Overlap of IgG4-related Disease and Primary Biliary Cirrhosis Complicated with Autoimmune Thrombocytopenia

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Abstract

A 63-year-old woman was referred to Fukushima Red Cross Hospital with an enlargement of the left submandibular gland and subcutaneous bleeding in the chest and legs. A diffuse enlargement of the pancreas was also detected by abdominal computed tomography, and laboratory data showed severe thrombocytopenia. She was diagnosed with IgG4-related disease (IgG4-RD) complicated with autoimmune thrombocytopenia and was treated with methylprednisolone, after which the number of platelets favorably increased. Further investigation for liver dysfunction revealed underlying primary biliary cirrhosis (PBC). We herein report a rare case of IgG4-RD overlapping PBC complicated with autoimmune thrombocytopenia.

Key words: IgG4-related disease, primary biliary cirrhosis, autoimmune thrombocytopenia, prednisolone

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Introduction

IgG4-related disease (IgG4-RD) was first reported in 1993 (1), and a new disease classification was established in Japan in 2011 (2). It is a novel clinical disease entity characterized by an elevated serum IgG4 concentration and tumefaction or tissue infiltration by IgG4-positive plasma cells (3-6). A certain proportion of patients formerly diagnosed with autoimmune pancreatitis (7, 8) or Mikulicz's disease (9, 10) are believed to have had IgG4-RD. Persistent enlargement of the lacrimal and salivary glands are characteristic of Mikulicz's disease. On the other hand, primary biliary cirrhosis (PBC) is a chronic cholestatic disease with a progressive course, characterized by the presence of serum anti-mitochondrial antibodies and histological nonsuppurative destructive cholangitis. PBC is an autoimmune liver disease distinct from IgG4-related sclerosing cholangitis (IgG4-

SC), which is classified as a biliary manifestation of IgG4-RD. However, a few cases of overlapping PBC and IgG4-SC have been reported (11-13). Although autoimmune thrombocytopenia is a well-defined autoimmune disease, its association with IgG4-RD or PBC is rare. We herein describe a rare case of overlapping IgG4-RD and PBC complicated with autoimmune thrombocytopenia and discuss the possible mechanisms underlying the association of these three diseases.

Case Report

A 63-year-old woman was referred to our department with an enlargement of the left submandibular gland and purpura on the chest and legs in December 2013. Her height and weight were 153 cm and 56 kg, respectively. The patient's vital signs were stable: blood pressure of 135/75 mmHg, heart rate of 73 beats per minute, body temperature

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Table. Laboratory Data at Admission.

Peripheral blood		Blood chemistry			
WBC	7,400 / μ L	T-bil	0.9 mg/dL	TP	8.8 g/dL
RBC	$4,840 \times 10^3$ / μ L	AST	35 IU/L	IgG	1,765 mg/dL
Hb	14.5 g/dL	ALT	36 IU/L	IgG4	160 mg/dL
Plt	4,000 / μ L	LDH	217 IU/L	IgA	269 mg/dL
Coagulation		ALP	671 IU/L	IgM	378 mg/dL
		γ -GTP	457 IU/L	C3	159 mg/dL
		P-AMY	27 IU/L	C4	20 mg/dL
		BUN	12.3 mg/dL	CH50	45 U/mL
PT	119.0 %	Cre	0.62 mg/dL		
PT-INR	0.96	Na	143 mmol/L	ANA(Discrete-Speckled)	640 $\times$
APTT	25.4 sec	K	3.9 mmol/L	Anti-PLT antibody	Slightly positive
Infection		Cl	105 mmol/L	Anti-SS-A antibody	negative
		PG	109 mg/dL	Anti-M2 antibody	214 U/mL
HBs-Ag	(-)				
HCV-Ab	(-)				
<i>H. Pylori</i> IgG	28 U/mL

Figure 1. CT of the submandibular gland. a: At admission: Non-contrast CT revealed enlargement of the bilateral submandibular gland. b: Two weeks after the beginning of PSL treatment: Contrast-enhanced CT revealed decreased swelling of the bilateral submandibular gland.

of 36.2°C, and SpO₂ of 100% (room air). Laboratory data (Table) showed severe thrombocytopenia, with a platelet (PLT) count of 4,000/ μ L. A coagulofibrinolysis test showed no abnormalities. Her anti-PLT antibody was slightly positive. The serum levels of γ -glutamyl transpeptidase (γ -GTP), alkaline phosphatase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were high. Serum IgG was elevated at 3,160 mg/dL, and IgG4 was elevated at 160 mg/dL (normal range 4.8-105 mg/dL). The serum IgM level was also elevated at 378 mg/dL (normal range 46-260 mg/dL). The complement level, which included C3, C4, and CH50, was not low. No apparent biliary dilatation or structural abnormalities of the liver were observed. However, anti-nuclear antibody was positive at 640 $\times$ in a discrete speckled pattern and cytoplasmic antibody was positive at 80 $\times$, while

anti-SS-A antibody was negative. *Helicobacter pylori* (*H. pylori*) IgG was positive at 28 U/mL. Computed tomography (CT) revealed the enlargement of both submandibular glands (Fig. 1a) and diffuse enlargement of the pancreas (Fig. 2a). The enlargement of the submandibular gland was bilateral, although only the left side was palpable. No dilation of the bile and pancreatic ducts was observed.

A differential count of the bone marrow fluid obtained by aspiration was within normal limits with a slight increase of megakaryocytes, although atypical cells were not detected (Fig. 3a). Immunostaining for CD138 and IgG4 did not reveal an increase of IgG4-producing plasma cells in the bone marrow (Fig. 3b and c). From these findings, IgG4-RD and autoimmune thrombocytopenia was diagnosed, although tumefaction or tissue infiltration by IgG4-positive plasma cells

Figure 2. CT of the pancreas. a: At admission: Contrast-enhanced CT revealed diffuse enlargement of the pancreas as shown by arrows. Dilation of both bile and pancreatic ducts was not seen. b: Two weeks after the beginning of PSL treatment: Contrast-enhanced CT revealed a decrease in diffuse swelling of the pancreas.

Figure 3. Pathological findings of the bone marrow fluid obtained by aspiration. a: Hematoxylin and Eosin staining (200×): A differential count of the bone marrow fluid was within normal limits with a slight increase in the number of megakaryocytes. Atypical cells were not detected. b: Immunostaining of CD138 (200×): The number of CD138-positive plasma cells was approximately 5%, which remained small. c: Immunostaining of IgG4 (200×): IgG4-producing plasma cells in the bone marrow were not detected.

Figure 4. The clinical course of the patient.

could not be established due to severe thrombocytopenia.

Treatment with methylprednisolone (mPSL) semi-pulse (500 mg/body/day) was started for autoimmune thrombocytopenia. Because the PLT count was elevated to more than $30 \times 10^3/\mu\text{L}$ within three days, we tapered mPSL to half the dose (Fig. 4a). Both total IgG and IgG4 were decreased to within normal limits within two months (Fig. 4b). Two weeks after beginning treatment with mPSL, CT showed a marked improvement of diffuse swelling of the bilateral submandibular gland and pancreas (Fig. 1b, 2b). The patient also received a *H. pylori* eradication 7-day course of triple therapy of lansoprazole, amoxicillin, and clarithromycin. Successful eradication was assessed by the urea breath test. Although the serum levels of AST, ALT, alkaline phosphatase (ALP), and γ -GTP decreased temporarily, γ -GTP did not decrease to within normal levels, and AST and ALT reelevated six months after beginning mPSL treatment (Fig. 4b). The serum level of anti-mitochondrial M2 (anti-M2) antibody was measured in order to determine a cause related to liver dysfunction, and it was elevated at 214 U/mL (normal range, <7 U/mL). Serologic markers for hepatitis B and C viruses were negative. Magnetic resonance cholangiopancreatography revealed neither strictures of the lower common bile duct nor a segmental stricture. Because underlying PBC was suspected at this time, a liver biopsy was

performed. The liver specimen showed infiltration of lymphocytes in the portal area but did not reveal any obvious bile duct destruction by lymphocytes. IgG4-positive plasma cells were not observed in this specimen. Early-phase chronic nonsuppurative destructive cholangitis (CNSDC) was suggested (Fig. 5). Liver dysfunction was improved by the administration of ursodeoxycholic acid after the liver biopsy.

Discussion

IgG4-RD and autoimmune thrombocytopenia (ITP) were initially diagnosed in this case. However, PBC was subsequently suspected after the administration of mPSL therapy. Although a liver biopsy did not reveal any obvious bile duct destruction due to CNSDC, PBC could be diagnosed by an elevated serum anti-M2 antibody level, which has a high sensitivity and specificity for the diagnosis of PBC (14, 15). Elevation of serum IgM also suggested the existence of PBC. Magnetic resonance cholangiopancreatography was performed, but the presence of IgG4-SC was not determined. IgG4-SC generally reveals segmental strictures, long strictures with prestenotic dilatation, and strictures of the lower common bile duct. Biliary strictures in IgG4-SC typically respond to steroids, and complete resolution of the strictures and/or normalization of liver tests has been observed in approximately two-thirds of patients, whereas improvement was seen in the remaining one-third (16). It was difficult to rule out the presence of IgG4-SC in our case treated by mPSL.

An underlying common pathogenesis may exist in these three diseases. First, regarding the common pathogenesis of PBC and IgG4-RD, Shimoda et al. (17) reported the role of Toll-like receptors and natural killer (NK) cells in PBC patients. Toll-like receptor 4 ligand (TLR4-L)-stimulated NK cells destroy autologous biliary epithelial cells in the presence of interferon (IFN)- α synthesized by TLR 3 ligand (TLR3-L)-stimulated monocytes. According to Shimoda et al., IFN- α production by hepatic monocytes was significantly increased in patients with PBC. The cytotoxic activity of hepatic NK cells was also increased compared to controls. However, this only occurred when the NK cells were prepared following ligation of both TLR3-L- and TLR4-L-stimulated liver mononuclear cells. On the other hand, Watanabe et al. (18) reported that activation of TLR and nucleotide-binding oligomerization domain-like receptors enhanced IgG4 responses in autoimmune pancreatitis. Stimulation with TLR3 and TLR4 ligands enhances IgG4 production by peripheral blood mononuclear cells in IgG4-RD patients. These consequences suggest a common pathogenesis in IgG4-RD and PBC through abnormal innate immune responses against microbial antigens.

Next, we examined the pathogenesis of PBC and autoimmune thrombocytopenia. Panzer et al. (19) reported that autoantibodies eluted from a patient with PBC and autoimmune thrombocytopenia, precipitate glycoprotein IIb/IIIa of

Figure 5. Pathological findings of the liver obtained by a needle biopsy. **a:** Hematoxylin and Eosin staining (200×). **b:** Elastica-Masson staining (200×). A liver specimen showed infiltration of lymphocytes in the portal area, but no obvious bile duct destruction by lymphocytes was found. There was slight deformation of the bile duct. Taken together, early phase chronic nonsuppurative destructive cholangitis (CNSDC) could not be ruled out.

autologous and allogeneic PLT and bind to an epitope of the rat 70-kDa mitochondrial protein M2. Furthermore, an *in silico* analysis of published peptide sequences of the mitochondrial protein and glycoprotein IIb/IIIa showed partial amino acid sequence homology, suggesting the possibility of a common antibody-binding site. From these findings, PBC-related autoantibodies might cross-react with PLT surface autoantigens in ITP (20).

A common underlying pathogenesis of IgG4-RD and autoimmune thrombocytopenia was considered. However, the role of the IgG4 antibody response in the immunopathogenesis of IgG4-RD is poorly understood. Moreover, serum IgG4 levels fluctuate along with the state of IgG4-RD. A few cases have been reported regarding the association between IgG4-RD, especially with autoimmune pancreatitis, and autoimmune thrombocytopenia (21-24). In the present case, no significant increase in the number of IgG4-positive cells or decrease in megakaryocytes in the bone marrow was observed. This suggests that the cause of thrombocytopenia was not a decrease in megakaryocytes in relation to the proliferation of IgG4-positive cells in the bone marrow, but the presence of anti-PLT antibody. Patients with ITP have anti-PLT autoantibodies that are most frequently directed against PLT glycoproteins IIb/IIIa or Ib/IX/V. Chan et al. (25) reported that the most common subclass of anti-IIb/IIIa antibodies in ITP patients was IgG1. Murase et al. (22) reported an IgG subclass of a PLT antibody to be potentially IgG4. While this suggested a role of IgG4 as an anti-PLT antibody in IgG4-RD patients, this finding is controversial because IgG4-RD is rarely complicated with thrombocytopenia.

H. pylori infection could also contribute to IgG4-RD and ITP. The peptide showed homology with an amino acid sequence between *H. pylori* α -carbonic anhydrase (α -CA) and human CA type II and between *H. pylori* plasminogen-binding protein and human ubiquitin-protein ligase E3 com-

ponent n-recogin 2, an enzyme highly expressed in acinar cells of the pancreas (26, 27). It has been suggested that this molecular mimicry can lead to immunological cross-reactivity. Furthermore, CagA antigen of *H. pylori* may induce anti-GPIIb/IIIa antibody production by a molecular mimicry mechanism (28), suggested a contribution to ITP. Although there is molecular mimicry between the urease beta antigen of *H. pylori* and E2 subunit of the pyruvate dehydrogenase complex of the major mitochondrial autoepitope, this homology does not lead to immunological cross-reactivity (29, 30).

In summary, it could be inferred that serum anti-M2 antibody from PBC or IgG4 from IgG4-RD recognized PLT as an antigen, subsequently inducing severe thrombocytopenia in our patient. In addition, IgG4 production may be enhanced by PBC. *H. pylori* infection could also contribute to IgG4-RD and ITP. We herein experienced a rare case of overlapping IgG4-RD and PBC complicated with autoimmune thrombocytopenia. A combination of these three diseases suggests that common immunogenetic factors might be involved in the development of IgG4-RD, PBC, and autoimmune thrombocytopenia.

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

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