>
<td>36.7±12.3</td>
</tr>
<tr>
<td>RF (IU/mL)</td>
<td>73 40 172 10 370</td>
<td>29.1±56.0</td>
</tr>
<tr>
<td>MPO-ANCA [EU]</td>
<td>222 720 262 279 640</td>
<td>All normal</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>138.5 512 6.0 85.9 78.9</td>
<td>2.5±3.3</td>
</tr>
</tbody>
</table>

Serologically, the mean total IgG level was 17.62±5.14 g/L in CSS and 26.78±16.07 g/L in MD. Both diseases presented with hypergammaglobulinemia. The three cases in CSS and 11 cases (21.6%) with MD showed elevated serum levels of IgE (normal range, <450 IU/mL). The serum IgE levels in CSS patients showed a tendency to be significantly higher than those in MD patients. As for the total complement activity, all cases with CSS presented with elevated levels of CH50 (normal range, 30.0-50.0 U/mL). On the other hand, 13 cases (25.5%) with MD disclosed hypocomplementemia. There were four RF-positive cases with CSS, and 13 RF-positive cases (25.5%) with MD. All patients with CSS had MPO-ANCA, but it was not detected in the MD patient group. CSS patients showed positive CRP (normal range, <3.0 mg/L), but there were only seven CRP-positive patients (13.7%) with MD.

With regard to IgG subclass, the amount of IgG1 and IgG4 was 8.22±3.64 g/L and 4.46±3.63 g/L in CSS patients, and 11.94±5.59 g/L and 8.91±7.33 g/L in MD patients. It tended to be elevated serum levels of IgG4 in the CSS group. The mean ratios of each IgG subclass to total IgG are shown in Fig. 1. Surprisingly, there were no significant differences in IgG subclasses between CSS and MD groups. The ratio of IgG1 and IgG4 to total IgG was 44.19±4.49% and 20.15±9.55% in CSS, and 41.13±6.29% and 25.76±9.89% in MD (Table 2).

Tissue specimens from the kidneys of 3 patients with CSS revealed infiltration of numerous IgG4-producing cells by anti-IgG4 antibody staining (Fig. 2).

**Discussion**

Recently, worldwide attention has been drawn to the new concept of 'systemic IgG4-related plasmacytic syndrome (SIPS) (3),' which originated from Japan. Until recently this disease was referred to by various names, 'IgG4-positive multi-organ lymphoproliferative syndrome (IgG4+MOLPS) (6),' 'IgG4-related sclerosing disease (10),' but we found and recognized that they were the same. Thus recently, the name was finally unified to 'IgG4-related disease' at the IgG4+MOLPS Study Group Meeting granted from Ministry of Health, Labour and Welfare, Japan. However recently, there is a tendency to label all pathogenesis, which presents with elevated levels of serum IgG4 and infiltration of IgG4-bearing plasmacyte in the involved organ, 'IgG4-related disease (11, 12).' The confusion occurs in the diagnosis and interpretation of this disease (13). We consider that there is 'IgG4-related disease' in a narrow and wide sense. Original 'IgG4-related disease,' called in a narrow sense, includes MD, autoimmune pancreatitis (AIP), and the diseases, which complicate them. The basic characteristics of these diseases, except IgG4, are the swelling of the in-
Figure 1. The ratio of each IgG subclass to total IgG in patients with Churg-Strauss syndrome and Mikulicz’s disease. The pattern of the ratio is similar in patients with Churg-Strauss syndrome and Mikulicz’s disease.

Figure 2. The renal specimen with anti-IgG4 antibody stain in a patient with Churg-Strauss syndrome. Renal biopsy revealed the infiltration of IgG4-bearing plasma cells in Churg-Strauss syndrome.

Figure 2. The renal specimen with anti-IgG4 antibody stain in a patient with Churg-Strauss syndrome. Renal biopsy revealed the infiltration of IgG4-bearing plasma cells in Churg-Strauss syndrome.

The detailed mechanism of the elevated levels of serum IgG4 in CSS and ‘IgG4-related disease’ in a narrow sense is still unknown. It has been considered that Th2 cytokines, such as interleukin (IL)-4, IL-5 and IL-10 are very important in allergy. It is known that they act on the proliferation and the induction of eosinophils, and the class switching to IgE (20, 21). IL-5 is the representative cytokine, which activates eosinophils (22), and the signals of IL-4/IL-13 and ligation of the CD40 induce IgE class switching (23, 24). Zen et al reported that the cytokines, mainly interleukin...
Table 3. The Clinical and Histological Characteristics of IgG4-related Disease

1. Elevated serum levels of IgG4
2. Pathological characteristics;
   1) Abundant infiltration of lymphocytes and plasma cells
   2) Infiltration of IgG4-positive plasmaocytes
   3) Infiltration of eosinophils
   4) Fibrosis around glands (Sclerosing lesions)
   5) Obstructive phlebitis
3. Efficacy of glucocorticoid for a short term
4. Spatio-temporal complications

(IL)-10, promote the production of IgG4 in AIP (9). As for IL-10, it decreases IL-4-induced IgE switching. IgE versus IgG4 production can be differentially regulated by IL-10 (25). On the other hand, Saito et al analyzed the cytokine profile in CSS and reported that IL-10 is reversely decreased in the active stage of CSS, and increased in the inactive stage (26, 27). CD4+ T cells from patients with active CSS rather tend toward Th17 (27). We do not know whether this difference leads to the difference between CSS and ‘IgG4-related disease’ in a narrow sense. The allergy, which is based on Th2 cytokines, is important, but it is suggested that the mechanism by which regulatory T cells are influential differs in the two diseases. To resolve this issue, we have to further analyze the relationship between IgG4 and cytokine profiles.

The novel findings were a shock to us, as we considered that only elevated levels of serum IgG4 and infiltration of IgG4-positive plasma cells in the involved organs were very important in diagnosing ‘IgG4-related disease.’ However we must remember that they simultaneously gave us a new start. In other words, ‘IgG4-related disease’ is carefully diagnosed based not only on IgG4 but also on other factors, such as physical findings and images. This study also suggested that IgG4 itself is not the fundamental cause of ‘IgG4-related disease.’ It may be true that the elevated levels of serum IgG4 and the infiltration of plasmaocytes with IgG4 are only part of the process of the some immunological reactions.

References