Successful Treatment of Autoimmune Pancreatitis Complicated with Autoimmune Thrombocytopenia and Interstitial Pneumonia by Prednisolone

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Abstract

We report the second patient diagnosed with autoimmune pancreatitis complicated with autoimmune thrombocytopenia and interstitial pneumonia. The patient was treated with prednisolone and responded favorably. We demonstrated that anti-platelet (PLT) antibody of the patient was IgG4 and that it may react with HLA, not specific antigen, on both pancreas and PLT.

Key words: autoimmune pancreatitis, autoimmune thrombocytopenia, interstitial pneumonia, immunoglobulin G4

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Introduction

An unusual form of pancreatitis called primary inflammatory sclerosis of the pancreas, which may result from an autoimmune mechanism, was first reported by Sarles et al (1). Toki et al (2) reported four cases of chronic pancreatitis showing diffuse irregular narrowing of the entire main pancreatic duct. Yoshida et al (3) summarized the clinical findings of the pancreatitis and proposed the concept of autoimmune pancreatitis (AIP). Since then many patients with AIP have been reported. However, a clear definition of its pathogenesis and pathophysiology remains to be determined. Since AIP dramatically responds to steroid therapy, precise diagnosis of AIP is necessary to avoid unnecessary surgery. Although the pathological criteria for the diagnosis of AIP is lymphoplasmacytic infiltration and fibrosis in the pancreas, it is usually difficult to take specimens from the pancreas. Therefore, AIP can be diagnosed using information from a combination of clinical, laboratory, and imaging studies. The Japan Pancreas Society has proposed “Diagnostic Criteria for Autoimmune Pancreatitis, 2002” (4, 5) however, the precision of the diagnostic criteria is low because emphasis is placed on the absence of pancreatic cancer and the value of serum IgG4 is not considered. Therefore, the diagnostic criteria of AIP were revised in 2006 as follows (6): (I) Clinical diagnostic criteria. (I-i) diffuse or segmental narrowing of the main pancreatic duct with irregular wall and diffuse or localized enlargement of the pancreas, (I-ii) high serum globulin, IgG, or IgG4, or the presence of autoantibodies, such as antinuclear antibodies and rheumatoid factor, (I-iii) marked interlobular fibrosis and prominent infiltration of lymphocytes and plasma cells in the periductal area, occasionally with lymphoid follicles in the pancreas. Diagnosis of AIP is established when criterion (I-i), together with criterion (I-ii) and/or (I-iii), are fulfilled. In the revised criteria, the relationship to extrapancreatic lesions and other associated disorders is also documented, that is, AIP may be associated with sclerosing cholangitis, sclerosing sialadenitis, or retroperitoneal fibrosis (7-12). Most AIP patients with sclerosing sialadenitis show negativity for anti-SS-A and anti-SS-B antibodies, which may suggest that AIP differs from

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Sjogren’s syndrome. Sclerosing cholangitis-like lesions accompanying AIP and primary sclerosing cholangitis respond to differentially to steroid therapy and have different prognoses, suggesting that they are not the same disorder.

**Materials and Methods**

Bone marrow biopsy specimen was immunostained with mouse anti-human IgG4 antibody, 1/500 dilution (The Binding Site, Birmingham, UK). Numbers of IgG4-positive cells per high-power field (HPF) (magnification, 400x) were counted in 10 HPFs that contained the denser lymphoplasmacytic infiltrate. Sensitized autoantibodies on the blood cells membrane from the patient were analyzed by flow cytometry (FACS). Blood cells (platelets, red blood cells, granulocytes and lymphocytes) were immunostained with anti-human IgG1 antibodies [HP6012 (Oxoid, Hampshire, UK)], anti-human IgG2 antibodies [HP6014 (Sigma, St. Louis, MO, USA)], anti-human IgG3 antibodies [HP6010 (Oxoid)], anti-human IgG4 antibodies (HP6025 (Sigma)], or phycoerythrin conjugated goat anti-human IgG antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and analyzed by FACS. Reaction of sensitized autoantibodies on RBC from the patient with the panel PLT or panel RBC was analyzed by FACS. Autoantibodies were eluted by Ortho DT solution II (Ortho-Clinical Diagnostics, Raritan, NJ, USA) from the patient’s RBC according to the manufacturer’s instruction and rinsed with PBS (DT dissociation method), and reactions of the elute with the panel PLT and panel RBC were analyzed by the FACS. Specificity of the autoantibodies on the patient’s PLT was examined by direct monoclonal antibody-specific immobilization of PLT antigens (direct MAIPA) (13). Specifically, sensitized antibodies bound to the specific molecules [glycoprotein (GP) Ib, GP Ib/IIIa, GP Ia/IIa, and HLA class-I] on the PLT were examined by ELISA. In more detail, washed PLT sensitized with autoantibodies were incubated with antiglycoprotein (GP) specific monoclonal antibodies (mAb): anti-GP Ib (AK2; Cymbus Biotechnology, Hampshire, UK), anti-GP Ib/IIIa (P2; Immunotech, Marseille, France), anti-GP Ia/IIa (4B4; Coulter, Miami, FL, USA), and anti-HLA class-I (w6/32; DAKO, Glostrup, Denmark). Following further washes, the PLT were solubilized and the lysates were added to microplate wells coated with goat anti-mouse IgG (Jackson ImmunoResearch Laboratories) to capture the mAb-GP-autoantibodies complexes. Human autoantibodies bound to GP were then revealed with HRP labeled goat anti-human IgG (Jackson ImmunoResearch Laboratories). The PLT from five healthy volunteers reacted with patient’s serum was immunostained with anti-human IgG4 antibodies [HP6025 (Sigma)] and FACS was performed.

**Case Report**

A 73-year-old man presented to another hospital with coughing and shortness of breath on exertion as chief complaint in August 2006. Chest X-rays revealed reticulonodular shadows in bilateral lower lung field. He was referred to our hospital for further examination in September 2006. On examination, he had fine inspiratory crepitations. Oxygen saturation was 95% on room air and pO2 was 77.8 mmHg on arterial blood gas analysis. Computed tomography (CT) revealed interstitial fibrosis of the bilateral lower lungs (Fig. 1-a). Pulmonary function tests showed a vital capacity (VC) of 88.9%, forced expiratory volume in 1 sec (FEV1) of 86%, and a carbon dioxide diffusing capacity (DLCO) of 1.54 mL/min/mmHg/L (35.7% of normal value). An increase in serum KL-6 (1,000 U/L) and SP-D (128 ng/mL) were recognized. He was diagnosed as interstitial pneumonia (IP) by the Department of Respiratory Medicine. Since the number of platelets (PLT) was decreased to 1.0x10^4/μL, and because petechiae were identified in the oral cavity and preordial area, he was referred to our department for further examination. His hemoglobin and PLT count were 12.9 g/dL and 1.2x10^4/μL, respectively. PLT associated antigen-IgG was positive (277 ng/10^7 cells) and half-life of PLT was short (5.1 days). Coagulofibrinolysis test showed no abnormalities. A differential count of bone marrow fluid obtained by aspiration was within normal ranges and atypical cells were not detected. Megakaryocytes appear to be hypolobulated. Karyotype of bone marrow nuclear cells was 46, XY. From these findings, autoimmune thrombocytopenia was suspected. Ultrasonography (US) revealed a hypoechoic lesion in the pancreas head (Fig. 1-b). CT demonstrated a hypovascular lesion in the pancreas head with dilatation of main pancreas duct in the body and tail (Fig. 1-c-i, ii). Endoscopic retrograde cholangiopancreatography (ERCP) revealed an irregular narrowing of the main pancreatic duct in the pancreatic head (Fig. 1-d). Serum levels of amylase (61.0 IU/L), lipase (11.5 U/L), CEA (1.4 ng/mL) and CA19-9 (4.3 U/mL) were within normal ranges. The serum IgG was elevated to 3,160 mg/dL, and IgG4 was also elevated to 468 mg/dL (<105 mg/dL). While anti-nuclear antibody (ANA)(1,280x) and Coombs test (Direct 4+, Indirect 1+) were positive, anti-ds DNA antibody, anti-Sm antibody, anti-Cardiolipin antibody, anti-RNP antibody, anti-SS-A antibody, anti-SS-B antibody, anti-Scl-70 antibody, anti-Jo-1 antibody, anti-Centromere antibody, anti-PR3-ANCA antibody, anti-MPO ANCA antibody, rheumatoid factor (RF) were all negative. Blood chemistry test showed no abnormalities. From these findings, autoimmune pancreatitis (AIP) was diagnosed according to the revised clinical diagnostic criteria for autoimmune pancreatitis of the Japan Pancreas Society (6). We next examined the extrapancreatic lesions of AIP (14). On sialography, branches of Stensen’s duct were small and inflow of contrasting agent was not sufficient to the periphery. Furthermore, small pooling of contrasting agent called pseudosialoectasis was demonstrated. These findings indicated the irregularity of stensen duct branches, and sialadenitis was diagnosed (Fig. 1-e)(15). Furthermore, IP was complicated in the patient as described above.

The treatment with prednisolone (PSL) (1 mg/kg/day, 55
mg/body/day) was started for immune thrombocytopenic purpura. Since the number of PLT was elevated to more than 20×10⁴/mL within 2 weeks, we tapered PSL to 5 mg/day (Fig. 2). Total IgG and IgG4 were decreased to normal ranges within 5 months. One month after the beginning of the treatment with PSL, US demonstrated that hypoechoic lesion became unclear (Fig. 3-a-i), and CT showed an atrophic change of the pancreas head (Fig. 3-a-ii, iii). Furthermore, reticular shadows in the lungs were also improved (Fig. 3-a-iv). Four months after the beginning of the treatment with PSL, hypoechoic mass in the pancreas could not be detected by US (Fig. 3-b-i) and atrophic change of pancreas head detected by CT remained unchanged from that observed after one month of PSL treatment (Fig. 3-b-ii, iii). The reticular shadows in the lungs also remained unchanged from that observed after one month of PSL treatment (Fig. 3-b-iv). To investigate whether IgG4 was also involved in the onset of diseases other than AIP in this case, we carried out immunohistochemistry for IgG4 in tissues other than the pancreas. However, since there was a bleeding diathesis due to thrombocytopenia, biopsy of the lung and salivary gland was not feasible. Bone marrow biopsy was instead performed. There was fewer than ten IgG4 positive cells/HPF in the bone marrow biopsy specimen, and no significant increase was observed (data not shown).

Next, we investigated whether the anti-PLT antibody that caused thrombocytopenia in this case was IgG4. First, sensitization of autoantibodies on the patient’s blood cells was examined by FACS. Sensitization of autoantibodies (IgG) on the membranes of PLT, granulocytes, and RBC was observed, and the IgG subclasses detected were IgG1 and IgG4, IgG1, and IgG1, respectively, which indicated that IgG4 was sensitized only on PLT (Fig. 4 and Table 1). On the other hand, no sensitized autoantibody was observed on lymphocytes. The patient’s autoantibody (IgG) isolated from RBC by the DT dissociation method was reacted with panel RBC and panel PLT. As a result, it was found that the patient’s autoantibody bound the panel RBC, but not the panel PLT. These data suggest that specificity of the autoantibody on the patient’s RBC was different from that of the patient’s PLT. Furthermore, to investigate whether autoantibodies bound to the surface of the PLT were those that specifically bound glycoprotein (GP) Ib, GP IIb/IIIa, GP Ia/IIa, and HLA class-I, direct MAIPA was performed. As a result, the autoantibodies did not bind these four molecules (data not shown).

Lastly, to investigate whether IgG4 recognized the common specific antigen of the pancreas and PLT in this case, the patient’s serum was reacted with PLT from five healthy volunteers and FACS was performed. No specific binding was observed (data not shown).

Discussion

To date, concurrent development of AIP and autoimmune thrombocytopenia has been reported in eight cases including the present case (16-19). In these cases, both PLT associated antigen IgG and total IgG were elevated, and IgG4 was elevated in the five cases that described its levels. PSL administration was the common treatment, and a marked improve-
ment was observed in the pancreatic lesion and the PLT count in all reports. To date, eight cases of concurrent AIP and interstitial pneumonitis (IP) have been reported (19-22), and thrombocytopenia was observed in only one case. Since AIP exhibited a variety of disease manifestations other than the pancreatic lesion and infiltration of plasma cells positive for IgG4 in various organs, it has recently been proposed that this disease is an IgG4-related sclerosing disease (14). The present case showed a concurrence of AIP, thrombocytopenia, IP, and sclerosing sialadenitis, and the findings were considered consistent with IgG4-related sclerosing disease.

Since it has been reported that plasma cells positive for IgG4 occur in lesions other than in the pancreas (23), bone marrow biopsy specimens were stained for IgG4. As a result, no significant increase in IgG4 positive cells was observed. These data suggest that the cause of thrombocytopenia is not a decrease in megakaryocytes in association with proliferation of IgG4 positive cells in the bone marrow, but the presence of anti-PLT antibody.

Next, we demonstrated that the IgG subclass of the PLT antibody detected in this study was potentially IgG4. Further, the antibody did not recognize the representative PLT antigens involved in the onset of idiopathic thrombocytopenic purpura (ITP) (24). We ruled out the possibility that

**Figure 2.** Clinical course. ANA: anti-nuclear antibody, PSL: prednisolone, WBC: white blood cells, Hb: hemoglobin, PLT: platelets

**Figure 3.** Radiological findings of the patient after prednisolone treatment. (a) After treatment with PSL for one month. (b) After treatment with PSL for four months. (i) Ultrasonography findings. (ii, iii) Abdominal computed tomography (CT) findings. (iv) Chest CT findings.
the IgG4 anti-PLT antibody in this case recognized a common specific antigen expressed in the pancreas and PLT. Meanwhile, recent reports showed that HLA DRB1*0405-DQB1*0401 carriers composed a large proportion of AIP cases and that *Helicobacter pylori* (HP) infection potentially induced AIP (15, 25). It is widely known that autoimmune thrombocytopenia is induced by HP infection (26). In the present case, HLA was not examined, but the 13C-urea breath test was positive. Therefore, we were interested in whether HP eradication was also effective for the immune thrombocytopenia associated with AIP. However, since the PLT count plummeted abruptly and a bleeding diathesis appeared, it was necessary to administer PSL to the patient.
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References


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