of serum IgG4 is frequently observed in patients with IgG4-SC, which responds dramatically to steroid therapy [4]. In contrast, even if the patients with PSC are medicated, it remains a progressive disease that involves the intra- and extra-hepatic bile ducts and leads to biliary cirrhosis. The effects of steroid therapy for PSC have been reported to be skeptical [5, 6] and liver transplantation is the only effective therapy. Histopathologically, lymphoplasmacytic and eosinophilic infiltration with mild fibrosis are seen in both IgG4-SC and PSC; and recent studies based on immunohistochemical findings of liver biopsy specimens report that IgG4-positive plasma cell infiltration is significantly more severe in IgG4-SC than in PSC [4, 7–11]. However, herein, we report 3 cases of PSC with an infiltration of abundant IgG4-positive plasma cells and ineffective steroid therapy.

Case report

Case 1

A 32-year-old man with elevated serum levels of hepatobiliary enzymes was admitted to our hospital. At the age of 22 years, the patient was diagnosed as PSC in other hospitals, and he had been treated with ursodeoxycholic acid. At the age of 24 years, he was found to have ulcerative colitis (UC). Physical examination at the time of admission revealed no significant findings except for jaundice. Laboratory examinations showed the following values (normal range): peripheral white cell count, 5700/μl (3500–8500); peripheral eosinocyte count, 251/μl (18–510); C-reactive protein, 1.1 mg/dl (<0.3); total bilirubin, 10.9 mg/dl (0.2–1.2); alkaline phosphatase, 2929 U/l (107–340); γ-glutamyl transpeptidase, 413 U/l (11–64); asparate aminotransferase, 133 U/l (13–35); alanine aminotransferase, 192 U/l (5–35); amylase, 127 U/l (37–125). Hepatitis B surface antigen and antibody to hepatitis C virus were negative. Serum IgG, IgG4, IgA, and IgM levels were 2104 mg/dl (870–1700), 96 mg/dl (4.8–105), 291 mg/dl (110–410), and 157 mg/dl (33–190), respectively. Rheumatoid factor was negative. Antinuclear antibody was positive. Among tumor markers, CEA was 1.8 ng/ml (<5.0) and CA19-9 was 237.2 U/ml (<37). Magnetic resonance cholangiopancreatography (MRCP) and endoscopic retrograde cholangiopancreatography (ERCP) revealed strictures of both the hepatic hilar region and the distal common bile duct and no narrowing of the main pancreatic duct (Fig. 1a, b). Cytology of bile juice was negative for malignancy. Histopathological examination by liver biopsy showed moderate lymphoplasmacytic and eosinophil infiltration with fibrosis in the enlarged portal area (Fig. 1c). Duct and ductular proliferation was conspicuous. Fibrous cholangitis (onion-skin fibrosis) was observed. These findings were compatible with PSC. The numbers of immunohistochemically identified IgG4-positive plasma cells were counted under five different high-power fields (hpf). Immunostaining study showed typical inflammation with abundant IgG4-positive plasma cells (126 cells/hpf) (Fig. 1d), a characteristic finding in IgG4-SC. His liver dysfunction was serious, with progressive ascites and jaundice, therefore it was determined that liver transplantation might be necessary.

Although oral steroid therapy requires a long period for drug tapering, steroid pulse therapy is a well-recognized alternative for refractory autoimmune pancreatitis without steroid tapering, as previously reported [12]. Therefore, we twice administrated steroid pulse therapy with 500 mg/day of methylprednisolone for 3 days/week. The hepato-biliary enzymes improved a little after steroid therapy, but MRCP revealed no improvements of strictures of the hilar and distal common bile ducts. Therefore, we strongly suspected PSC. Two months later, we decided on liver transplantation with consent of the patient and his family. Histopathological findings of the liver after transplantation showed severe lymphoplasmacytic and eosinophil infiltration with fibrosis in the enlarged portal area (Fig. 2a, b). Duct and ductular proliferation was conspicuous, and onion-skin fibrosis was observed, which suggested typical advanced PSC findings. Histopathological findings of the pancreas biopsy during the operation showed infiltration of mononuclear cells around the pancreatic duct (Fig. 2c) with an infiltration of abundant IgG4-positive plasma cells (Fig. 2d), but did not show LPSP.

Case 2

A 74-year-old woman was admitted to our hospital with liver dysfunction. Laboratory examinations showed the following values (normal range): peripheral white cell count, 8900/μl (3500–8500); C-reactive protein, 2.19 mg/dl (<0.3); total bilirubin, 0.6 mg/dl (0.2–1.2); alkaline phosphatase, 1544 U/l (107–340); γ-glutamyl transpeptidase, 1030 U/l (11–64); asparate aminotransferase, 130 U/l (13–35); alanine aminotransferase, 140 U/l (5–35); amylase, 44 U/l (37–125). Hepatitis B surface antigen and antibody to hepatitis C virus were negative. Serum IgG, IgM, and IgE levels were 1960 mg/dl (870–1700), 77 mg/dl (33–190), and 370 (0–320), respectively. Antinuclear antibody was positive. Antimitochondrial antibody was negative. Among tumor markers, CEA, CA19-9, and soluble interleukin 2 receptor (sIL-2R) were 2.1 ng/ml (<5.0), 18.5 U/ml (<37), and 611 U/ml (<650), respectively. Abdominal computed tomography (CT) showed dilatation of common bile duct (Fig. 3a) and no significant pancreatic lesions (Fig. 3b). ERCP revealed irregular narrowing of the

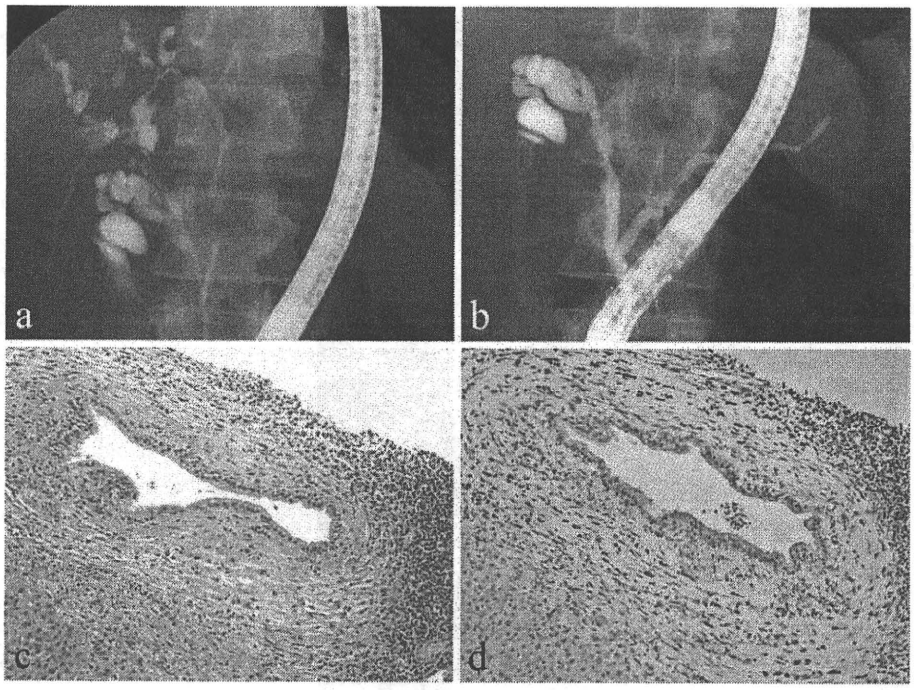


Fig. 1 ERCP images and histopathological findings of case 1 on clinical onset. ERCP revealed strictures of the hepatic hilar area (**a**) and the distal common bile duct, and no narrowing of the main pancreatic duct (**b**). Histopathological findings of the liver biopsy showed a moderate lymphoplasmacytic and eosinophil infiltration with fibrosis, and fibrous cholangitis (onion-skin fibrosis) (H&E staining ×200, **c**). IgG4-immunostaining of the liver biopsy showed infiltration of abundant IgG4-positive plasma cells (×200, **d**)

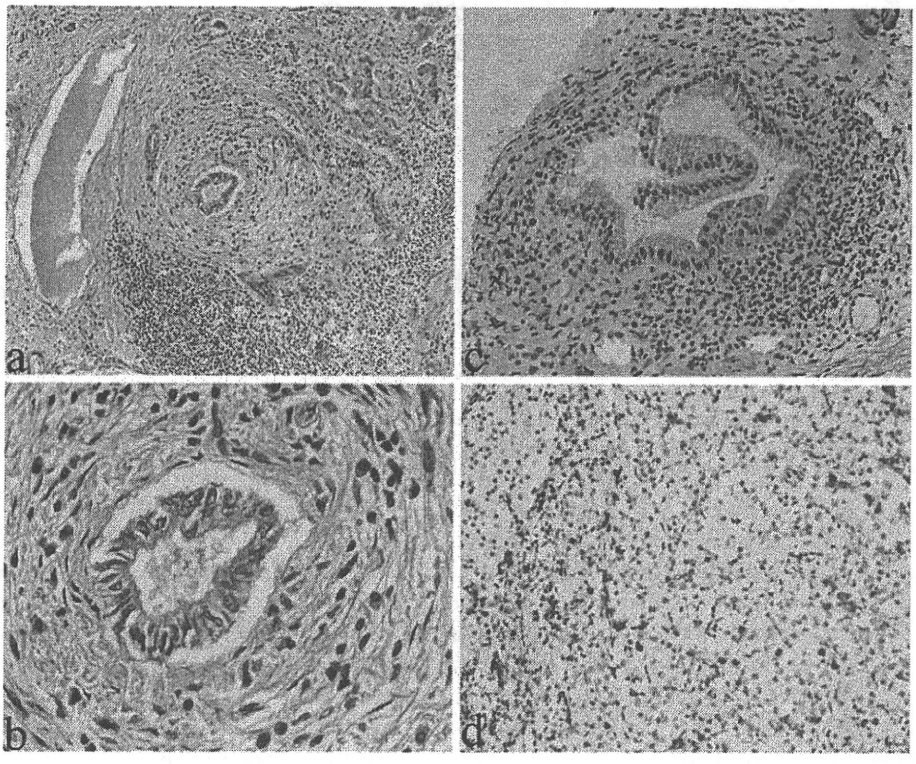


Fig. 2 Histopathological findings of case 1 on transplantation. Histopathological findings of liver after transplantation showed severe lymphoplasmacytic and eosinophil infiltration with fibrosis in the enlarged portal area (H&E staining ×100, **a**; ×400, **b**). Duct and ductular proliferation was conspicuous, and onion-skin fibrosis was observed. Histopathological findings of the pancreas biopsy during operation showed that infiltration of mononuclear cells around pancreatic duct (H&E staining ×200, **c**). IgG4-immunostaining of the pancreas biopsy during operation showed infiltration of abundant IgG4-positive plasma cells (×200, **d**)

intrahepatic bile ducts (Fig. 3c) and no narrowing of the main pancreatic duct (Fig. 3d). Intraductal ultrasonography (IDUS) detected wall thickness of the intrahepatic and common bile ducts. Cytology of bile juice was negative for malignancy. She was diagnosed with PSC and treated with ursodeoxycholic acid.

Four months after clinical onset, the patient was referred to our hospital for further evaluation of recurrent

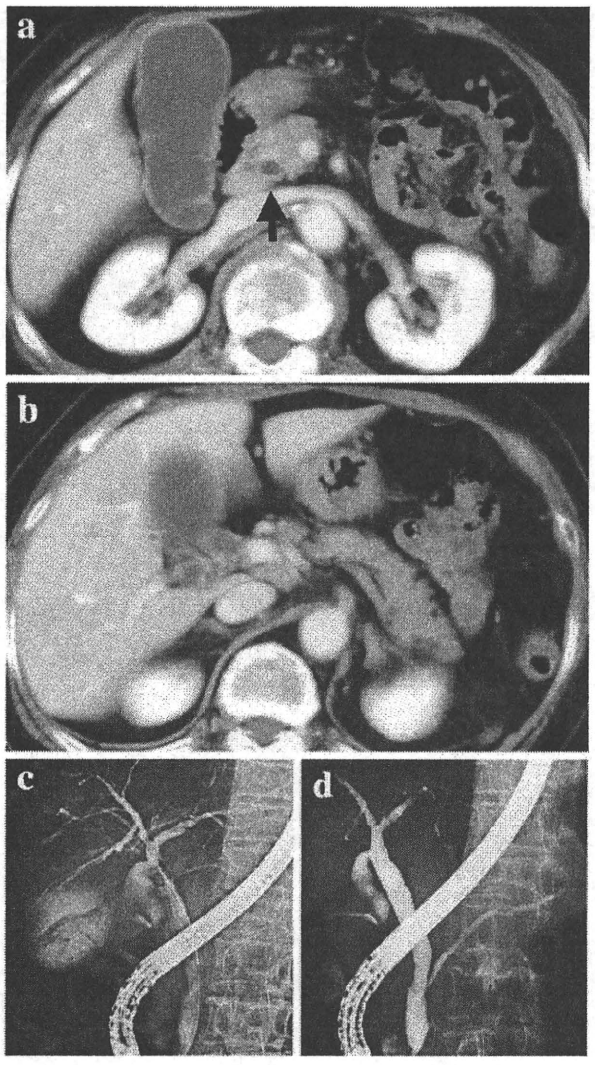


Fig. 3 Abdominal computed tomography (CT) images of case 2 on clinical onset. Abdominal CT showed a dilatation of common bile duct (**a**) and no significant pancreatic lesions (**b**), such as swelling of the pancreas. ERCP revealed irregular narrowing of the intrahepatic bile duct (**c**), and no narrowing of the main pancreatic duct (**d**)

obstructive jaundice. Laboratory tests showed elevations of IgG4 to 206 mg/dl (4.8–105). Histopathological examination by liver biopsy showed moderate lymphoplasmacytic infiltration with fibrosis and fibrotic change surrounding the bile ducts in the enlarged portal area, which is compatible with PSC (Fig. 4a). However, an inflammation with abundant IgG4-positive plasma cells (16 cells/hpf) (Fig. 1d), a characteristic finding in IgG4-SC, was also found. Then, we suspected IgG4-SC, and steroid therapy was initiated at a dose of 30 mg/day. The dose of steroid was reduced by 5 mg/week until it reached 10 mg/day. MRCP revealed no improvements of the irregular narrowing of the intrahepatic lesion and the common bile duct after steroid therapy. One year later, her liver dysfunction developed into liver cirrhosis.

Case 3

The patient was a 23-year-old woman who was admitted to our hospital with the complaint of jaundice. ERCP revealed stricture of the lower common bile duct, irregular dilatation after confluent strictures, and many small defects in intrahepatic bile ducts (Fig. 5a). Endoscopic naso-biliary drainage (ENBD) was performed. The pancreatic-duct image showed no narrowing of the main pancreatic duct. Cytology of bile juice was negative for malignancy. Physical examination revealed no significant findings except for jaundice. Laboratory examinations showed the following values (normal range): peripheral white cell count, 10100/μl (3000–8500); peripheral eosinocyte count, 91/μl; C-reactive protein, 0.09 mg/dl (<0.3); total bilirubin, 5.0 mg/dl (0.2–1.2); alkaline phosphatase, 1750 U/l (107–323); γ-glutamyl transpeptidase, 211 U/l (8–45); asparate aminotransferase, 90 U/l (12–31); alanine aminotransferase, 101 U/l (6–24); amylase, 37 U/l (32–112). Hepatitis B surface antigen and antibody to hepatitis C virus were negative. Serum IgG, IgA, and IgM levels were 2570 mg/dl (1092–1577), 208 mg/dl (134–287), and 363 mg/dl (60–161), respectively. Rheumatoid factor, antinuclear antibody, and antimitochondrial antibody were negative. The irregular dilatation of bile ducts improved 5 months after a drainage procedure with a biliary plastic stent, but irregular narrowing of the intrahepatic bile ducts persisted (Fig. 5b). She was diagnosed with PSC and treated with ursodeoxycholic acid.

Three years after clinical onset, the patient was referred to our hospital for further evaluation of recurrent obstructive jaundice. Histopathological examination by liver biopsy showed an infiltration of lymphocytes and ductular proliferation in the portal area (Fig. 6a), and a few IgG4-positive plasma cells (1 cell/hpf) were detected (Fig. 6b). Laboratory tests showed elevations of IgG4 to 313 mg/dl (4.8–105). Therefore, we suspected IgG4-SC and steroid therapy was initiated at the dose of 30 mg/day. The dose of steroid was reduced by 5 mg/day biweekly until it reached 10 mg/day. MRCP revealed no improvements of strictures of the intrahepatic and the common bile ducts after steroid therapy.

Discussion

Sarles et al. [13] observed the first case of pancreatitis with hypergammaglobulinemia, and Yoshida et al. first proposed the concept of autoimmune pancreatitis (AIP) in 1995 [14], in which patients show diffusely enlarged

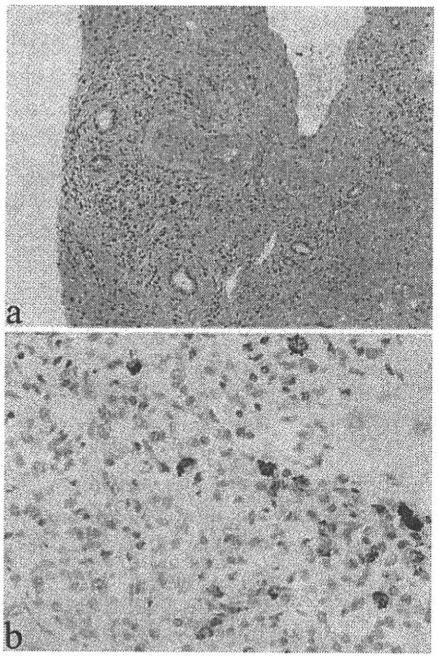
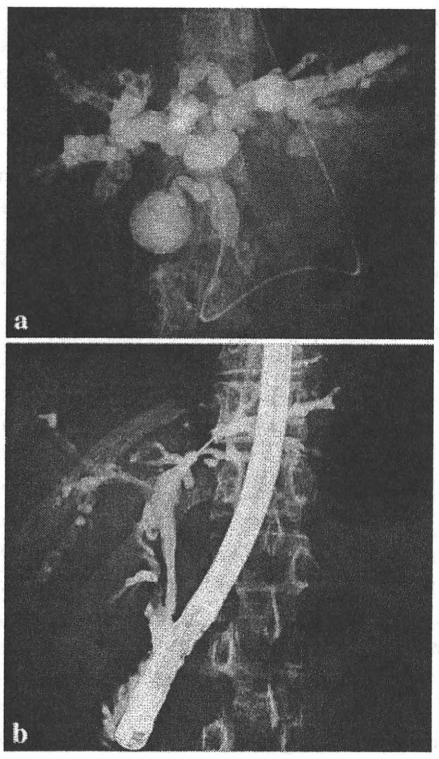


Fig. 4 Histopathological findings of the liver (case 2). Histopathological examination by liver biopsy showed moderate lymphoplasmacytic infiltration with fibrosis and fibrotic change surrounding the bile ducts in the enlarged portal area (H&E staining ×100, **a**). IgG4-immunostaining of the liver specimens showed infiltration of abundant IgG4-positive plasma cells (×400, **b**)



◀ **Fig. 5** Endoscopic nasobiliary drainage (ENBD) cholangiogram and ERCP image of case 3 on clinical onset. Cholangiography through an ENBD tube revealed a stricture of the lower common bile duct, irregular dilatation after confluent strictures, and many small defects in intrahepatic bile ducts (**a**). ERCP after a drainage procedure with a biliary plastic stent revealed improvement of bile duct dilatation, but irregular narrowing of the intrahepatic bile ducts persisted (**b**)

pancreas, narrowing pancreatogram, increased serum IgG, presence of autoantibodies, fibrotic changes with lymphocytic infiltration, and steroidal efficacy. Thereafter, many AIP cases have been reported by Japanese investigators, and AIP has been accepted as a new clinical entity [15–17]. Patients with AIP often show discomfort in the epigastrium, obstructive jaundice due to bile-duct stricture, and diabetes mellitus. AIP is more common in middle-aged and elderly men. Patients with AIP often also have extrapancreatic lesions such as biliary lesions, sialoadenitis, retroperitoneal fibrosis, enlarged celiac and hilar lymph nodes, chronic thyroiditis, and interstitial nephritis [18–23], which suggests that AIP may be a systemic disorder. In 2001, Hamano et al. [24] reported that patients with AIP have high serum IgG4 concentrations. Kamisawa et al. [1] proposed IgG4-related sclerosing disease. Recently, IgG4-SC was recognized as a disease entity characterized by

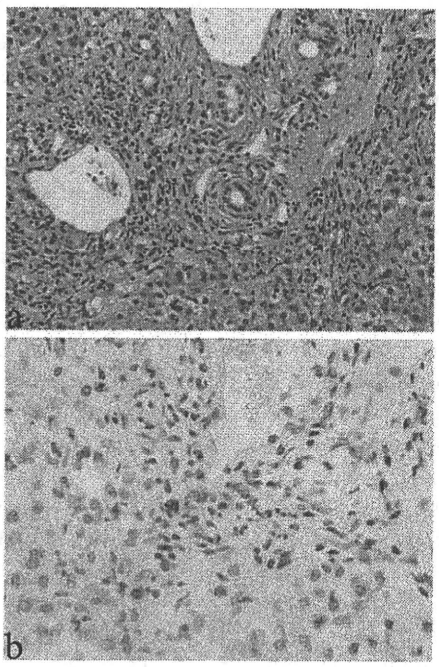


Fig. 6 Histopathological findings of the liver (case 3). Histopathological examination by liver biopsy showed moderate lymphoplasmacytic infiltration and ductular proliferation in the enlarged portal area (H&E staining ×200, **a**). IgG4-immunostaining of the liver specimens showed infiltration of few IgG4-positive plasma cells (×400, **b**)

sclerosing inflammation with an infiltration of abundant IgG4-positive plasma cells, and AIP was associated in most cases. Before establishing the concept of AIP, IgG4-SC used to be misdiagnosed as PSC complicating chronic pancreatitis. Therefore, differential diagnosis between IgG4-SC and PSC is important, because the effective treatments and the prognoses are different. Although IgG4-SC is usually associated with pancreatic lesions, a few patients with IgG4-SC have shown little pancreatic change or other organ involvement [25, 26]. The correct diagnosis of such cases is difficult.

In this study, we presented 3 PSC cases with elevated serum IgG4 levels and/or infiltration of abundant IgG4-positive plasma cells in the liver, which usually support the diagnosis of IgG4-SC. In the 3 cases presented, abdominal ultrasound and abdominal CT scan did not show inflammatory swelling of the whole pancreas, and ERCP did not show strictures over one-third of the main pancreatic duct (MPD), which is characteristic of AIP [27]. Cholangiography in these 3 patients showed strictures of the intrahepatic and common bile ducts, and no narrowing of the MPD. After steroid therapy, strictures of the intrahepatic and common bile ducts were not improved on MRCP images. These findings supported the diagnosis of PSC. In these 3 patients, however, there were findings atypical for PSC. First, the serum IgG4 concentrations in cases 2 and 3 were elevated. The Japanese criteria of AIP contain three approaches: pancreatic imaging, laboratory data, and histopathology [18]: (1) Pancreatic image examinations show the narrowing of the main pancreatic duct and enlargement of pancreas which are characteristic of the disease; (2) Laboratory data show the presence of autoantibodies, or elevated levels of serum gammaglobulin, IgG, or IgG4; (3) Histopathological examinations of the pancreas show fibrosis and pronounced infiltration of cells, mainly lymphocytes and plasmacytes. For a diagnosis, criterion (1) must be present, together with criterion (2) and/or (3). However, it is necessary to exclude malignant diseases such as pancreatic or biliary cancers. In the diagnostic criteria of Korea [28] and Asia [29], apparent pancreatic lesions comparable with AIP must be present for a diagnosis of AIP. Two patients (cases 2 and 3) did not fulfill the diagnostic Japanese, Korean, and Asian criteria, because they had no apparent pancreatic lesions comparable with AIP. Secondly, infiltration of abundant IgG4-positive plasma cells in the liver specimens was found in cases 1 and 2. IgG4 immunostaining showing >10 IgG4-positive plasma cells/hpf is suggestive of AIP in the HISORt criteria by the Mayo Clinic [30] and Korean criteria [28]. The presence of IgG4-SC in the HISORt criteria can be diagnosed in patients with effective steroid therapy. Two patients (cases 1 and 2) did not fulfill the HISORt criteria because they had no response to steroid therapy.

The role of IgG4 in patients with PSC has been used to differentiate clinical syndromes of atypical PSC cases. In 1991, Kawaguchi et al. [31] first described clinical and pathological features of variant cases of PSC, which were later known as sclerosing cholangitis complicated with autoimmune pancreatitis (AIP). In 1995, Takikawa et al. [32] analyzed 192 cases of Japanese PSC and found two peaks in the age distribution. Some cases in elderly patients were complicated with chronic pancreatitis, which was regarded as sclerosing cholangitis complicated with autoimmune pancreatitis. The patients in cases 1 and 3 were young, and case 2 was an elderly woman. In 2004, Takikawa et al. [33] analyzed 269 additional cases of Japanese PSC and showed that 7% of these cases had AIP. In a recent study, Mendes et al. [34] have reported that 12 (9%) of 127 PSC patients had elevated serum IgG4 levels. These patients also had significantly higher levels of ALP and total bilirubin, and higher PSC Mayo risk scores. Mendes's study also reveals that IgG4-SC may have been included among PSC cases in the United States. There may possibly be disease entities such as overlap syndrome. In our cases, it is difficult to differentiate IgG4-SC from PSC on cholangiographic and immunohistochemical findings. The findings of elevated serum IgG4 levels and/or an infiltration of abundant IgG4-positive plasma cells in the liver usually support the diagnosis of IgG4-SC. On the other hand, the patients of cases 1 and 3 were younger, and the patient of case 1 was associated with UC. These clinical characteristics may be compatible with PSC. In a recent study, Kawabe et al. have reported an advanced state of biliary cirrhosis and atrophic pancreas but did not reveal typical imaging findings of AIP and AIP-related sclerosing cholangitis [35]. Hamano et al. have reported 3 patients with IgG4-SC who had no apparent pancreatic lesions comparable with AIP [26]. These cases were improved only by steroid therapy or drainage. In this study, however, our 3 patients with sclerosing cholangitis who had no apparent pancreatic lesions comparable with AIP did not respond to steroid therapy. Some AIP patients may develop pancreatic stones and the conventional type of chronic pancreatitis [36, 37]. Though there may be a possibility that far advanced stages of AIP with sclerosing cholangitis who had no pancreatic lesions might not respond to steroid therapy, the long-term untreated prognosis of AIP still remains unclear. Therefore, further studies are necessary. Finally, we diagnosed our 3 patients as PSC according to the commonly used diagnostic criteria for PSC [38]. They did not fulfill all criteria, and histopathological finding of the pancreas in case 1 did not show so-called LPSP. Here, our cases showed elevated serum IgG4 levels and/or an infiltration of abundant IgG4-positive plasma cells in patients with PSC, which do not respond to steroid therapy. Therefore, it is necessary to be aware of the possibility of

PSC with these findings to correctly differentiate PSC from IgG4-SC. The mechanisms of increased serum IgG4 and the role of the infiltrated IgG4-positive plasma cells in the portal area still remain unclear at this time. Recent studies of immune tolerance and allergy show that high dose antigen exposures cause immune deviation both of Th2 response in favor of Th0/Th1, and in the generation of IL-10- and TGF-β-producing regulatory T cells [39], though our 3 patients were not found to have allergic disease. Additionally, IL-10 induces preferential switching of B cell response in favor of producing IgG4 antibodies, and possibly IgA antibodies under the influence of TGF-β [40]. Our previous data [41] and others [11] showed that IL-10 secreted from increased inducible peripheral regulatory T cells may be involved in switching B cells to produce IgG4-positive cells and increased serum IgG4 in IgG4-related sclerosing pancreatitis (autoimmune pancreatitis) or IgG4-related sclerosing cholangitis, but not in PSC [11]. These findings suggested that increased IgG4 may be reactive and involved in the pathophysiology of IgG4-related diseases, but not in the pathogenesis. Further studies are necessary to clarify the role of IgG4.

In conclusion, some of the patients with PSC show elevated serum IgG4 levels and/or an infiltration of abundant IgG4-positive plasma cells, and do not respond to steroid therapy.

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Analysis of Humoral Immune Response in Experimental Autoimmune Pancreatitis in Mice

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Objectives: To study the autoimmune response in MRL/Mp mice, which spontaneously develop pancreatitis in the exocrine pancreatic tissue.

Methods: Six-week-old female mice were injected intraperitoneally with polyinosinic polycytidylic acid at a dose of 5 mg/kg of body weight twice a week for up to 12 weeks. The mice were serially killed, and the severity of their pancreatitis was graded with a histological scoring system. Immunohistological examinations were performed, and the serum levels of autoantibodies were measured by enzyme-linked immunosorbent assay.

Results: The administration of polyinosinic polycytidylic acid accelerated the development of pancreatitis, with abundant infiltration of $B220^+$ B cells and $CD138^+$ plasmacytes. Various autoantibodies directed against autoantigens, including carbonic anhydrase II and lactoferrin, were detected but none against glutamic acid decarboxylase. Of these, autoantibodies directed against the pancreatic secretory trypsin inhibitor (PSTI; 91.7%) were more prevalent than those against carbonic anhydrase II (33.3%) or lactoferrin (45.8%). Determination of the epitope of the anti-PSTI antibody showed that most immunoreactivity was directed at the site on PSTI that is active in the suppression of trypsin activity.

Conclusions: The autoimmune response to PSTI protein may induce a failure of PSTI activity, resulting in the activation of trypsinogen and the subsequent disease progression.

Key Words: autoimmune pancreatitis, autoantibody, pancreatic secretory trypsin inhibitor

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Chronic pancreatitis is characterized by chronic inflammation and progressive fibrosis of the pancreas, which leads to irreversible pancreatic dysfunction and, finally, to pancreatic insufficiency. Although the cause of chronic pancreatitis is frequently associated with excessive alcohol use and gall stones, approximately 30% to 40% of cases are idiopathic.[1] Since Sarles et al[2] reported a case of chronic pancreatitis with hyperglobulinemia, an increasing number of similar cases have been documented, with or without other autoimmune diseases. Many such cases of pancreatitis are associated with increased immunoglobulin G (IgG), IgG4, or autoantibody production and are highly responsive to steroid treatment. These findings suggest that an autoimmune mechanism is involved in the development of pancreatitis. Recent studies have demonstrated the frequent cooccurrence of extrapancreatic lesions, such as sclerosing cholangitis, sclerosing sialoadentitis, interstitial nephritis, or retroperitoneal fibrosis,[3] suggesting that autoimmune pancreatitis (AIP) is a discrete form of pancreatitis.[4,5]

A series of diagnostic criteria have been proposed by researchers in several countries in an attempt to differentiate AIP from other forms of chronic pancreatitis or pancreatic cancer. These criteria are based on a combination of findings from imaging, laboratory testing, and histological analysis.[6–8] A typical radiological image shows narrowing of the main pancreatic duct and enlargement of the pancreas. Laboratory data show abnormally elevated levels of serum γ-globulin, IgG, or IgG4, or the presence of autoantibodies. Histopathological analysis of the pancreas demonstrates marked fibrosis and prominent infiltration by lymphocytes and plasma cells. Recently, widespread awareness of the disease and the proposed diagnostic criteria has resulted in an increasing number of patients with AIP reported throughout the world.

However, little is known about the precise pathogenesis of AIP, although reports have shown that the disease is associated with the progressive infiltration of lymphocytes and plasmacytes, predominantly localized to ductal structures, in addition to varying degrees of parenchymal and acinar destruction.[9] The natural course of the disease is also as yet unknown. The disease may remain largely asymptomatic for prolonged periods, and symptoms develop in patients with advanced stages of the disease. Therefore, the early immune response underlying the pathogenesis of AIP is difficult to study in patients with the disease. A serological hallmark of AIP is considered to be elevated levels of IgG and IgG4 and the presence of autoantibodies against antigens such as carbonic anhydrase II (CA-II), lactoferrin (LF), pancreatic secretory trypsin inhibitor (PSTI), and nuclear antigens,[10–14] although these autoantibodies are not found in all patients with AIP and their role in its pathogenesis is not fully understood.

To investigate the autoimmune mechanism involved in the development of human AIP, several animal models have been studied that develop AIP-like pancreatic lesions spontaneously or after immunization with exogenous antigens.[15–21] Among these, MRL/Mp mice spontaneously develop pancreatitis via an autoimmune mechanism at 34 weeks of age or older.[15] The administration of polyinosinic polycytidylic acid (poly I:C), a synthetic double-stranded RNA, accelerates the development of the disease, with an incidence of 100% at 18 weeks without any

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other severe autoimmune diseases.[19] Although the pancreatitis in MRL/Mp mice has been shown to be cell mediated, the humoral autoimmune response has not been fully investigated. In this study, we investigated the autoimmune response in MRL/Mp mice treated with poly I:C, with a specific focus on AIP-related autoantibody production.

MATERIALS AND METHODS

Mice

Female MRL/Mp mice were purchased from the Jackson Laboratory (Bar Harbor, Me). Wild-type female C57BL/6 mice were purchased from Japan SLC (Shizuoka, Japan). All mice were bred at the animal facility of Kyoto University under specific-pathogen–free conditions.

Induction of Pancreatitis

The 6-week-old female MRL/Mp mice were injected intraperitoneally with poly I:C (Sigma Chemical Co, St Louis, Mo) at a dose of 5 mg/kg of body weight twice a week for up to 12 weeks. The control mice were injected with phosphate-buffered saline (PBS). All experiments were conducted with the approval of the Ethics Committee for the Use of Experimental Animals of Kyoto University.

Histological Examination

The mice were killed at the age of 12 or 18 weeks. Blood was collected, and sera were stored at −20°C until use. Several tissues, including the pancreas, the liver, the salivary gland, and the kidney, were removed for histopathological examination. The tissues were fixed in 10% phosphate-buffered formaldehyde (pH 7.2) and embedded in paraffin. The sections were stained with hematoxylin and eosin and examined histopathologically under a light microscope. The severity of the pancreatitis was scored on a 0 to 4 scale based on the histopathological scoring system described by Kanno et al[15]: 0, pancreas without mononuclear cell infiltration; 1, mononuclear cell aggregation and/or infiltration within the interstitium, with no parenchymal destruction; 2, focal parenchymal destruction with mononuclear cell infiltration; 3, diffuse parenchymal destruction but some intact parenchymal residue retained; and 4, almost all pancreatic tissue, except the pancreatic islets, destroyed or replaced with

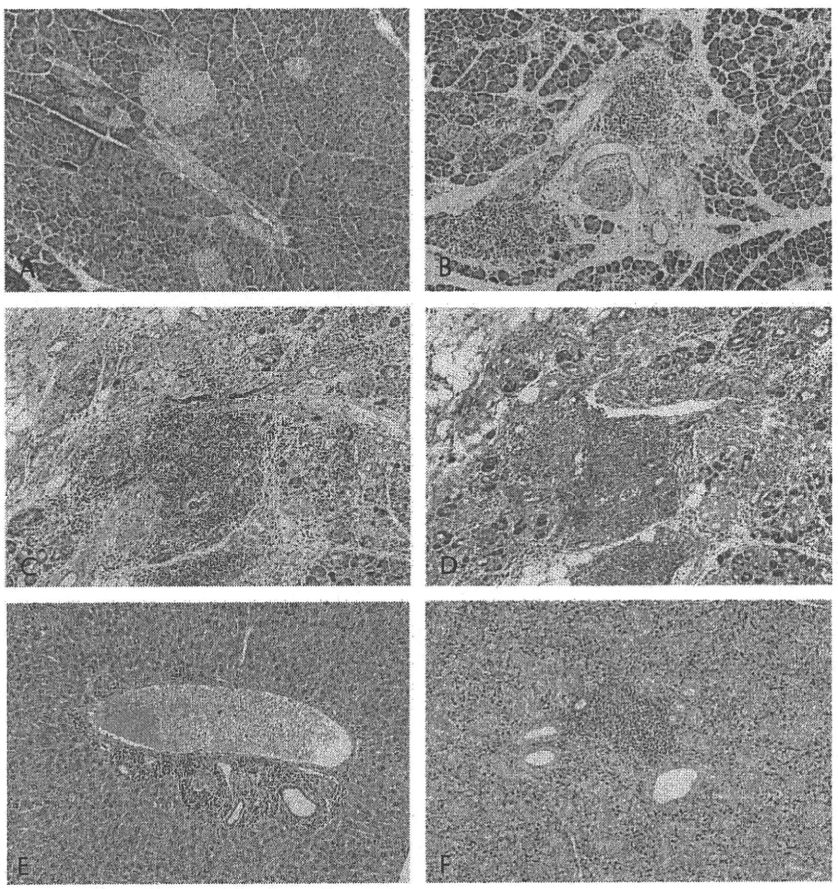

FIGURE 1. Histopathological examination of the pancreas, the liver, and the submandibular salivary gland. Representative pancreatic sections stained with hematoxylin and eosin or Azan: 12-week treatment with PBS (A), 6-week treatment with poly I:C (B), 12-week treatment with poly I:C (C), and 12-week treatment with poly I:C (Azan staining) (D). Liver (E) and submandibular salivary gland sections (F) after treatment with poly I:C for 12 weeks. After the mice were injected with poly I:C for 6 weeks, interstitial edema and moderate inflammatory cell infiltration of the pancreas were observed. After treatment for 12 weeks, marked inflammatory cell infiltration with severe destruction of the acini, irregular fibrosis, and fatty changes of the pancreas was seen. Mononuclear cell infiltration was observed around some of the portal areas of the liver. Periductular infiltration by mononuclear cells was scattered in the submandibular salivary gland (original magnification ×100).

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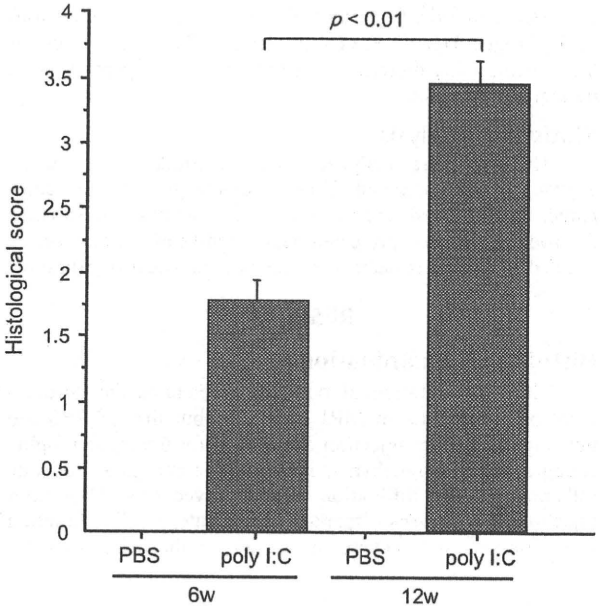

FIGURE 2. Histological scoring of pancreatitis. The severity of pancreatitis was scored on a 0 to 4 scale based on a histopathological scoring system. The histological scores for pancreatitis increased according to the duration of the treatment (6 weeks, 1.8 ± 0.4, 12 weeks, 3.5 ± 0.7). The results are expressed as mean ± SD.

fibrosis or adipose tissue. The maximum score was used as the grade of pancreatitis in each mouse. To estimate the incidence of pancreatitis, mice with pancreatic lesions that were scored 2 or higher were defined as positive. The fibrosis of the sections was also examined histologically with Azan staining.

Immunohistochemistry

Immunohistochemical staining was performed on 5-μm-thick, formalin-fixed, paraffin-embedded tissue sections. The sections were deparaffinized in xylene, rehydrated in graded alcohol, and washed in PBS. Antigen retrieval was accomplished by microwave irradiation. After the serial sections had been blocked in 1.5% normal rabbit serum for 15 minutes at room temperature, they were incubated for 1 hour at room temperature with 1 of the following primary antibodies at a dilution of 1:50: rat anti–mouse B220 monoclonal antibody (mAb), rat anti-mouse CD4 mAb, rat anti–mouse CD8 mAb, or rat anti–mouse CD138 mAb (all from BD Bioscience, San Jose, Calif). After brief rinsing, the sections were treated with biotinylated rabbit anti–rat IgG secondary antibody (Serotec Inc, Kidlington, Oxford, United Kingdom) for 30 minutes at room temperature, rinsed, and incubated with peroxidase-conjugated avidin-biotin complex (ABC Elite kit; Vector Laboratories, Burlingame, Calif) for 30 minutes at room temperature. The peroxidase activity was visualized by the application of a fresh mixture of 3,3′-diaminobenzidine and 0.005% H_2O_2 in Tris-buffered saline (0.05 mol/L, pH 7.6). The sections were then counterstained with hematoxylin, dehydrated, cleared, and mounted.

Measurement of Autoantibodies

To evaluate the humoral immunity against PSTI, CA-II, and LF, the levels of specific autoantibodies in the sera were quantified with enzyme-linked immunosorbent assays (ELISAs) according to a previous report,[12] with minor modifications. Microtiter plates (Maxi Sorp; Nalge Nunc International, Roskilde, Denmark) were coated with 50 μL of a 20-μg/mL solution of bovine PSTI, CA-II, or LF (Sigma Chemical Co) and incubated overnight at 4°C. The plates were then incubated with 200 μL of 10% skim milk in PBS containing 0.05% Tween 20 (PBST) to block nonspecific binding and rinsed 4 times with PBST. The murine sera, at a dilution of 1:40, were tested in duplicate for 2 hours at room temperature. The optimal dilution

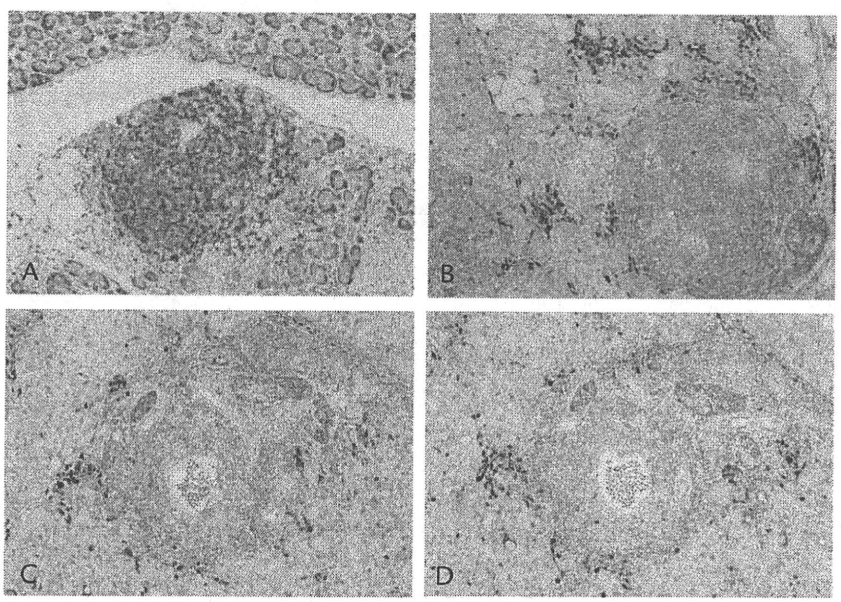

FIGURE 3. Immunohistochemical analysis of the pancreas. Representative pancreatic sections from mice treated with poly I:C for 6 weeks: anti-B220 (A), anti-CD138 (B), anti-CD4 (C), and anti-CD8 staining (D). $B220^+$ mononuclear cells infiltrated into the pancreatic parenchyma and formed lymphoid follicles at the periductal region of the pancreatic ducts. $CD138^+$ cells infiltrated around the follicles and interstitium, in addition to $CD4^+$ and $CD8^+$ T cells (original magnification ×200).

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of the sera (1:40) was determined in preliminary experiments in which 4-fold serial dilutions of the sera (1:40 to 1:640) were tested for the detection of autoantibodies by ELISA. After the samples were washed with PBST, the bound antibodies were reacted specifically with diluted (1:4000) goat anti–mouse IgG antibody conjugated with horseradish peroxidase (Serotec Inc, Raleigh, NC) for 2 hours at room temperature. After 4 washes with PBST, the plates were incubated with 50 μL of 0.4-mg/mL *o*-phenylene diamine in 0.1-mol/L citrate phosphate buffer (pH 5.0) with H_2O_2 for 15 minutes at room temperature. The reaction was terminated by the addition of 50 μL of 2 N H_2SO_4, and the absorbance was determined at an optical density (OD) of 490 nm. Positive results were defined as OD values greater than the mean plus 3 SDs (mean + 3 SDs) of the values obtained for the sera of untreated C57BL/6 mice. Anti–glutamic acid decarboxylase (GAD) antibody was measured by a commercial laboratory (SRL Inc, Osaka, Japan).

Measurement of Serum IgG Subclasses

To clarify which IgG subclass was predominant in mice with AIP, we measured the levels of the IgG1, IgG2a, IgG2b, and IgG3 subclasses using the Mouse IgG1, IgG2a, IgG2b, and IgG3 ELISA Quantitation kits (Bethyl Laboratories Inc, Montgomery, Tex), according to the manufacturer's instructions. Briefly, microtiter plates (Maxi Sorp) were coated with 100 μL of capture antibody for 1 hour at room temperature. After the plates were blocked with blocking solution, the sera (serially diluted to fall within the concentration range of the standards) were added to the plates, and the plates were incubated for 1 hour at room temperature. After the plates were washed, the bound IgG was reacted specifically for 1 hour at room temperature with the diluted detection antibody conjugated with horseradish peroxidase. After the plates were washed with PBST, they were incubated with 100 μL of tetramethylbenzidine/H_2O_2 (R&D Systems, Inc, Minneapolis, Minn) for 15 minutes at room temperature. The reaction was terminated by the addition of 100 μL of 2 N H_2SO_4, and the absorbance was determined at an OD of 450 nm. The levels of the IgG subclasses were calculated from the respective standard curves.

Immunoglobulin G Subclass and Epitope Mapping of Anti-PSTI Antibody

To determine the IgG subclass of the serum anti-PSTI antibodies, an ELISA was performed using bovine PSTI as the antigen. The murine sera were tested in duplicate for 1 hour at room temperature at a dilution of 1:40. The bound anti-PSTI antibody was reacted with optimally diluted (1:5000 to 1:100,000) goat anti–mouse IgG1, IgG2a, IgG2b, or IgG3 antibody conjugated with horseradish peroxidase (Bethyl Laboratories Inc). The plates were incubated with 100 μL of tetramethylbenzidine/H_2O_2 (R&D Systems, Inc) for 15 minutes at room temperature. The reaction was terminated by the addition of 100 μL of 2 N H_2SO_4, and the absorbance was determined at an OD of 450 nm as described previously. To define the epitopic region for the serum anti-PSTI antibodies, we synthesized overlapping peptides that covered the entire amino acid sequence of the serine protease inhibitor, Kazal type 3 (Spink3), a mouse homologue of bovine PSTI, consisting of the 56 amino acids following the secretion signal sequence composed of the first 24 amino acids. The first peptide included amino acids 1 to 25, KVTGKEASCHDAVAGCPRIYDPVCG; the second peptide included amino acids 17 to 41, PRIYDPV CGTDGITYANECVLCFEN; and the third peptide included amino acids 32 to 56, ANECVLCFENRKRIEPVLIRKGGPC. The peptides with the previously mentioned sequences were synthesized to order (immunological purity and no conjugation) by Invitrogen Japan KK (Tokyo, Japan). The serum levels of the autoantibodies directed against these synthetic peptides were measured by ELISA.

Statistical Analysis

The data were analyzed using the Student *t* test when 2 groups were compared. When multiple groups were compared, the data were examined by 1-way analysis of variance followed by Fisher protected least significant difference. A 2-tailed $P < 0.5$ was deemed to indicate statistical significance.

RESULTS

Histological Examination

The administration of poly I:C accelerated the development of pancreatitis in MRL/Mp mice, but that of PBS did not (Fig. 1A). After injection of poly I:C for 6 weeks, atrophic changes in the parenchyma, interstitial edema, and moderate inflammatory cell infiltration were observed (Fig. 1B). After injection for 12 weeks, marked inflammatory cell infiltration was observed, with severe destruction of the acini, irregular

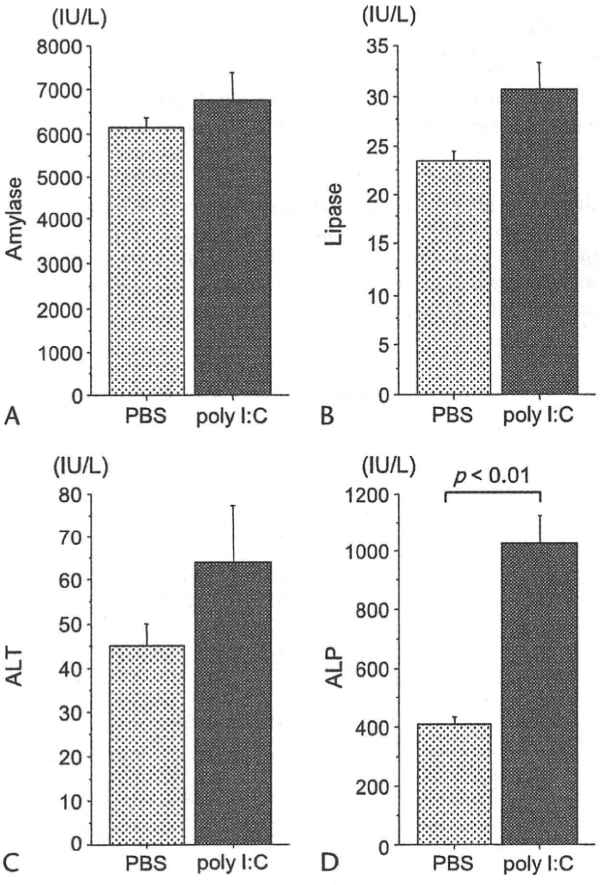

FIGURE 4. Serum pancreaticobiliary enzymes of the control mice and mice treated with poly I:C for 12 weeks: amylase (A), lipase (B), alanine aminotransferase (ALT) (C), and alkaline phosphatase (ALP) (D). There was no significant increase in the serum amylase, the lipase, or the ALT levels in the mice treated with poly I:C relative to those treated with PBS. The serum levels of alkaline phosphatase were significantly higher in the poly I:C–treated mice. The results are expressed as mean ± SD.

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fibrosis, and fatty changes (Figs. 1C, D). Some of the acinar cells also showed vacuolar changes in their cytosol (cellular vacuolization). The histological scores for pancreatitis increased with the duration of the treatment (6 weeks, 1.8 ± 0.4, 12 weeks, 3.5 ± 0.7; Fig. 2). However, the endocrine glands showed few changes, and the tissues were well preserved. In the liver, mononuclear cell infiltration was observed around some of the portal areas, indicating the coexistence of cholangitis (Fig. 1E). Mild periductul infiltration by mononuclear cells was also observed in the submandibular salivary gland (Fig. 1F).

Immunohistochemistry

Immunohistochemistry showed that most of the infiltrates in the pancreatic parenchyma were $B220^+$ mononuclear cells, which infiltrated the pancreatic parenchyma and formed lymphoid follicles in the periductal regions of the pancreatic ducts (Fig. 3A). $CD138^+$ plasmacytes infiltrated around the follicles and interstitium (Fig. 3B). $CD4^+$ and $CD8^+$ T cells were mainly observed around the follicles (Figs. 3C, D).

Blood Chemistry

There were no significant increases in serum amylase, lipase, or alanine aminotransferase levels in mice treated with poly I:C relative to those in mice treated with PBS (Fig. 4). The serum levels of alkaline phosphatase in the poly I:C–treated mice were significantly higher than those in the control mice ($P < 0.01$), which is consistent with the histopathological finding of cholangitis in the livers of mice treated with poly I:C.

Measurement of Serum IgG Subclasses

The IgG subclass levels in the murine sera were measured by ELISA. The levels of the IgG subclasses in the control mice (n = 6) and mice treated with poly I:C (n = 6) were as follows (mg/dL): IgG1, 133.5 ± 25.8 and 363.5 ± 44.6, respectively; IgG2a, 255.2 ± 125.4 and 560.2 ± 211.6, respectively; IgG2b, 24.1 ± 13.8 and 85.8 ± 21.7, respectively; and IgG3, 776.7 ± 635.3 and 1177.4 ± 462.3, respectively (Fig. 5). The levels of IgG1 and IgG2b were significantly higher in the poly I:C–treated mice than in the control mice. The serum IgG2a and IgG3 levels were elevated in the poly I:C–treated mice, but they were not significantly higher than those of the control mice.

Autoantibody Production

The OD values for autoantibodies directed against CA-II in the poly I:C–administered mice (0.745 ± 0.222) were significantly higher than those in the PBS-treated mice (0.371 ± 0.299). Similarly, the titers of the anti-LF antibodies in the poly I:C–administered mice (0.613 ± 0.191) were higher than those in the control mice (0.288 ± 0.231). The titers of the anti-PSTI antibody in the poly I:C–administered mice (0.489 ± 0.177) were also higher than those in the PBS-treated mice (0.289 ± 0.271; Figs. 6A–C). When the cutoff index was set at a value equivalent to the mean absorbance + 3 SDs of the values for untreated C57BL/6 mice (0.347), 91.7% (22/24) of the poly I:C–treated mice were positive for anti-PSTI antibody. This is in contrast to the relatively low frequencies of anti–CA-II (33.3%, 8/24) and

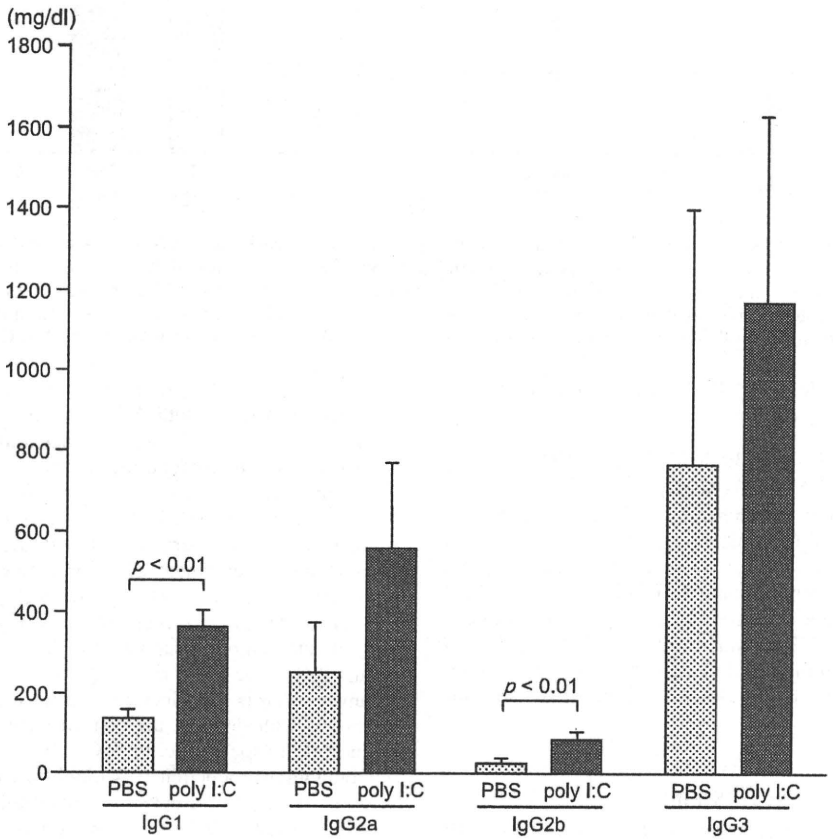

FIGURE 5. Measurement of serum IgG subclasses. The levels of the IgG subclasses were quantified by ELISA. The amounts of IgG1 and IgG2b were significantly higher in the poly I:C–treated mice than in the control mice ($P < 0.01$). The serum IgG2a and IgG3 levels were elevated in the poly I:C–treated mice but were not significantly higher than those in the control mice. The results are expressed as mean ± SD.

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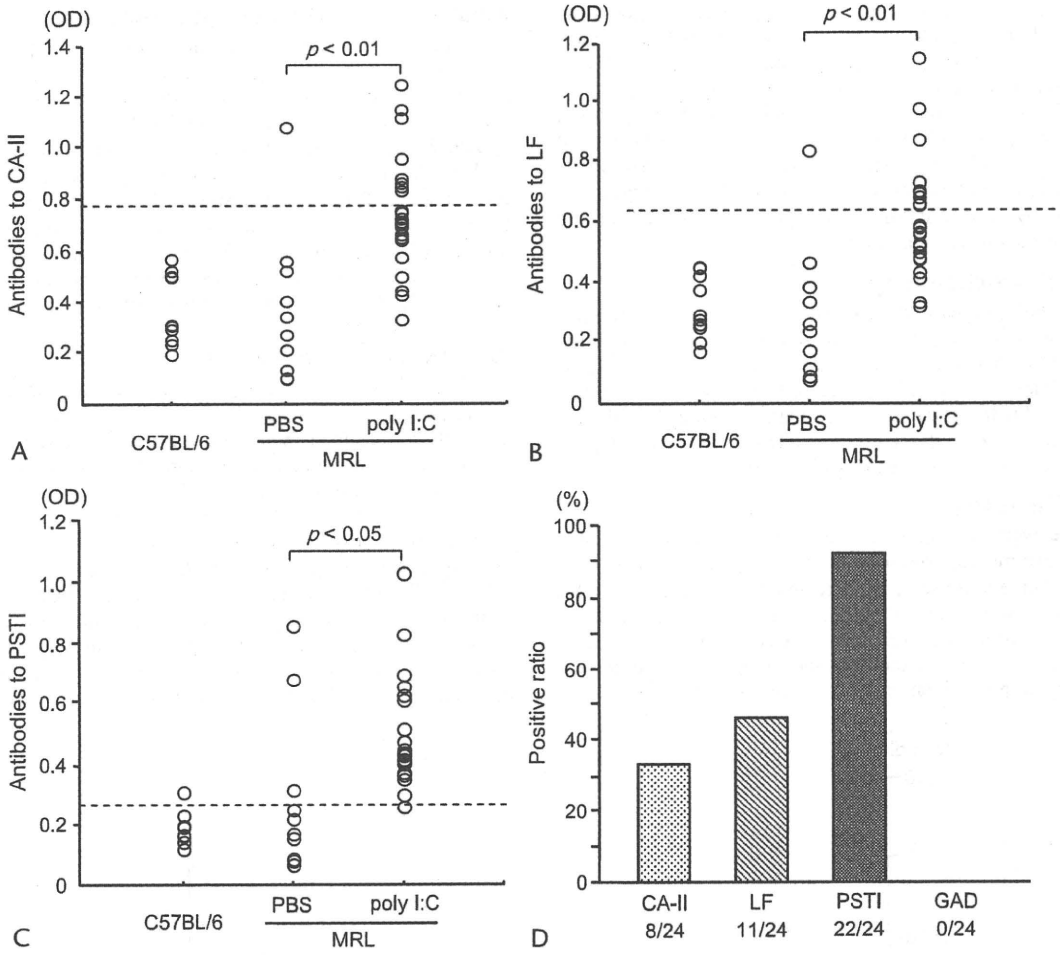

FIGURE 6. Titers of autoantibodies in the sera of mice treated with poly I:C for 12 weeks: anti–CA-II (A), anti-LF (B), anti-PSTI antibodies (C), and prevalence of autoantibodies (D). The titers of these antibodies were all significantly higher than those of the control mice. When the cutoff values were equivalent to the means + 3 SDs of the values for the untreated C57BL/6 mice, the prevalence of the anti-PSTI antibody (91.7%) was higher than that of the anti–CA-II (33.3%) and anti-LF antibodies (45.8%). No anti-GAD antibodies were detected in the mice with AIP. The dotted line indicates the means + 3 SDs of the values for the untreated C57BL/6 mice.

anti-LF antibodies (45.8%, 11/24). No sera were positive for anti-GAD antibody (Fig. 6D).

Immunoglobulin G Subclass and Epitope Mapping of Anti-PSTI Antibody

Anti-PSTI antibody was shown by ELISA to be predominantly present as the IgG2a subclass (data not shown). The reactivity of the sera from the AIP mice was tested against overlapping peptides corresponding to PSTI. When the cutoff index was set at a value equivalent to the mean absorbance + 3 SDs of untreated C57BL/6 mice, 82.3% (20/24) and 75% (18/24) of the sera from poly I:C–treated mice reacted with peptides 1 and 2, respectively. However, none of the sera reacted with peptide 3.

DISCUSSION

Although the clinical and histopathological features of AIP have been well documented, the precise pathogenesis of the disease is poorly understood.[22] This is partly because of the difficulty in making a diagnosis and obtaining pancreatic samples in the early stage of the disease. Therefore, the early cellular events underlying the pathogenesis of the disease are difficult to clarify in patients with AIP. To overcome the difficulty, we used MRL/Mp mice, in which pancreatitis spontaneously develops via an autoimmune mechanism.[15] The development of pancreatitis was accelerated by the administration of poly I:C, a synthetic double-stranded RNA.[19] Because of its structural resemblance to double-stranded viral RNA, poly I:C should accelerate the development of autoimmune diseases in several animals with genetically susceptible backgrounds.[23,24]

The observation of increased serum IgG levels or the presence of autoantibodies supports the diagnosis, whereas elevated serum IgG4 levels are nearly diagnostic.[25,26] We found that serum IgG levels were increased in mice treated with poly I:C compared with those of the control mice. However, we found no increase in specific serum IgG subclass levels, although immunoglobulin production was highly increased by the administration of poly I:C, a polyclonal activator of B cells.

Interestingly, various autoantibodies, including anti–CA-II antibody and anti-LF antibody, were also detected in the MRL/Mp mice with pancreatitis, confirming the hypothesis that CA-II and LF are target antigens in autoimmune-mediated pancreatitis as reported previously.[10,12,14] In addition to the

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production of autoantibodies directed against CA-II and LF, we found that the prevalence of anti-PSTI antibodies was markedly increased relative to the autoantibodies directed against CA-II or LF. This is in contrast to the prevalence of anti-PSTI antibodies in human AIP, in which anti-PSTI antibodies are detected in 30% to 40% of patients. This may be explained by a difference in genetic background because MRL mice are considered genetically homogeneous, whereas humans constitute a genetically heterogeneous population. Therefore, although the analysis of a mouse model is useful for studying the pathogenesis of AIP, the results obtained may not be applicable to all patients with AIP.

In our previous study, anti-PSTI antibodies in the sera of patients with AIP were of the IgG1 subclass, not of the IgG4 subclass. Moreover, there was no significant correlation between the serum levels of IgG4 and anti-PSTI IgG antibodies in patients with AIP. This is in contrast to a recent study that found a strong association between increased serum IgG4 and anti–CA-II antibody levels in patients with AIP.[26] In the mice with pancreatitis, anti-PSTI antibodies were of the IgG2a subclass, not of the IgG1 or IgG4 subclass, although the roles of the corresponding IgG subclasses are considered to be different between mice and human.

Pancreatic secretory trypsin inhibitor (serine protease inhibitor, Kazal type 1), a 56–amino acid peptide, is synthesized in pancreatic acinar cells and colocalizes with trypsinogen granules. It inhibits approximately 20% of trypsin activity within the pancreas by physically blocking the active site on trypsin.[27,28] In addition to its protective role in acinar cells, PSTI inhibits the activation of trypsinogen in the pancreatic duct.[29] Such protective role of PSTI has been demonstrated in several animal models of experimental pancreatitis.[29–32] Recent studies have suggested that N34S, an exonic mutation of PSTI, is closely associated with the pathogenesis of hereditary pancreatitis and idiopathic chronic pancreatitis.[33,34]

However, the role of serum anti-PSTI antibodies in the development of human AIP has not yet been determined. One possibility is that anti-PSTI antibodies neutralize and inhibit the action of PSTI, resulting in the excessive activation of trypsin in the pancreas. To clarify the role of anti-PSTI in the pathogenesis of AIP, we synthesized overlapping peptides of the serine protease inhibitor, Kazal type 3 (Spink3), which is considered to be homologous to human PSTI, and investigated the epitopic region of the anti-PSTI antibodies. Interestingly, the sera of mice with pancreatitis reacted with synthetic peptide 1 (amino acids 1–25) and peptide 2 (amino acids 17–43) but not to peptide 3 (amino acids 32–56). Because previous studies have reported that the active site of PSTI necessary for its binding to trypsin occurs in the amino acid sequence 18 to 21,[35] such skewed reactivity may indicate that the anti-PSTI antibody inhibits the activity of PSTI in vivo. Alternatively, the presence of anti-PSTI antibody may merely be a secondary immune response against antigens released from the destroyed pancreatic tissue. It is necessary to study whether the anti-PSTI antibody inhibits the function of PSTI, leading to the progression of pancreatitis, although the cell-mediated immune response is considered to play the major role in the pathogenesis of murine AIP.[15] This would clarify the autoimmunity in human AIP, in which both humoral and cellular immune response may be involved.[36]

In conclusion, we have demonstrated the increased production of various autoantibodies in mice with AIP. Notably, autoantibody production directed against PSTI was more prevalent than that against CA-II and LF, and this immunoreactivity was predominantly directed to the active site of PSTI. These findings suggest that the autoimmune response to PSTI protein accelerates AIP disease progression through the inhibition of PSTI activity.

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REVIEW

Japanese consensus guidelines for management of autoimmune pancreatitis: I. Concept and diagnosis of autoimmune pancreatitis

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Abstract As the number of patients with autoimmune pancreatitis (AIP) is increasing in Japan, practical guidelines for managing AIP need to be established. Three committees [the professional committee for developing clinical questions (CQs) and statements by Japanese specialists, the expert panelist committee for rating statements by the modified Delphi method, and the evaluating committee of moderators] were organized. Fifteen AIP specialists extracted specific clinical statements from a total of 871 articles in the literature using a PubMed search (1963–2008) and a secondary database, and developed the CQs and statements. The expert panelists individually rated these clinical statements using a modified Delphi approach in which a clinical statement receiving a median score greater than 7 on a 9-point scale from the panel was regarded as valid. The professional committee developed 13, 6, 6, and 11 CQs and statements for the concept and diagnosis, extra-pancreatic lesions, differential diagnosis and treatment, respectively. The expert panelists regarded them as valid after two-round modified Delphi approaches. After evaluation by the moderators, the Japanese clinical guidelines for AIP were established. The digest versions of the present guidelines have been published in the official journal of the Japan Pancreas Society, "*Pancreas.*" Full versions divided into three series are scheduled to be published in the present and followings two issues in the *Journal of Gastroenterology* with approval of Professor Go VLW, the Editor-in-Chief of "*Pancreas.*"

Keywords Autoimmune pancreatitis · Guideline · Diagnosis · Treatment · Delphi method

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Introduction

Autoimmune pancreatitis (AIP) is accepted worldwide as a distinctive type of pancreatitis [1–4]. It is suspected that the pathogenesis of AIP involves autoimmune mechanisms. In addition to pancreatitis, patients with AIP often develop extra-pancreatic lesions such as biliary lesions, sialadenitis, retroperitoneal fibrosis, enlarged celiac and hilar lymph nodes, chronic thyroiditis, and interstitial nephritis, suggesting that AIP may be a systemic disorder [5–7]. Although the pathogenesis is still unclear, the most important issue in the management of AIP is to differentiate it from pancreatic and biliary malignancy. Recently, various diagnostic criteria for AIP have been proposed, including those of Japan [8], Korea [9, 10], the Mayo Clinic [11], and Asia [12]. As a

systemic corticosteroid is usually effective, the steroid effect is included in the diagnostic criteria proposed by Korea and the Mayo Clinic. Although Japanese criteria do not recommend facile therapeutic use of steroids [8], Asian criteria proposed by the Japan-Korea joint symposium permit it only when recommended by experts after a full negative workup of malignancy [12]. Although the numbers of patients with AIP are increasing in Japan, the clinical evidence is limited. Therefore, practical guidelines for managing AIP are needed. Most of the evidence levels of the specific clinical statements from 871 articles extracted from a Pub Med search (1963–2008) and from a secondary database were lower than the grade III proposed by the Agency for Health Care Policy and Research in 1993. Therefore, we have developed "the Japanese Consensus Guidelines for AIP" according to the modified Delphi approach [13–15]. This method, which provides panelists with the opportunity to discuss their judgments between the ratings' rounds, is suitable for the development of consensus guideline statements.

To establish consensus guidelines, three committees (the professional committee for developing clinical questions and statements by Japanese specialists concerning AIP, the expert panelist committee for rating statements using the modified Delphi method, and the evaluating committee of moderators) were organized (Table 1). In brief, during the first phase, 15 specialists (11 pancreatologists, two radiologists, one expert of respiratory system, and one pathologist), who were selected from the members of the Research Committee for Intractable Pancreatic Diseases, supported by the Ministry of Health, Labor, and Welfare of Japan, developed 36 clinical questions (CQs) and statements for (1) the concept and diagnosis (13 CQS), (2) extra-pancreatic lesions (6 CQs), (3) the differential diagnosis (6 CQs), and (4) treatment (11 CQs) based on the selected papers as described above. In the second phase, the expert panelists (ten pancreatologists) individually rated these clinical statements for appropriateness, and discussed areas of disagreement and uncertainty. Ratings of appropriate methods for management of AIP were developed using a modified Delphi approach [13–15]. Rating was on a 9-point scale, with 1 being highly inappropriate and 9 being highly appropriate. A clinical statement receiving a median score greater than 7 from the panel was regarded as valid. In the third phase, the specialists revised some of the clinical statements after discussion with expert panelists. During the third phase, the revised clinical statements were rated again. Based on the two-round modified Delphi approach, guideline statements for diagnosis and management of AIP were developed. In addition to the specialist and expert panels, the moderators comprised one pancreatologist, one surgeon, one pathologist, and one internist who were also familiar with epidemiology and the modified Delphi approach. The moderators searched and reviewed the literature, collected clinical statements from the literature as well as from the professional group's survey, facilitated the panelist meetings, and analyzed the data obtained using the modified Delphi approach. Because available clinical evidence regarding diagnosis and management of AIP is limited, we could not set a suitable recommendation level for some clinical statements. In the present consensus-based guidelines, the statements for clinical practice receiving a score of 9 and less than 9 were evaluated as level A–D (Table 2).

The digest versions of the present guidelines have been published in the official journal of the Japan Pancreas Society, "*Pancreas*" [16]. Full versions divided into three series are scheduled to be published in the represent and the following two issues of the *Journal of Gastroenterology* with approval of Prof. VLW Go, the Editor-in-Chief of the "*Pancreas*."

Table 1 Committee members for developing consensus-based guidelines for AIP

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The expert panelist committee for rating statements by the modified Delphi method	Toru Shimosegawa, Tetsuhide Ito, Kazuo Inui, Hirotaka Ohara, Kazuichi Okazaki, Shigeyuki Kawa, Terumi Kamisawa, Shigeki Tanaka, Takayoshi Nishino, Isao Nishimori
The evaluating committee	Masao Tanaka, Keiko Shiratori, Koichi Suda, Toshimasa Nishiyama

Table 2 Consensus-based recommendation levels

Level A	Recommendation that procedure or treatment is useful or effective
Level B	Recommendation in favor of procedure or treatment being useful or effective
Level C	Recommendation's usefulness or efficacy less well established
Level D	Recommendation that procedure or treatment is not useful or effective, but may be harmful

Clinical questions and statements

I. Concept and diagnosis

CQ-I-1. What is "autoimmune pancreatitis" (AIP)?

- It is a unique form of pancreatitis that shows evidence of possible involvement of autoimmune mechanisms such as hypergammaglobulinemia, increased serum levels of IgG, increased serum levels of IgG4, or presence of autoantibodies, and effective response to steroid therapy.
- Autoimmune pancreatitis (AIP), as commonly observed in Japan, shows symptoms of lymphoplasmacytic sclerosing pancreatitis (LPSP) characterized by pronounced infiltration of lymphocytes and plasmacytes, infiltration of IgG4-positive plasmacytes, storiform fibrosis, and obliterative phlebitis.
- However, idiopathic duct-centric chronic pancreatitis (IDCP) or granulocyte epithelial lesions (GEL), commonly seen in Europe and the US, show neutrophilic lesions and therefore are different conditions than AIP.
- AIP may be a systemic disorder associated with pancreatic lesions, since the following disease concepts have also been proposed: IgG4-related sclerosing disorders, systemic IgG4-related plasmacytic syndrome (SIPS), or IgG4-positive multi-organ lymphoproliferative syndrome (IgG4-MOLPS).

Description Autoimmune pancreatitis is a disease concept originally proposed in Japan [1]. Because its characteristics are associated with evidence of possible involvement of autoimmune mechanisms such as hypergammaglobulinemia, increased serum levels of IgG, increased levels of IgG4 or presence of autoantibodies, and effective response to steroid therapy, the disease is defined as pancreatitis in which pathogenesis could possibly involve autoimmune mechanisms [1, 2, 8, 17, 18]. In Japan, it is commonly observed in elderly males and is comparable to lymphoplasmacytic sclerosing pancreatitis (LPSP), which is characterized by histopathological findings of abundant infiltration of lymphocytes and plasmacytes, infiltration of IgG4-positive plasmacytes, storiform fibrosis, and obstructive phlebitis [19]. Cases in young patients associated with ulcerative colitis, commonly reported in Europe and the US, show pathological neutrophilic lesions and are called idiopathic duct-centric chronic pancreatitis (IDCP) [20] or granulocyte epithelial lesions (GEL). Although their imaging findings show resemblance to those of AIP, there are not enough serological findings, so it is highly possible that their pathological conditions are different from AIP [21]. Since most cases in Japan show a diffusely enlarged pancreas and narrowing of the main pancreatic duct, it is believed that typical AIP lesions spread to over one-third of the pancreas; however, there are also cases of localized lesions or mass-forming types [8]. Upper abdominal discomfort, obstructive jaundice due to the stenosis of the biliary duct, and diabetes mellitus are the clinical features often observed [2]. Although the long-term prognosis of AIP is not clear, the formation of pancreatic stones has been reported. AIP is occasionally associated with lesions of organs other than the pancreas (sclerosing cholangitis, sclerosing sialadenitis, retroperitoneal fibrosis, enlarged celiac and hilar lymph nodes, chronic thyroiditis, interstitial nephritis, etc.), suggesting that it may be a systemic disorder. Therefore, the following concepts have been proposed: IgG4-related systemic sclerosing disease [5], systemic IgG4-related plasmacytic syndrome (SIPS) [6], and IgG4-positive multi-organ lymphoproliferative syndrome (IgG4-MOLPS) [7]. Because in most cases sialadenitis is found to be negative for both the anti-SSA antibody and anti-SSB antibody, which are distinctive to Sjögren's syndrome [2], and the histopathological images show pronounced infiltration of IgG4-positive plasmacytes seen in Mikulicz's disease and Kűttner's tumor, AIP is considered to be different from typical Sjögren's syndrome. Since sclerosing cholangitis-like lesions seen in patients with AIP show different responses to steroids and different prognosis from those with primary sclerosing cholangitis (PSC), and AIP is characterized by the infiltration of IgG4-producing plasmacytes, the two diseases are considered to be different pathological conditions.

CQ-I-2. Are there characteristic clinical symptoms of AIP?

- There are no specific symptoms seen in patients with AIP. However, in many cases, the patients show minor to no abdominal pain, obstructive jaundice, symptoms of diabetes mellitus, or accompanying extra-pancreatic lesions.

Description Patients with AIP do not show the type of severe abdominal pain seen in those with acute pancreatitis or with acute exacerbation of chronic pancreatitis; abdominal pain is mild to almost none, if it even exists [2, 22–25]. There have been a few cases reported where the disease started as acute pancreatitis or severe pancreatitis [26, 27]. One-third to one-half of the patients show obstructive jaundice or mild abdominal pain, and 15% have back pain or weight loss [22, 27] (Table 3). More than half of the cases are associated with sclerosing cholangitis, diabetes mellitus, sclerosing sialoadenitis/dacryoadenitis, or retroperitoneal fibrosis, showing, in some cases, obstructive jaundice, polydipsia/polyuria or malaise, xerostomia/xerophthalmia, or hydronephrosis, respectively [7].

Table 3 Clinical symptoms of AIP

Obstructive jaundice	33–59%
Abdominal pain	32%
Back pain	15%
Body weight loss	15%
Anorexia	9%
General fatigue	9%
Abnormal stool	7%
Fever	6%
No symptoms	15%

Modified from refs. [22, 24, 25, 28, 30]

CQ-I-3. How is AIP found?

- In many cases, patients go to see doctors with complaints such as minor abdominal pain, general malaise, jaundice, or dry mouth.
- In many cases, AIP is found when patients showing increased levels of biliary enzymes, obstructive jaundice, or diabetes mellitus are tested for pancreatic or biliary duct cancers in the differential diagnosis.
- In many cases, the enlarged pancreas demonstrated by abdominal ultrasonography leads to the detection of AIP.

Description In more than half of the cases, patients visit the hospital for symptoms such as minor abdominal pain, general malaise, jaundice, or dry mouth [1, 2, 6, 7, 22, 24, 26, 28]. A urine test or general blood biochemical test shows abnormal levels of pancreatic or biliary enzymes, or in some cases an increased level of CA19-9; pancreatic parenchymal imaging such as abdominal ultrasonography, CT, or MRI shows a diffusely or locally enlarged pancreas, or a pancreatic mass in some cases. In many cases the disease is found in the course of thedifferential diagnosis against pancreatic or biliary cancers [1, 2, 22–24, 28]. AIP is also found during the close examination of extra-pancreatic lesions; for example, during the differential diagnosis against primary sclerosing cholangitis (PSC); in examination in suspicion of Sjögren's syndrome by a head and neck otolaryngologist, ophthalmologist, or collagen disease-rheumatologist; or in examination for retroperitoneal fibrosis by a urologist. The rate of association with other autoimmune diseases is not clear; however, there have been reports, mainly in Europe and the US, of cases associated with juvenile ulcerative colitis showing evidence of idiopathic duct-centric chronic pancreatitis (IDCP) [20] or granulocyte epithelial lesion (GEL) [21]. Conversely, cases associated with ulcerative colitis or primary biliary cirrhosis are rarely seen in Japan [28].

CQ-I-4. What are the characteristic blood-biochemical or immunological findings in AIP?

- Although there are no disease-specific blood-biochemical findings, increased serum levels of pancreatic enzymes, biliary enzymes, and total bilirubin are commonly observed in AIP.
- Serum levels of IgG4 have the highest diagnostic value as a single serological diagnostic method among all the available ones; however, it is not disease specific.
- The combination of non-specific antibodies, such as serum IgG, antinuclear antibodies, or rheumatoid factor, shows sensitivity and specificity equivalent to IgG4.

Description Most AIP cases are discovered when patients show increased levels of biliary enzymes, obstructive jaundice, diabetes mellitus, etc., which are usually reflected in biochemical tests. Abnormal biliary findings are seen in many cases; 60–82% of cases exhibit an increase of biliary enzymes; 39–62% of cases exhibit an increase of total bilirubin, etc. [28–31]. Compared to cases of acute pancreatitis or acute exacerbation of chronic pancreatitis, the occurrence rate of abnormal levels of serum pancreatic enzymes is lower, 36–64% [28, 29], and the levels rarely become abnormally high. There have been reports of increased levels of peripheral eosinophil granulocytes [28] and activated T-lymphocytes (CD4-positive, CD8-positive) [29].

Immunological examinations show high incidences of hypergammaglobulinemia (43%), increased levels of serum IgG (62–80%), increased levels of serum IgG4 (68–92%) [2, 28, 31], antinuclear antibodies (40–64%), rheumatoid factor (25%), etc. [28, 29], although these are not disease-specific. Some reports have shown the presence of autoantibodies, such as anti-carbonic anhydrase II antibodies (55%) or anti-lactoferrin antibodies (75%), in patients with AIP in high frequency, although they generally cannot be tested [28, 29]. Anti-SSA/B antibodies or anti-mitochondrial antibodies, on the other hand, are rarely seen [28, 29]. Among all serological diagnostic methods, an increased level of serum IgG4 has the highest diagnostic value as a single method because of its sensitivity (80%) and its specificity (98%) in differentiating from pancreatic cancer; however, it is not disease specific. The sensitivity and specificity of serum IgG are 70 and 75%, respectively, and the positive ratios of antinuclear antibodies and rheumatoid factor are 60 and 20–30%, respectively. Even when IgG is combined with antinuclear antibodies or rheumatoid factor, the sensitivity is 91%, but the specificity is 61%; the specificity is lower than that for IgG4; however, the

sensitivity is equivalent to that for IgG [6, 31, 41] (refer to CQ-II-2-2).

CQ-I-5. Are there pancreatic exocrine and endocrine dysfunctions?

- Autoimmune pancreatitis is often associated with pancreatic exocrine dysfunction and endocrine dysfunctions (diabetes mellitus); occurrence ratios are about 80 and 70%, respectively.

Description Autoimmune pancreatitis is in many cases associated with pancreatic exocrine dysfunction and endocrine dysfunction (diabetes mellitus). According to the fact-finding survey conducted in 2000 by the Ministry of Health and Welfare Investigation Research Committee for Intractable Pancreas Disease, 80.6% of the cases studied showed abnormal pancreatic exocrine function [in which the abnormality is defined as 70% or lower secretion in the BT-PABA (PFD test)], and 70.0% of the cases showed exocrine dysfunction (as determined by the secretin test), comparable to that in confirmed cases of chronic pancreatitis. On the other hand, 77.0% of the cases were reported to be associated with diabetes mellitus [32]. Studies by individual medical facilities reported that 83–88% of the cases were associated with secretion dysfunction and 42–78% with diabetes mellitus [32–35]. The diabetes mellitus accompanying AIP was analyzed in detail in the national fact-finding survey conducted in 2006 [30, 32]. Among those AIP patients who sought medical attention during the 1-year period of 2002, 66.5% of cases were found to be associated with diabetes mellitus; of these patients, 33.3% had diabetes mellitus prior to the onset of AIP, and 51.6% started developing diabetes mellitus around the same time as the onset of pancreatitis. Among those patients having diabetes mellitus, 14% developed diabetes after steroid treatment [30, 32], suggesting that such diabetes may be caused by long-term steroid treatment. There are some cases where pancreatic endocrine dysfunction was improved by steroid treatment; however, since not all cases improved, it can be stated that medical conditions that have progressed far enough to cause some degree of organic change cannot be reversed (refer to CQ-IV-9).

In AIP, the mechanism of pathogenesis of pancreatic exocrine dysfunction is assumed to involve the following: decreased secretion of pancreatic enzymes associated with collapsed ancinar cells caused by pronounced cellular infiltration mainly of plasmacytes and fibrosis, and obstructed flow of pancreatic juice due to inflammatory cell infiltration around the pancreatic ducts and subsequent narrowing of the pancreatic ducts [34–37]. In contrast, the mechanism of pathogenesis of diabetes mellitus is assumed to be affected by both of the following disorders [35, 37]: obstructed blood flow of endocrine glands (islets of Langerhans) associated with the fibrosis of exocrine glands and damaged islets of Langerhans due to the spreading of inflammation [2, 38]. Future discussions, however, are necessary [37].

CQ-I-6. What are the characteristic findings of abdominal ultrasonography in AIP?

- Abdominal ultrasonography is effective for the diagnosis of AIP (level of recommendation: A).
- Ultrasonographic findings in patients with AIP are characterized by a diffusely or locally enlarged pancreas with low echo; a diffusely enlarged pancreas is called a "sausage-like" pancreas (level of recommendation: A).

Description The Clinical Diagnostic Criteria for Autoimmune Pancreatitis 2006 [17, 39] defines that a "diffusely or locally enlarged pancreas is detected by abdominal US, X-ray, or MRI." Ultrasonography is the initial clinical examination serving as the tool to diagnose AIP. In some cases, patients are found to have AIP during their physical examinations [40].

A diffusely enlarged pancreas appears as a low-echo area in general (Fig. 1) and has a so-called "sausage-like" appearance [41]. No dilatation of the main pancreatic duct is seen in most cases. The enlarged area shows a low echo image, in some cases with scattered high echo spots [42]. In the case of a locally enlarged pancreas, it becomes an issue to distinguish it from pancreatic cancer or mass-forming pancreatitis with the differential diagnosis. Although dilatation of the main pancreatic duct is not seen in most cases, some patients may show minor dilation, which makes the differential diagnosis difficult. Conversely, if the main duct is found to penetrate through the mass (Fig. 2), it (the duct-penetrating sign) may be a useful sign that can be used for the differential diagnosis against pancreatic cancer [43, 44]. In some cases, there may be many low echo mass images in the pancreatic parenchyma (Fig. 3), which makes it difficult to differentiate AIP from malignant lymphoma or metastatic pancreatic tumors.

Some patients with AIP show thickened bile duct walls; the occurrence rate has been reported to be about 60% [45]. A thickened bile duct wall is characterized by layered or parenchymal low-echo wall thickening [46]. There have been some cases where the thick wall centering around the extrahepatic bile duct extends over to the intrahepatic bile duct or gallbladder [45–47]. The wall thickening has been studied in detail with intraductal ultrasonography (IDUS) [48]. Although wall thickening of narrowed areas is not clear, since areas other than the narrowed area show thickening of the internal low echo layer while maintaining the high echo image for the outer, it is assumed that the thickening is happening on the bile duct wall itself [49].