Student's t-test. In all tests, corrected P values of < 0.05 were considered statistically significant.

Results

Clinical manifestations

After 6 months' treatment, oral prednisolone had been administered to 19 patients in both groups [pulse group: 9 of 11 vs. oral group: 10 of 10; difference not significant (N.S.)], and the median dosage of prednisolone in each group was 10 mg/day (pulse group: 2.5–12.5 vs. oral group: 5–12.5; N.S.). Two patients in the pulse group dropped out of the maintenance therapy. Neither group showed severe or lethal complications.

In both groups, extrapancreatic lesions other than the bile duct lesions were observed in 10 patients (pulse group: 5 of 11 vs. oral group: 5 of 10; N.S.) (Tables 1, 2). No exacerbation of the extrapancreatic lesions was found following either of the treatments (data not shown). Laboratory findings including immunoglobulin, autoantibody, and exocrine function at the treatment start are listed in Tables 1 and 2.

Immunoglobulin

At the beginning of treatment, in both groups, abnormal serum immunoglobulin-G4 (IgG4) values were observed in all patients (Tables 1, 2), and abnormal serum immunoglobulin-G (IgG) values were observed in 13 patients (pulse group: 6 of 11 vs. oral group: 7 of 10; N.S.) (Tables 1, 2). Normalization of the IgG value was shown in all these patients within 6 months (data not shown).

Liver function

At the beginning of treatment, abnormal γ-GTP values were revealed in both groups, in a total of 18 patients (pulse group: 11 of 11 vs. oral group: 7 of 10; N.S.). In the pulse group, the median γ-GTP levels fell, from 222 IU/L (range 65–1,352) at the beginning of treatment to 92 IU/L (22–679) ($P < 0.01$) after 2 weeks of pulse therapy (Fig. 1a), and to 36 IU/L (19–556) ($P < 0.01$) after 8 weeks of pulse therapy (Fig. 1a). In the oral group, however, the median γ-GTP fell insignificantly after 2 weeks of prednisolone treatment, from 149 IU/L (range 67–380) at the beginning of treatment to 125 IU/L (63–274) ($P = 0.083$) (Fig. 1b), although the level fell significantly to 72 IU/L (29–114) ($P = 0.027$) after 8 weeks of prednisolone treatment (Fig. 1b). When limiting results to the patients who showed diffuse pancreatic swelling, γ-GTP was significantly improved in the pulse group after 2 weeks ($P = 0.043$) and 8 weeks ($P = 0.043$) of pulse therapy (Fig. 1c), whereas the improvement in the γ-GTP level was insignificant in the oral group after 2 weeks ($P = 0.27$) and 8 weeks ($P = 0.27$) of prednisolone treatment (Fig. 1d).

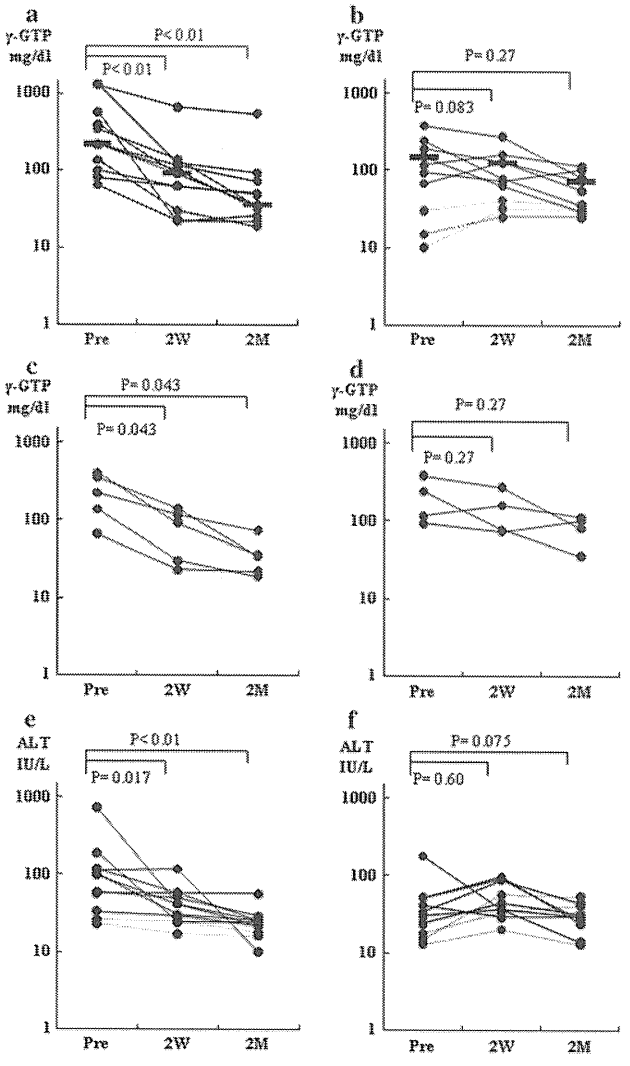

Fig. 1 γ-Guanosine triphosphate (*γ-GTP*) and alanine aminotransferase (*ALT*) changes after steroids. The serum levels of γ-GTP after steroid pulse therapy (**a**), after oral steroid therapy (**b**), those of ALT after steroid pulse therapy (**e**) and those after oral steroid therapy (**f**) were monitored on day 0 (*Pre*), and 2 weeks (*W*) and 8 weeks after therapy. To evaluate the therapeutic effect strictly, the patients (*dotted lines*) who did not show an abnormal value during the clinical course were excluded from this analysis. The serum levels of γ-GTP after 2 weeks on steroids and those of ALT after 2 and 8 weeks on steroids were significantly improved in the pulse group, compared with the oral group. When limiting the patients to those who showed diffuse pancreatic swelling, the serum level of γ-GTP was significantly improved in the pulse group after 2 and 8 weeks of pulse therapy (**c**), compared with the oral group (**d**). *M* Months

At the beginning of treatment, abnormal ALT values were revealed in both groups, in a total of 16 patients

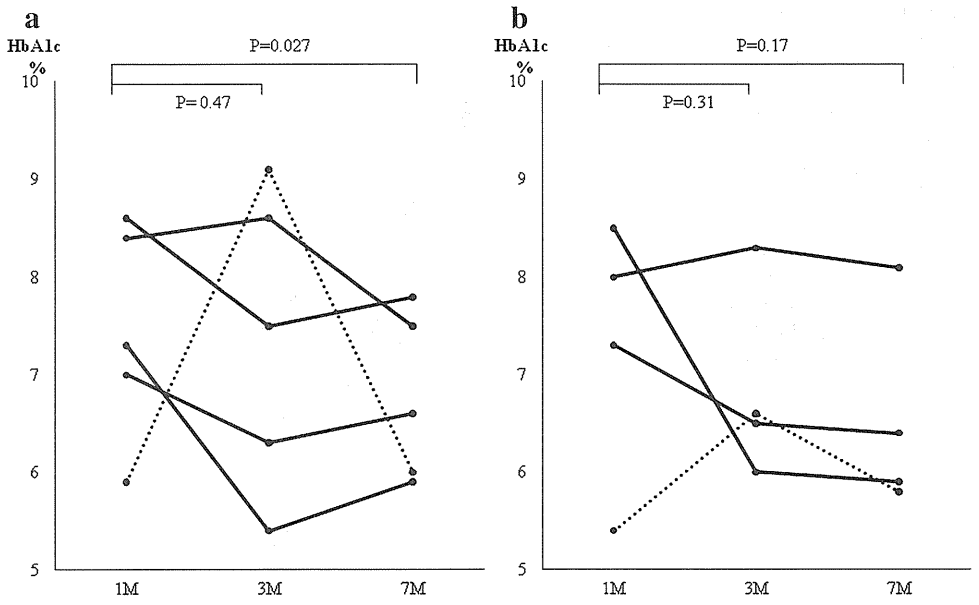

Fig. 2 Glycosylated hemoglobin (*HbA1C*) changes after steroid therapy. The levels of HbA1c after steroid pulse therapy (**a**) and oral steroid therapy (**b**) were monitored at months 1, 3, and 7 after therapy, which closely reflect glucose tolerance at months 0, 2, and 6, respectively. To evaluate the therapeutic effect strictly, patients who did not show abnormal values during the clinical course were excluded from this analysis. *Dotted lines* represent the patients who developed glucose intolerance after 2 months of steroid therapy. The level of HbA1c at month 7 on steroids tended to be improved in the pulse group, compared with the oral group

(pulse group: 10 of 11 vs. oral group: 6 of 10; N.S.). In the pulse group, the median ALT level fell from 100 IU/L (range 33–721) at the beginning of treatment to 41 IU/L (24–117) ($P = 0.017$) after 2 weeks of pulse therapy (Fig. 1e), and to 25 IU/L (10–57) ($P < 0.01$) after 8 weeks of pulse therapy (Fig. 1e). In the oral group, however, the median ALT rose transiently from 46.5 IU/L (range 30–178) at the beginning of treatment to 62 IU/L (29–96) ($P = 0.60$) after 2 weeks of prednisolone (Fig. 1f), but improved to 28 IU/L (14–44) ($P = 0.075$) after 8 weeks of prednisolone treatment (Fig. 1f).

Endocrine function

Before steroid treatment, diabetes mellitus was seen in 7 patients (pulse group: 4 of 11 vs. oral group: 3 of 10; N.S.) (Tables 1, 2). One patient in each group developed glucose intolerance after 2 months of steroid therapy. Including these patients, all the patients with impaired glucose tolerance were treated with dietary measures and received medical therapy while the steroid therapy was maintained. Neither group showed significant improvement in glucose tolerance after 2 months, but at 6 months, the pulse group had improved significantly ($P = 0.027$), whereas the oral group had not ($P = 0.17$) (Fig. 2).

Pancreas size

Both groups evidenced pancreatic swelling: diffuse pancreatic swelling was observed in 10 patients (pulse group: 5 of 11 vs. oral group: 5 of 10; N.S.) and focal swelling was observed in 11 patients (pulse group: 6 of 11 vs. oral group: 5 of 10; N.S.) (Tables 1, 2). The change in pancreas size

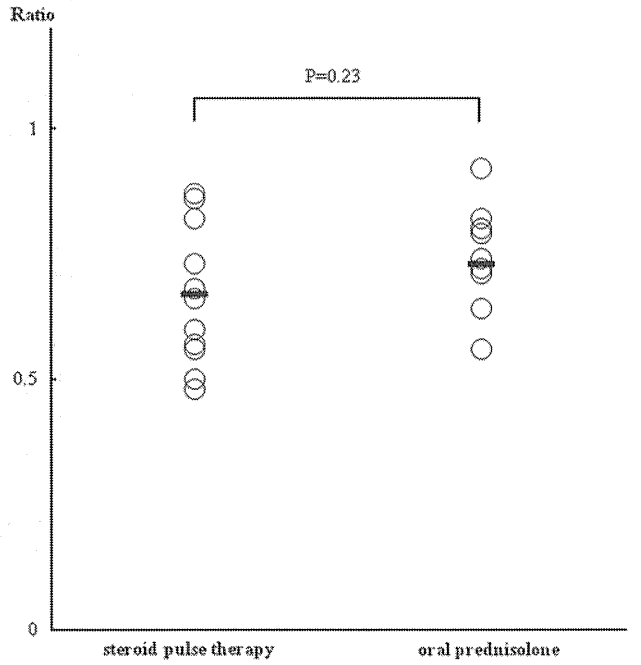

Fig. 3 Pancreas morphology changes after 2 weeks on steroids. Morphological changes of the pancreas after 2 weeks of steroid pulse therapy (*left*) and oral steroid therapy (*right*) were scored. The width of the pancreas along its longest axis was measured on computed tomography (CT) or magnetic resonance imaging (MRI) and compared with the transverse diameter of the vertebral body, referred to in the method of Heuck et al. [29]. The pancreas size on the first image was defined as 100%, and the ratio after 2 weeks with each treatment was measured in the same manner. The *black bars* represent the mean in each group. The two groups showed no significant difference in morphological change of the pancreas after 2 weeks

after 2 weeks showed no significant difference between the groups after treatment (pulse group: 67% vs. oral group: 73.4%, $P = 0.23$) (Fig. 3).

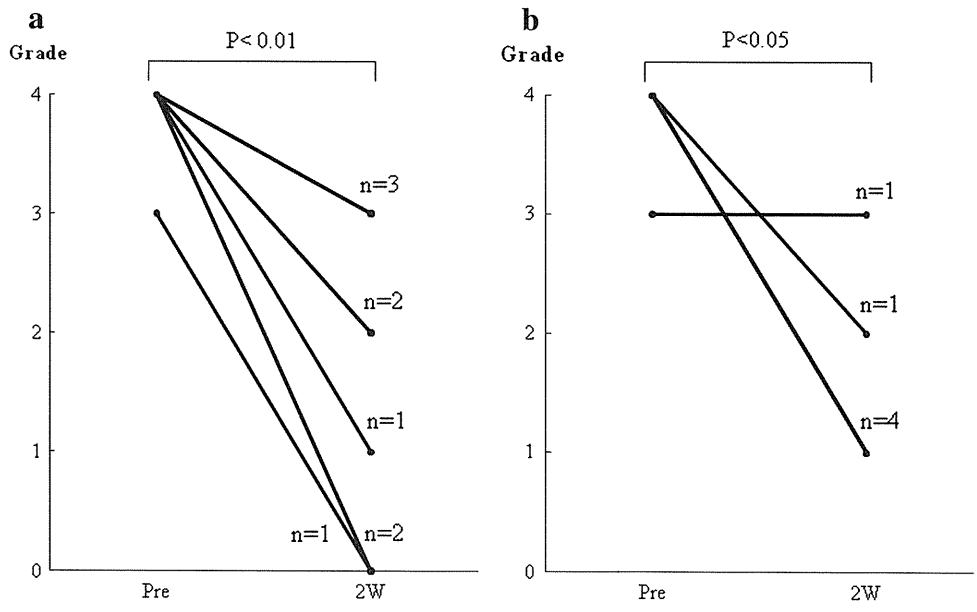

Fig. 4 Lower bile duct stricture changes after steroids. The changes in lower bile duct stricture in the pulse group (**a**) and the oral group (**b**) on day 0 and 2 weeks after therapy were scored as follows: 0 = absent, 1 = <0–25%, 2 = <25–50%, 3 = <50–75%, 4 = <75–100%, referred to in the method of Craig et al. [30]. Bile duct stenosis in the distal third part was significantly improved after 2 weeks in each group

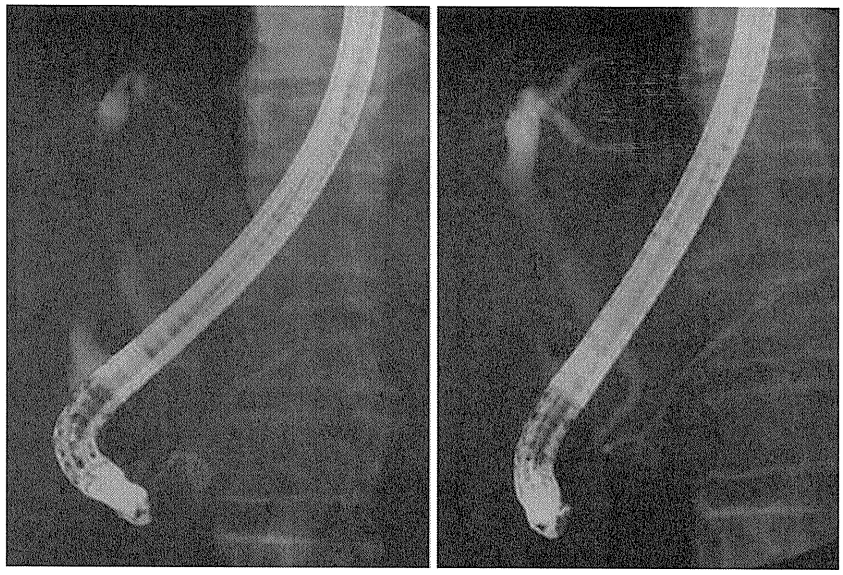

Fig. 5 Endoscopic retrograde cholangiopancreatography (ERCP) images of the impact of steroid pulse therapy on refractory autoimmune pancreatitis (AIP). Although oral prednisolone was commenced, it had had no effect on the biliary stenosis (*left*). Two courses of steroid pulse therapy ameliorated the stenosis dramatically (*right*) (Case 10; reference [31])

Bile duct lesion

After 2 weeks on steroids, significant improvement of lower bile duct stricture was shown in both groups (pulse group: $P < 0.01$, and oral group: $P < 0.05$) (Fig. 4). However, there was one patient (case 10) whose lower bile duct stricture did not improve following oral prednisone treatment, but showed definite improvement with steroid pulse therapy [31] (Fig. 5).

Discussion

Several cases have been reported of pancreatic cancer or bile duct cancer concurrent with AIP [16–19]; because the image findings of AIP often mimic pancreatobiliary malignancies, it is extremely crucial to distinguish AIP from cancers, although this can be difficult [12–15].

In the Asian diagnostic criteria proposed by the Japan-Korea symposium on autoimmune pancreatitis [11], the use of steroids as diagnostic treatment was allowed only when the imaging findings were compatible with AIP and only after there was a negative result for malignancy work-up. Cases of diagnostic treatment using steroids will doubtless increase in future. The usefulness of a 2-week conventional oral steroid diagnostic treatment was also proposed by Moon et al. [20]. However, oral steroid therapy requires a long period for drug tapering, because any patient who has received a glucocorticoid in doses equivalent to at least 20 mg a day of prednisone for >5 days is at risk of

secondary adrenal insufficiency due to hypothalamic–pituitary–adrenal suppression [21–23]. Diagnostic treatment with an oral steroid may cause an undesirable effect when surgical resection is required. Although one article reported that azathioprine was used for refractory AIP [32], it is causative of acute pancreatitis [33]. Therefore, a safe and simple alternative to oral steroid treatment is needed for refractory AIP [34].

Steroid pulse therapy is a well known alternative to oral steroid treatment for autoimmune disorders; it requires no drug tapering [28], and we have already reported cases where steroid pulse therapy was effective for AIP [35], although comparative studies of conventional oral steroid therapy and steroid pulse therapy have not been reported. Here we report that steroid pulse therapy is an effective alternative to oral steroid for the initial treatment of AIP.

The efficacy of oral steroid therapy for AIP is well known, and the improvement of AIP in patients treated with steroids for 2 weeks can be shown in radiographic findings [5, 20, 36]. Our data on pancreas size after 2 weeks did not show a significant difference between oral steroid and pulse therapy. Both therapies were effective for alleviating lower bile duct stricture in the short term and for resolving abnormal IgG values. However, the short-term change in γ-GTP showed significant improvement in the steroid pulse therapy group, but not in the oral steroid group. In one patient, lower bile duct stricture was improved by steroid pulse therapy, although it had not been improved by oral steroids. These findings suggest that steroid pulse therapy may prevent patients from having unnecessary major operations for benign bile duct lesions which do not respond to oral steroid treatment.

Steroid therapy is reported to be effective in approximately half of AIP patients with accompanying diabetes mellitus [37, 38]. The mechanism of steroid action in the recovery of endocrine function in patients with AIP is unclear, but Tanaka et al. [39] have suggested that steroids can suppress the release of cytokines produced by inflammatory cells, and enable islet regeneration and eventual restoration of insulin secretion. However, we have reported that conventional oral steroids did not improve diabetes mellitus in the long term [40]. Although the accumulation of a larger numbers of cases is needed, our study of glycosylated hemoglobin values showed that steroid pulse therapy tended to be more effective than oral steroids for improving glucose tolerance after 6 months of treatment. In our protocol, there was a decisive difference between the two therapies just for the initial 2 weeks, because 20 mg/day of oral prednisolone was prescribed as maintenance therapy after the 2 weeks of steroid pulse therapy. We presume that the steroid pulse therapy was better than the oral steroid therapy for improving glucose tolerance because of its stronger cytokine suppression.

In summary, steroid pulse therapy is an effective alternative initial treatment for AIP, and surpasses conventional oral steroid therapy in the improvement of bile duct lesions. When a differential diagnosis between AIP and pancreatic cancer is difficult clinically or when bile duct lesions do not respond to oral steroid treatment, we recommend steroid pulse therapy as an alternative to oral steroid therapy. In future, the accumulation of a larger number of patients receiving steroid pulse therapy is needed, and prospective studies will be required.

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Recent Concepts of Autoimmune Pancreatitis and IgG4-Related Disease

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Abstract Recent studies suggested the existence of two subtypes of autoimmune pancreatitis (AIP): type 1 related with IgG4 (lymphoplasmacytic sclerosing pancreatitis; LPSP) and type 2 related with a granulocytic epithelial lesion (idiopathic duct-centric chronic pancreatitis; IDCP). Apart from type 2 AIP, the pathological features of type 1 AIP with increased serum IgG4/IgE levels, abundant infiltration of IgG4+ plasmacytes and lymphocytes, fibrosis, and steroid responsiveness are suggestive of abnormal immunity such as allergy or autoimmunity. Moreover, the patients with type 1 AIP often have extrapancreatic lesions such as sclerosing cholangitis, sclerosing sialadenitis, or retroperitoneal fibrosis showing similar pathological features. Based on these findings, many synonyms have been proposed for these conditions, such as "multifocal idiopathic fibrosclerosis", "IgG4-related autoimmune disease", "IgG4-related sclerosing disease", "IgG4-related plasmacytic disease", and "IgG4-related multiorgan lymphoproliferative syndrome", all of which may refer to the same conditions. Therefore, the Japanese Research Committee for "Systemic IgG4-related Sclerosing Disease" proposed a disease concept and clinical diagnostic criteria based on the concept of multifocal fibrosclerosis in 2009, in which the term "IgG4-related disease" was appointed as a minimal consensus on these conditions. Although the significance of IgG4 in the development of "IgG4-related disease" remains unclear, we have proposed a hypothesis for the development of type 1 AIP, one of the IgG4-related disease. The concept and diagnostic criteria of "IgG4-related disease" will be changed in accordance with future studies.

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Keywords IgG4 · IgG4-related disease · Autoimmune pancreatitis · Mikulicz disease · Regulatory T cell (Treg)

Abbreviations

AIP	Autoimmune pancreatitis
ANA	Anti-nuclear antibody
CA-II	Carbonic anhydrase-II
CTLA-4	Cytotoxic T lymphocyte antigen-4
ERCP	Endoscopic retrograde cholangiopancreatography
FCRL	Fc-receptor-like
IFN-γ	Interferon-γ
IL-4	Interleukin-4
LF	Lactoferrin
LPSP	Lymphoplasmacytic sclerosing pancreatitis
MD	Mikulicz disease
MHC	Major histocompatibility complex
MOLPS	Multiorgan lymphoproliferative disease
PBP	Plasminogen-binding protein
SjS	Sjögren's syndrome
PSC	Primary sclerosing cholangitis
RF	Rheumatoid factor
SIPS	IgG4-systemic plasmacytic syndrome
SLE	Systemic lupus erythematosus
Treg	Regulatory T cell
UBR2	Ubiquitin-protein ligase E3 component n-recognin 2

Introduction

In 1961, Sarles et al. first observed a case of particular pancreatitis with hypergammaglobulinemia [1]. Yoshida et al. first proposed the concept of autoimmune pancreatitis (AIP) [2]. Hamano et al. reported the increased serum levels of IgG4 in Japanese patients with AIP [3]. Thereafter, many studies of AIP have been reported, mainly by

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Japanese investigators. The histopathological findings of AIP are characterized by the periductal localization of predominantly CD4-positive T cells, IgG4-positive plasma cells, storiform fibrosis with acinar cell atrophy frequently resulting in stenosis of the main pancreatic duct, and obliterative fibrosis [4–6], which is also called lymphoplasmacytic sclerosing pancreatitis (LPSP) [7]. In 2003, Kamisawa et al. [8] suggested that AIP is a systemic sclerosing disease based on the findings that the pancreas and other involved organs have fibrosis with abundant infiltration of IgG4-positive plasma cells, which is similar to the concept of multifocal fibrosclerosis proposed by Comings et al. [9]. Further, histological and clinical profiling of patients with "AIP" reveals two distinct subtypes, type 1 and type 2 AIP [10]. Type 1 AIP is classified as a pancreatic manifestation of IgG4-related disease, probably a systemic disease with an autoimmune process, whereas type 2 AIP is supposed to be a specific pancreatic disease with occasional coexistence with ulcerative colitis.

On the other hand, patients with Mikulicz's disease (MD), classified as an atypical type of Sjögren's syndrome, who usually have bilateral, painless, and symmetrical swelling of the lachrymal, parotid, and submandibular glands [11], show elevated serum levels of IgG4, infiltration of IgG4-positive plasma cells into the glands, and recovery of secretion with steroid treatment. Similar to AIP, these patients often show other systemic organ involvement such as AIP, sclerosing cholangitis, retroperitoneal fibrosis, enlarged celiac and hilar lymph nodes, chronic thyroiditis, interstitial nephritis, and so on [4–6, 12]. Recently, MD has been considered to be completely different from Sjögren's syndrome because of lacking anti-SS-A/Ro or anti-SS-B/La antibodies and showing steroid responsiveness [2–6]. The steroid responses and the prognoses of AIP patients with sclerosing cholangitis differ from patients with primary sclerosing cholangitis (PSC), which suggests different pathological conditions. These findings led us to the concept of "IgG4-related disease" such as IgG4-related systemic sclerosing disease [8, 13], systemic IgG4-related plasmacytic syndrome (SIPS) [14], or IgG4-positive multiorgan lymphoproliferative syndrome (IgG4-MOLPS) [15]. Although pathogenesis or pathophysiology remains unclear, we will discuss the most recent advances in the concept of AIP and a novel concept of "IgG4-related disease."

Recent Concepts of AIP: Subtypes

Recent studies have revealed that "AIP" manifests two distinct subtypes, type 1 and type 2 AIP [10] (Table 1). In type 1 AIP, whose histologic description is called LPSP, the pancreatic histopathology shows the following characteristic features: (a) abundant infiltration of plasma cells (IgG4+ cells; >10/hpf, 40% > IgG4/IgG cells) and lymphocytes, (b) peculiar storiform or swirling fibrosis, and (c) perivenular infiltration with lymphocytes and plasma cells often leading to obliterative phlebitis. Clinically, type 1 AIP seems to be the pancreatic manifestation of the recently proposed IgG4-related disease, characterized by swelling of the pancreas, elevated serum IgG4 levels, and extrapancreatic

Table 1 Subtypes of autoimmune pancreatitis

Subtype of AIP	Type 1	Type 2
Other nomenclatures	AIP without GEL	AIP with GEL
	IgG4-related	IgG4-unrelated
	LPSP	IDCP
Prevalence	Asia>USA, EU	EU>USA>Asia
Age	High aged	Younger
Gender	Male ≫ female	Male=female (NS)
Symptoms	Obstructive jaundice, rare abdominal pain	Often obstructive jaundice abdominal pain like acute pancreatitis
Pancreas images	Swelling (diffuse/segmental/focal)/mass forming	Swelling (diffuse/segmental/focal)/mass forming
Serology	High serum IgG, IgG4, autoAbs (+)	Normal IgG, normal IgG4, autoAbs (−)
OOI	Sclerosing cholangitis	Unrelated with OOI
	Sclerosing sialadenitis	
	Reteroperitoneal fibrosis	
	Others	
Ulcerative colitis	Rare	Often
Steroid	Responsive	Responsive
Relapse	High rate	Rare

OOI other organ involvement

lesions (e.g., sclerosing cholangitis, sclerosing sialadenitis, and retroperitoneal fibrosis) associated with infiltration of abundant IgG4+ plasma cells (Figs. 1 and 2). Patients with type 1 AIP often have obstructive jaundice in elderly males, and the pancreatic and extrapancreatic manifestations respond to steroid therapy.

On the other hand, type 2 AIP was proposed from histological examination of the resected pancreata of patients with chronic non-alcoholic pancreatitis by American and European pathologists, who reported another histopathological pattern named as idiopathic duct-centric pancreatitis (IDCP) or AIP with granulocytic epithelial lesion (GEL) [16, 17]. The most characteristic feature of type 2 AIP is the GEL often with destruction and obliteration of the pancreatic duct (Fig. 3). Type 2 AIP has swelling of the pancreas, but none or very few IgG4-positive plasma cells, and clinical features show a distinctly different profile associated with no serum IgG4, IgG elevation, presence of autoantibodies, or other organ involvement except for inflammatory bowel disease (approximately 30%). Although it is still in debate as to whether type 2 AIP should be classified as an autoimmune disease or not, the nomenclature of the two subtypes is generally accepted in the meeting of the International Association of Pancreatology held at Fukuoka in 2010.

Extrapancreatic Lesions

A variety of extrapancreatic lesions in patients with AIP have been noted, including lachrymal and salivary gland lesions [18], pulmonary lesions including hilar lymphadenopathy [19], sclerosing cholangitis [20, 21], retroperitoneal fibrosis [22], and tubulointerstitial nephritis [23, 24]. Associations were also reported with hypophysitis [25], chronic thyroiditis [26], and prostatitis [27]. Other extrapancreatic involvements have been reported in a few cases [28–30]. Though it is not certain that all of them have a relation with AIP, extrapancreatic lesions are prevalent in the systemic organs (Table 2) [24–36], suggesting that type 1 AIP, but not type 2 AIP, may be a pancreatic manifestation of IgG4-related disease. The extrapancreatic lesions appear synchronously or metachronously with the pancreatic lesion(s), share the same pathological conditions, and show favorable response to steroid therapy; these characteristics suggest a common pathophysiological background. The lesions are usually detected by imaging and blood tests (CT, MRI, gallium scintigraphy, FDG-PET, and IgG4); however, these should be confirmed by histological findings. Extrapancreatic lesions sometimes mimic, or are misdiagnosed as, primary lesions of the corresponding organs: lachrymal and salivary gland lesions for Sjögren's syndrome, respiratory lesions for sarcoidosis, and sclerosing cholangitis for PSC. Therefore, it is necessary to differentiate between IgG4-related diseases and inherent diseases of the corresponding organs. The patients with IgG4-related sialodacryoadenitis, synonymous with IgG4-related Mikulicz's disease [11, 36], have usually symmetrical enlargement of salivary and lacrimal glands. The IgG4-related central nervous system lesions include infundibulohypophysitis, hypertrophic pachymeningitis, intracranial inflammatory pseudotumor, and orbital pseudotumor [18–36].

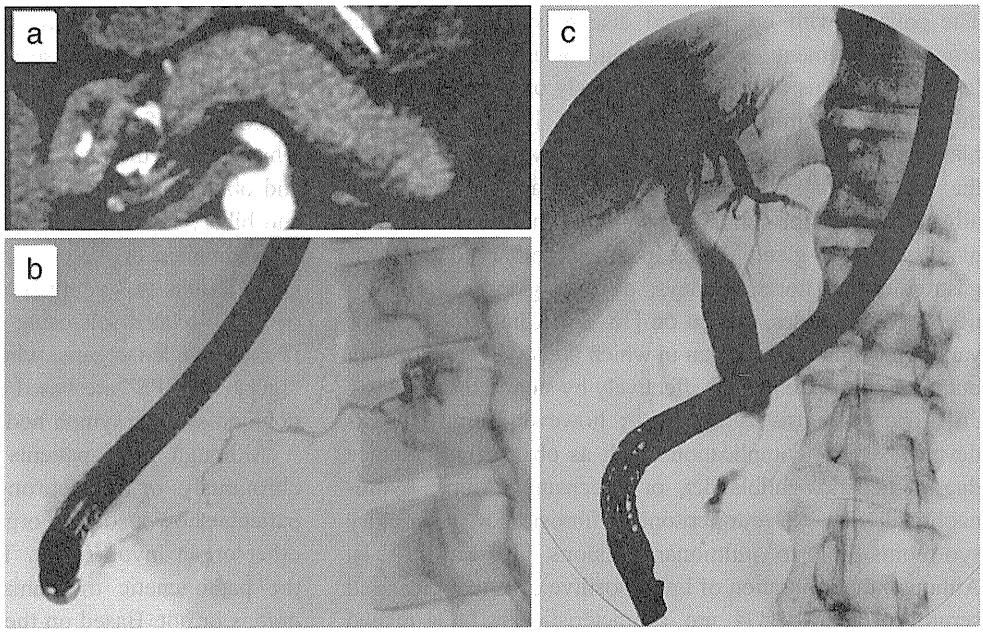

Fig. 1 Pancreas images of type 1 AIP. **a** Swelling of the pancreas with hepatic phase enhancement and low-density capsule like rim. **b** Pancreatogram shows diffusely irregular narrowing of the main pancreatic duct. **c** Cholangiogram shows stenosis of the biliary duct

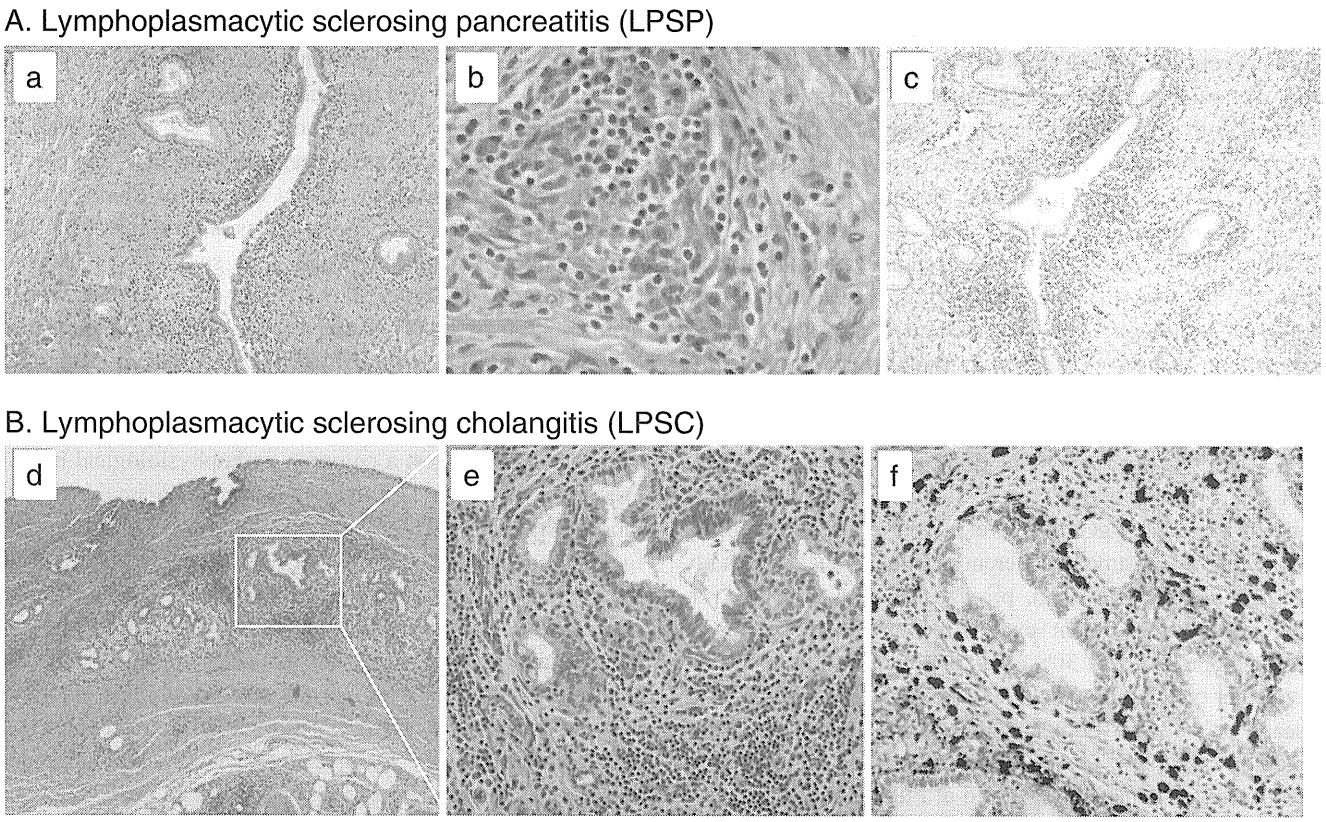

Fig. 2 Histopathology of type 1 AIP. **a** Lymphoplasmacytic sclerosing pancreatitis (*LPSP*). Histopathological findings show abundant infiltration of plasmacytes and lymphocytes mainly around the pancreatic duct and fibrosis (**a**) and obliterative phlebitis (**b**). Immunohistological findings show abundant infiltration of IgG4-positive plasma cells (**c**). **b** Lymphoplasmacytic sclerosing cholangitis (*LPSC*). Histopathological findings show abundant infiltration of infiltration of plasmacytes and lymphocytes and fibrosis (**a**, **b**). Immunohistological findings show abundant infiltration of IgG4-positive plasma cells (**c**)

The Concept of IgG4-Related Disease and Proposal of the Clinical Diagnostic Criteria

The patients with IgG4-related disease show diffuse/focal organ enlargement, mass forming, or nodular/thickened lesions in various organs, synchronously or metachronously, due to the prominent infiltration of lymphocytes and plasmacytes with fibrosis [18–36]; however, the causes of the disease are still not clear. The organs known to be affected include the central nervous system, lacrimal/salivary glands, thyroid gland, lungs, pancreas, biliary duct, liver, gastrointestinal tracts, kidneys, prostate gland, retroperitoneum, lymph nodes, and so on [18–36]. Clinical symptoms vary depending on the organ in which the lesions are located, but many cases are treated effectively by steroid therapy [18–36]. The prognosis is not clear; however, some patients develop serious complications such as obstructive jaundice due to hepatic, gallbladder, or pancreatic lesions, hydronephrosis due to retroperitoneal fibrosis, or respiratory symptoms due to pulmonary lesions [13–16, 21–24]. Although the infiltration of IgG4-positive cells and increased serum levels of IgG4 are characteristic in IgG4-related disease, the severity of fibrosis seems to be different among the individual involved organs. These conditions are quite similar to multifocal idiopathic fibrosclerosis (MIF) [9].

In addition to MIF, there are many synonyms, such as IgG4-related autoimmune disease [8], "IgG4-related sclerosing disease" [13], SIPS [14], and "IgG4+MOLPS" [15], all of which may refer to the same conditions. It has been debated which term is the most appropriate or not. Storiform fibrosis and obliterative phlebitis are characteristic in the pancreatic and biliary tract lesions, but the degree varies depending on the individual organs, e.g. very seldom in lacrimal/salivary gland lesions or lymph node lesions. Then, the nomenclature of "IgG4-related sclerosing disease" is mainly based on the fibrous swollen organs, whereas those of "IgG4-SIPS" and "IgG4+MOLPS" are based on lymphoplasmacytic proliferation and swollen lymph nodes without fibrosis.

Although most patients have multiorgan lesions synchronously or metachronously, about 10–20% of the patients show a solitary organ involved without confirming other organ involvement. Therefore, it is unclear whether the pathogenetic mechanism is same among individual organs or not. Based on these findings, the members of the

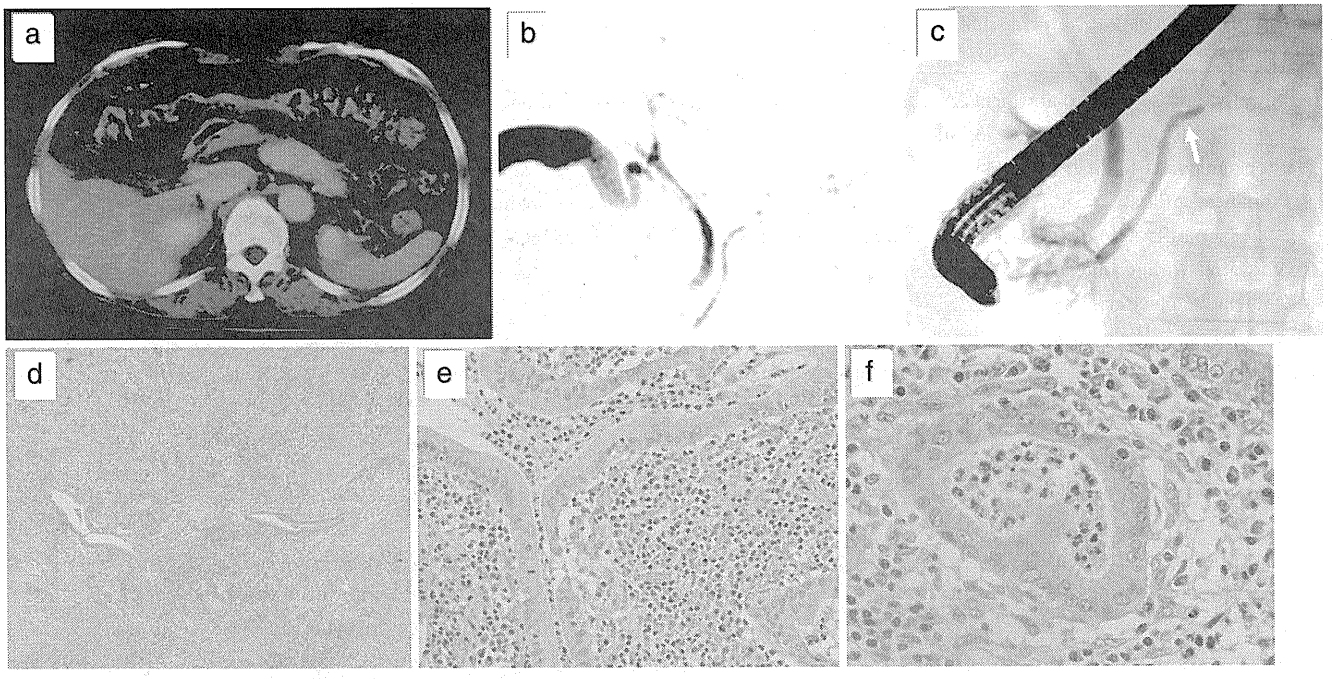

Fig. 3 Pancreas images and histopathology of type 2 AIP CT shows swelling of the pancreas (**a**). MRCP (**b**) and ERCP (**c**) show obstruction of the main pancreatic duct. Histopathological findings show fibrosis, abundant infiltration with granulocytes (**d–f**) cited from ref. [88]

Japanese Research Committees for "systemic IgG4-related sclerosing disease" (chaired by Prof. Okazaki K) [35] and "IgG4-MOLPS" (chaired by Prof. Umehara H) [36], both of which were supported by the "Research for Intractable Disease Program from the Ministry of Health, Labor and Welfare of Japan", have agreed that the term "IgG4-related disease" is appointed as minimally accepting these conditions at this moment. To study these conditions, the Japanese Research Committee for "systemic IgG4-related sclerosing disease" (chaired by Prof. Okazaki K) proposed a disease concept and clinical diagnostic criteria of "systemic IgG4-related sclerosing disease" in 2009 (Table 3) [35]. However, the concept and diagnostic criteria should be changed in accordance with the findings of the future studies.

Pathogenesis and Pathophysiology of AIP and "IgG4-Related Disease"

The pathogenesis and pathophysiology of AIP have been studied mainly from immunological approaches and focused mainly on IgG4-related type 1 AIP because few evidences of abnormal immunity have been reported in type 2 AIP.

Immunogenetic Factors

Immunogenetic factors have been present in a few series of AIP, but not conclusive. Susceptibility to AIP may be

Table 2 Extrapancreatic lesions complicated with autoimmune pancreatitis (from [31])

Close association
Lachrymal gland inflammation
Sialoadenitis
Hilar lymphadenopathy
Interstitial pneumonitis
Sclerosing cholangitis
Retroperitoneal fibrosis
Tubulointerstitial nephritis
Possible association
Hypophysitis
Autoimmune neurosensory hearing loss
Uveitis
Chronic thyroiditis
Pseudotumor (breast, lung, liver)
Gastric ulcer
Swelling of papilla of Vater
IgG4 hepatopathy
Aortitis
Prostatitis
Schonlein–Henoch purpura
Autoimmune thrombocytopenia

Table 3 Clinical diagnostic criteria 2009 for "IgG4-Related Disease" (proposed by the Japanese Research Committee for "Systemic IgG4-related Sclerosing Disease"; [35])

1. Clinically, diffuse/focal enlargement, or mass forming, nodular/thickened lesions in one or more organs
2. Elevated levels of serum IgG4 (>135 mg/dl)
3. Histopathological findings
① Prominent infiltration and fibrosis of lymphocytes and plasmacytes, but no neutrophilic infiltration
② Abundant infiltration of IgG4-positive plasmacytes (>10/hpf) and/or the ratio of IgG4/IgG-positive cells (>40%)
③ Storiform/swirling fibrosis
④ Obliterative phlebitis
Diagnosis of IgG4-related disease: 1+2, 1+3 ①②, 2+3 ①②, or 3 ①②③④
The following cases must be excluded from the diagnosis: malignant tumors developed in organs (e.g., cancers, malignant lymphomas) or similar diseases (e.g., Sjögren's syndrome, primary sclerosing cholangitis), bronchial asthma, and Castleman's disease

associated with immunogenetic factors such as the class II antigen of the major histocompatibility complex (MHC), polymorphism of nuclear factor-κB and Fc-receptor-like (FCRL) 3 genes expressed on B cells [37, 38]. Two studies of HLA association with AIP have been reported from the Japanese [37] and Korean groups [38]. In the Japanese patients with AIP, HLA haplotype DRB1*0405-DQB1*0401 (class II), and ABCF1 proximal to C3-2-11, telomeric of HLA-E (class I), are susceptible to AIP [37], but not so with the Korean patients [38]. However, substitution of aspartic acid to nonaspartic acid at DQβ1 may be a predictive factor for the relapse of AIP in Korean patients [38]. FCRL3 polymorphisms are linked to various autoimmune diseases, such as rheumatoid arthritis, autoimmune thyroid disease, and systemic lupus erythematosus (SLE) in the Japanese population [39, 40]. However, Fc-receptor-like 3 gene polymorphisms are not correlated with the DRB1*0405-DQB1*0401 haplotype, suggesting that while both are related to AIP susceptibility in the Japanese population, they are part of the distinct underlying mechanisms of disease development [39, 40].

A few immunogenetic studies for innate or acquired immunity have been reported. Innate immunity is important in the development of acquired immunity or autoimmune diseases. Although polymorphisms in the toll-like receptor-4 gene have been linked with several autoimmune and allergic diseases, this gene seems not to play an important role in the development of AIP [41]. On the other hand, an inhibitory molecule, cytotoxic T lymphocyte antigen-4 (*CTLA-4*; CD152), expressed on the activated memory T cells and $CD4^{+}CD25^{+}$ regulatory T cells (Tregs), was independently reported as a susceptibility factor for AIP in the Taiwanese [42] and Japanese population [43]. CTLA-4 acts as a negative regulator of T cell responses by competing with the CD28 molecule for engagement with the B7 molecules CD80 and CD86 on antigen-presenting cells [43]. Umemura et al. [43] reported that the 3′ untranslated region of *CTLA-4*+6230 SNP plays a pivotal role in both susceptibility (+6230G/G genotype) to and protection (haplotype of the +6230A allele) from AIP, while exon 1+49 SNP is not associated with AIP in the Japanese patients. They also found that +49A/A or +6230A/A genotypes may be associated with recurrence of the disease, which is observed in Graves' disease, type 1 diabetes, and clearance of hepatitis B virus [44]. On the other hand, Chan et al. [43] have reported that *CTLA-4* SNPs have shown significantly higher frequencies of the +49G allele in patients with AIP than in controls, but not with other subtypes of chronic pancreatitis. Chan et al. also reported that tumor necrosis factor (TNF)-alpha promoter 863A was associated with a significantly higher risk of AIP. Racial and geographical differences may be associated with SNPs of the different locus of CTLA-4 [42]. The soluble isoform of CTLA4 (sCTLA4) is reported to be elevated in patients with autoimmune diseases, such as autoimmune thyroid disease, SLE, and myasthenia gravis [43]. Therefore, the sCLTA4 molecule may have a dual role of maintaining self-tolerance and enhancing immune responses by blocking the interaction of CD80 on antigen-presenting cells and CTLA4 on T cells.

Immunoglobulin Subclasses and IgG4

In healthy subjects, IgG1 usually accounts for most of the total IgG [45]. Generally, the amount of IgG4 does not vary with sex or age, and the quantity of IgG4 as well as the IgG4/total IgG ratio tends to remain constant [45]. The ratios for each IgG subclass are 65% of IgG1, 25% of IgG2, 6% of IgG3, and 4% of IgG4 [45]. In IgG4-related diseases, total IgG, IgG1, IgG2, IgG4, and IgE are usually increased compared with healthy subjects, while IgM, IgA, and the ratios of IgG to IgM or IgA are decreased compared with normal or other control diseases [3, 14, 15, 46] (Table 4). Ratios of IgG subclasses other than IgG4 are somewhat different among individual diseases; in AIP, all subclasses (IgG1–G4) of IgG increased compared with other types of pancreatitis. In contrast, IgG_1 and IgG_3 in MD are

Table 4 Immunoglobulin subclasses in IgG4-related disease

	Year		Number	IgG	IgG1	IgG2	IgG3	IgG4(/IgG)	IgM	IgA	IgE	IC (μg/ml)
Hamano et al.	2001	AIP		2,389	NT	NT	NT	742 (28%)	NT	NT	NT	30
		Control										
Yamamoto et al.	2006	MD	16	3,226.9	1,256.4 (41.5%)		NT	1,111 (28.6%)				
		SS	16	2,398	1,624.9 (73.0%)		NT	88.8 (2.8%)				
		Normal	–[a]		65%	25%	6%	4%				
Masaki et al.	2008	MD	64	2,960.1	1,153.3	786.5	57.6	697.7	63	194.7	307.4	
		SS	31	2,473.1	1,437.1	566.6	81.9	23.5	147.3	389.7	15.3	
Taguchi et al.	2009	AIP	20	2,556	NT	NT	NT	762	85	213	NT	
		CP	21	1,245*	NT	NT	NT	NT	122	294	NT	

AIP autoimmune pancreatitis, *MD* Mikulicz disease, *SS* Sjögren's syndrome, *CP* chronic pancreatitis, *IC* immune complex

[a] [45]

significantly lower in negative correlations with IgG4 than in typical SS.

Although the association of IgE-mediated allergy and IgG4 antibodies is well-known [47], IgG4 characteristics are still poorly understood. Basically IgG4 has non-acting characteristics for immune responses involved in a continuous process referred to as "Fab-arm exchange" by swapping a heavy chain and attached light chain (half-molecule) with a heavy-light chain pair from another molecule [48], which results usually in asymmetric antibodies with two different antigen-combining sites. While these modified antibodies are heterobivalent, they behave as monovalent antibodies [48] (Fig. 4a). Another aspect of IgG4 mimics IgG rheumatoid factor (RF) activity by interacting with IgG on a solid support [49] (Fig. 4b). In contrast to conventional RF, which binds via its variable domains, the activity of IgG4 is located in its constant domains, but inefficient in activating potentially dangerous effector systems due to its low affinity for C1q and the classical Fcγ-receptors.

The Complement System

Patients in active stages of AIP occasionally show decreased complement (C3, C4) with elevated circulating immune complex as well as serum levels of IgG4 and the IgG4 subclass of immune complexes [3, 50]. However, a recent study showed that the classical pathway of complement activation through IgG1 may be involved in the development of AIP rather than mannose-binding lectin or alternative pathways through IgG4 [51]. Moreover, IgG4 bound to other isotypes such as IgG1, 2, and 3 with an Fc–Fc interaction immune complex in patients with AIP [49] and then IgG4 may contribute to the clearance of immune complexes or termination of the inflammatory process by preventing the formation of large immune complexes with blocking Fc-mediated effector functions of IgG1. Compared with SLE, tubulointerstitial nephritis (TIN) is more often observed in renal lesions of IgG4-related disease. But in acute TIN associated with AIP, deposition of immune

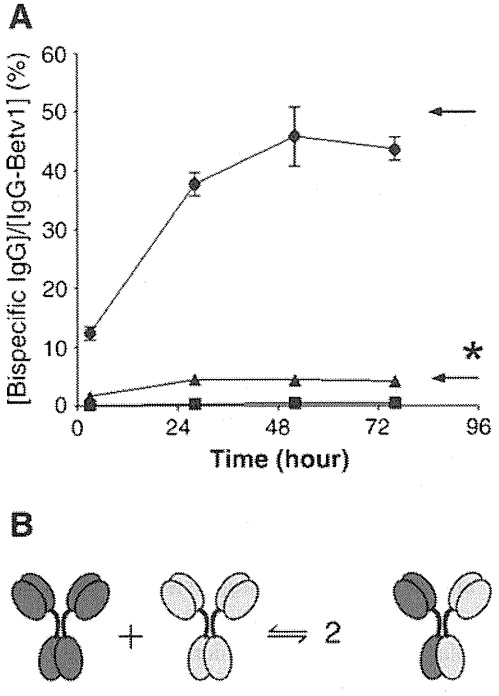

Fig. 4 Characteristic forms of IgG4. **a** Schematic representation of the generation of bispecific IgG4 antibodies by the exchange of half-molecules ("Fab-arm exchange"; cited from [46]). IgG4 Fab arm exchange occurs by the exchange of a heavy chain-light chain pair (half-molecule) of one IgG4 molecule with that of another IgG4 molecule. The IgG4 molecule may thereby acquire two distinct Fab arms and become bispecific. The Fc structure remains essentially unchanged apart from potential changes due to differences in glycosylation or allotype. Fab arm exchange is proposed to be stochastic and dynamic. **b** On the *left*: IgG4 Fc interacts with Ig Fc. On the *right*: IgM RF recognizes IgG in a "classical" Fab-Fc recognition (cited from [47])

complex (IgG and C3) was observed in the glomerular basement membrane but not in the tubular basement membrane, which suggested that membranous glomerulonephritis is also associated with severe TIN associated with IgG4-related disease [24].

Autoantibodies

Patients with IgG4-related diseases generally show several autoantibodies in addition to increased IgG and IgG4 [4, 5]. Although some patients with IgG4-related disease have non-specific antibodies such as an anti-nuclear antibody, there is scarce association of IgG4-related disease and well-known autoimmune diseases such as Sjögren's syndrome and SLE. From the view of IgG4 function, the big mystery is whether IgG4-related disease is an autoimmune or an allergic disease. However, the occasional coexistence of other organ involvement leads us to the concept that there may be common target antigens in the involved organs such as the pancreas, salivary glands, biliary tract, lungs, renal tubules, and so on. Although disease-specific antibodies have not been identified at this moment, several disease-related antibodies such as anti-lactoferrin (LF) [52, 53], anti-carbonic anhydrase (CA)-II [52–55], anti-CA-IV [56], anti-pancreatic secretory trypsin inhibitor (PSTI) [57], anti-amylase-alpha [58], anti-HSP-10 [59], and anti-plasminogen-binding protein (PBP) peptide autoantibodies [60] have been reported. Although the patients show increased serum levels of IgG4, the major subclass of these autoantibodies is not necessarily IgG4, but often IgG1 [57]. CA-II [53], CA-IV [56], LF [53], and PSTI [54] are distributed in the ductal cells of several exocrine organs, including the pancreas, salivary glands, biliary duct, lungs, renal tubules, etc. [52, 53]. Although not all peptides have been studied, immunization with CA-II or LF induced systemic lesions such as pancreatitis, sialadenitis, cholangitis, and interstitial nephritis in the mice models similar to human IgG4-related diseases [61, 62]. The high prevalence of the above antibodies suggests that they may be candidates for the target antigens in AIP [53].

Molecular mimicry among microbes and target antigens may be a possible mechanism for breaking down immune tolerance. The hypothesis is based on the concept that infectious agents share one or more epitopes with self-components, or infectious agents cause bystander activation of immune cells with autoaggressive potential [63–65]. Guarneri and colleagues showed significant homology between human CA-II and alpha-CA of *Helicobacter pylori*, a fundamental enzyme for bacterial survival and proliferation in the stomach [65]. Moreover, the homologous segments contain the binding motif of DRB1*0405, which confers a risk for AIP development [65]. The PBP peptide newly identified in European patients with AIP shows homology with an amino acid sequence of PBP of *H. pylori* and with ubiquitin-protein ligase E3 component n-recognin 2, an enzyme highly expressed in acinar cells of the pancreas, while European patients with AIP did not necessarily show LPSP as the typical histopathology of type 1 AIP in IgG4-related diseases [65]. These findings suggest that gastric *H. pylori* infection might trigger AIP in genetically predisposed subjects [63–65].

Diabetes mellitus complications exist in 43~68% of AIP patients, but autoantibodies against glutamic acid decarboxylase, beta-cell, or tyrosine phosphatase-like protein [62] associated type 1A DM are rarely observed. These findings suggest that islet cells may not be targeted in the development of DM associated with AIP.

No disease-specific autoantibodies have been identified in IgG4-related disease. The scarce association of IgG4-related disease and well-known autoimmune diseases such as Sjögren's syndrome and SLE must be discussed.

Th1 and Th2 Immune Balance

The effector cells in IgG4-related diseases have been poorly understood. The presence of autoantibodies, the predominant infiltration of $CD4^+$ and $CD8^+$ T cells, and the expression of HLA-DR antigens in the pancreas [52] suggest that an immunological mechanism may be involved in the development of AIP as well as the infiltration of plasmacytes and B cells. $CD4^+$ T cells differentiate from naïve T cells (Th0) to Th1, Th2, Th17, and Treg cells [66]. IL-12 induces Th1 cells, which produce IL-2, TNF-alpha, and IFN-gamma; mediate cellular immunity, macrophage activation, cytotoxicity; and help for B cell production of opsonizing and complement fixing antibodies [4]. IL-4 induces Th2 cells which produce IL-4, IL-5, IL-6, and IL-10, promoting humoral and allergic responses [4]. Transforming growth factor (TGF)-β, IL-6, IL-21, and IL-23 induce Th17 cells, which secrete IL-17, and may be involved in inflammation in mice [67].

In some patients with AIP, Th1 cells are predominant over Th2 type cells in the periphery [53, 68]. On the other hand, a Th2 type immune reaction is induced in the livers of IgG4-related sclerosing cholangitis patients as well as the Th1 responses [69]. The discrepancy may be explained by the shift of Th2 cells from the periphery to local tissues, or by different disease stages. Mice models with depletion of Tregs by neonatally thymectomy (nTx) support the hypothesis that Th1 cells act mainly as effectors in the initial early stage [70]. In Sjögren's syndrome [71] and PSC [72], the major infiltrating cells in the tissue are $CD4^+HLA\text{-}DR^+$ Th1 cells, although $CD8^+$ and B cells are also present. Similar to Sjögren's syndrome, Th1 cytokines may be essential in the induction of

AIP, while Th2 cytokines may be involved in the progression of the disease process, especially the maturation and proliferation of local B cells and plasmacytes [4].

Regulatory T Cells

From naïve Th0 cells, TGF-β can induce $CD4^+CD25^+$ Tregs, which have a potent inhibitory function via the transcription factor Foxp3 to $CD4^+$ T cell-mediated immune responses such as Th1, Th2, and Th17 [67]. Foxp3 is a member of the forkhead/winged-helix family of transcriptional regulators and functions as the master regulator in the development and function of Tregs. This suppressive function is mediated by TGF-β and IL-10, and/or cell-to-cell contact via ligation of CTLA-4. Recent studies clarified several subtypes of Tregs [73]. Tregs originating in the thymus are naturally occurring $CD4^+CD25^+$ Tregs, which are different from adaptive Tregs induced in the periphery by different antigens [73]. As Tregs expressing Foxp3 are critical in the transfer of immune tolerance, Treg deficiency induced various autoimmune diseases in animal experimental models [67]. However, in humans, an increased prevalence of circulating $CD4^+CD25^+$ T cells or a similar level of peripheral $CD4^+CD25^+$ T cells was observed in patients with rheumatoid arthritis, Sjögren syndrome, and inflammatory bowel disease, compared with healthy controls [74]. Therefore, the evidence of decreased circulating Tregs as shown in the animal studies may not be a general finding in human autoimmune diseases. In IgG4-related diseases, the role of Tregs remains unclear. In AIP, in addition to increased soluble *CTLA4*, circulatory naïve ($CD45RA^+$) Tregs are significantly decreased in the peripheral blood of patients with AIP, whereas memory ($CD45RA^-$) Tregs in major population are significantly increased [75]. In addition, prominent infiltration of Tregs with upregulation of IL-10 is observed in the liver of IgG4-related sclerosing cholangitis patients [53]. These findings suggest that increased memory Tregs in the periphery and local tissues may be inhibitory immune responses against inflammation in the patients with AIP, although decreased naïve Tregs may be pathogenetic.

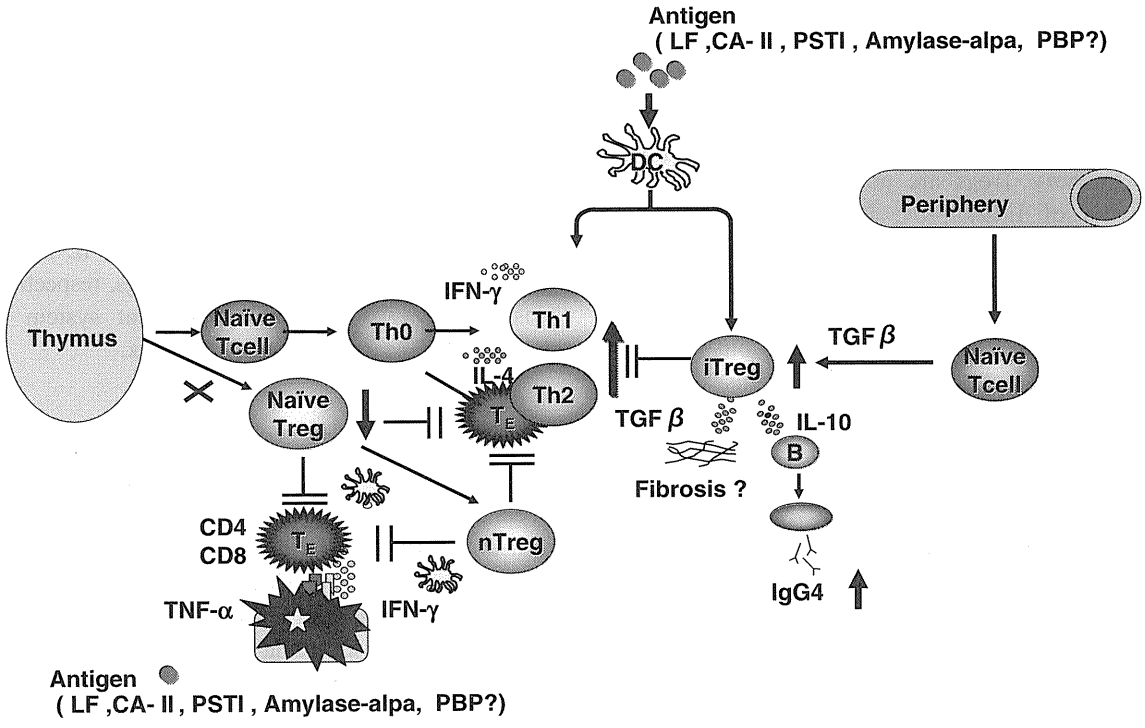

Fig. 5 Hypothesis for the pathogenesis of AIP and IgG4-related disease. In the central tolerance, naïve, and natural regulatory T cells (*Tregs*) derived from the thymus suppress autoreactive CD4 or CD8 cells in the normal state. In the IgG4-related disease, the basic concept is the biphasic mechanism of "induction" and "progression". Initial response to self-antigens (LF, CA-II, CA-IV, PSTI, amylase-alpha, PBP peptide of *H. pylori*, etc.) might be induced by decreased naïve-Tregs. Th2 immune responses followed by Th1 type immune response with release of proinflammatory cytokines (IFN-γ, IL-1beta, IL-2, TNF-α). In progression, Th2 type immune responses with producing IgG, IgG4 and autoantibodies may be involved in pathophysiology. IgG4 and fibrosis may be regulated by increased IL-10 and TGF-β secreted from inducible memory Tregs, respectively. *iTreg* inducible Treg, *TE* effector T cell, *nTreg* natural Treg

Possible Role of IgG4 in "IgG4-Related Disease"

IgG4 seems to be associated with a pathogenic effect in a few situations. In pemphigus, recognition of skin autoantigens (desmogleins) by IgG4 is at the origin of the disease process [76]. IgG4 Fc–Fc binding may have a pathological role within the inflammatory process, or even induce inflammation through aggregation of immunoglobulins like a mouse lupus model [77]. Although some preliminary reports for AIP suggested the presence of autoantibodies against the systemic distributed antigens described above, it remains unclear whether IgG4 type autoantibodies have a direct role in the pathogenesis of IgG4-related diseases or not. To date, there have been few reports indicating IgG4 deposition in IgG4-related renal diseases [24]. Therefore, in some IgG4-related diseases, the infiltration of IgG4+ plasma cells might have an association with pathological roles similar to pemphigoid diseases through IgG4 Fc–IgG Fc binding.

On the other hand, IgG4 is associated with several clinical conditions and generally considered to be a benign, non-pathogenic antibody [78]. Some of these associations suggest a protective effect, such as in allergen-specific immunotherapy, tolerance induction after food avoidance [79], and protection from allergic effects during parasitosis [80, 81]. Recent data on regulating IgG4 showed that IgG4-related diseases may reflect an excessive production of anti-inflammatory cytokines such as IL-10 triggering an overwhelming expansion of IgG4-producing plasma cells. In AIP, increased peripheral inducible memory Tregs are positively correlated with serum levels of IgG4 [75]. In addition, prominent infiltration of Tregs upregulated IL-10 in the livers of patients with IgG4-related sclerosing cholangitis [79]. These findings suggest that IgG4 or IgG4-immune complexes do not act as a pathogenetic factor but not as an anti-inflammatory factor in IgG4-related diseases [49]. Further studies are necessary for clarifying the role of IgG4 in IgG4-related diseases.

Our hypothesis for the Pathogenesis of AIP as "IgG4-Related Disease"

In nTx-BALB/c mice models immunized with CA-II or LF, the $CD4^+$ T cells predominantly infiltrate in pancreatitis, sialoadenitis, and cholangitis over B cells, which is similar to human AIP [70]. These findings suggested that depletion of naïve Tregs in the periphery [82] and MHC class II restricted autoreactive $CD4^+$ T cells, which escape from the positive selection in the thymus, may take important roles in the induction of systemic organ lesions. These $CD4^+$ T cells probably induce macrophage activation and further proinflammatory reactions during the early stage of AIP as direct cytotoxicity effects through Fas ligand expression [83]. On the other hand, $CD8^+$ T cells may play roles as effector cells in the MHC class II-deficient mouse [84] or WBN/Kob rat models [85]. WBN/Kob rats with congenital decreased peripheral Tregs spontaneously develop sialadenitis, thyroiditis, sclerotic cholangitis, and tubulointerstitial nephritis. Although target antigens remain unclear, $CD8^+$ cells also seem to be effectors. Although rodents lack IgG4 subclass, the deposits of tissue-specific IgG2b, in electrophoretic position similar to human IgG4, were observed in the injured pancreas and lachrymal glands in WBN/Kob rats [85]. These animal models suggest that although $CD8^+$ T cells may be partially involved, $CD4^+$ T cells take major roles in the development of experimental systemic lesions, which is similar to human IgG4-related diseases [4, 53], although the counterpart of IgG4 in mice IgG subclasses has not been identified. As TGF-β is an important regulating factor in maintaining immune homeostasis [86], TGF-β dominant negative mutant mice suggested that loss of TGF-β signaling may contribute to autoimmune pancreatitis [87].

From the above findings, we propose a hypothesis for the pathogenesis of AIP (Fig. 5). The basic concept is the biphasic mechanism of "induction" and "progression". An initial response to self-antigens (LF, CA-II, CA-IV, PSTI, amylase-alpha, PBP peptide of *H. pylori*, etc.) might be induced by decreased naïve Tregs followed by a Th1 type immune response with the release of proinflammatory cytokines (IFN-γ, IL-1beta, IL-2, TNF-α). In progression, Th2 type immune responses with producing IgG, IgG4, and autoantibodies may be involved in pathophysiology. IgG4 and fibrosis may be regulated by increased IL-10 and TGF-β secreted from inducible memory Tregs, respectively. The classical pathway of the complement system may be activated by the IgG1 immune complex.

Conclusion

In conclusion, recent advances support the concept of IgG4-related disease, a unique clinical entity as a systemic disease. As Tregs seem to take important roles in progression as well as induction of the disease, further studies are necessary to clarify the pathogenesis including genetic backgrounds, disease-specific antigens, and the role of IgG4.

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Analysis of regulatory T cells and IgG4-positive plasma cells among patients of IgG4-related sclerosing cholangitis and autoimmune liver diseases

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Abstract
Objectives Patients with autoimmune pancreatitis (AIP) characteristically show elevated serum levels of immunoglobulin G4 (IgG4) and abundant infiltration of IgG4-positive plasmacytes in the involved organs. The most common involved organ showing extrapancreatic lesions is the bile duct, which exhibits sclerosing cholangitis (SC). However, the role of IgG4 in the development of IgG4-related SC (IgG4-SC) remains unclear. To clarify the role of IgG4 in IgG4-SC, we have performed an immunohistochemical analysis of the bile duct.
Methods Laboratory and immunohistochemical findings of liver biopsy specimens obtained from patients with IgG4-SC, primary sclerosing cholangitis (PSC), autoimmune hepatitis (AIH), and primary biliary cirrhosis (PBC) were compared. The biopsy specimens were first stained with anti-IgG1, anti-IgG4, and anti-Foxp3 (forkhead box P3) antibodies, and the ratio of IgG4-, IgG1-, and Foxp3-positive cells, respectively, to infiltrated mononuclear cells (IgG4/Mono, IgG1/Mono, Foxp3/Mono) was assessed.
Results The ratio of IgG4/IgG1-positive plasma cells was significantly higher in specimens obtained from patients with IgG4-SC than in those from patients with PSC, AIH, and PBC. The Foxp3/Mono ratio in patients with PBC was significantly higher than that in patients with IgG4-SC and PSC. In patients with IgG4-SC, the number of Foxp3-positive cells was significantly correlated with the number of IgG4-positive cells; in the other patient groups, there was no correlation.
Conclusions The IgG4/IgG1 ratio in the liver may be a useful marker for differential diagnosis of IgG4-SC and PSC. In IgG4-SC, abundant infiltration of regulatory T cells (Tregs) may affect the switching of B cells to IgG4-producing plasmacytes, and there is a possibility that the function of Tregs is different in IgG4-SC and other liver diseases (PSC, AIH, and PBC).

Keywords Autoimmune pancreatitis · IgG4 · IgG4-related diseases · Regulatory T cell · Sclerosing cholangitis

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Introduction

Sarles et al. [1] reported the first case of pancreatitis with hypergammaglobulinemia in 1961. This was followed by the occasional report of the coexistence of pancreatitis with other autoimmune diseases. In 1995, Yoshida et al. [2] proposed the concept of autoimmune pancreatitis (AIP): a clinical condition in which patients show a diffusely enlarged pancreas, narrowing pancreatogram, increased serum immunoglobulin (Ig)G, presence of autoantibodies, fibrotic changes with lymphocytic infiltration, and steroidal

efficacy. Many AIP cases have since been reported by Japanese investigators, and AIP has been accepted as a new clinical entity [3, 4]. Patients with AIP often show discomfort in the epigastrium, obstructive jaundice due to bile-duct stricture, and diabetes mellitus. It is more common among 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 [5–8], which suggests that AIP may be a systemic disorder. In 2001, Hamano et al. [9] reported that patients with AIP have high serum IgG4 concentrations. In 2006, Kamisawa et al. [10] proposed the existence of IgG4-related sclerosing disease, which has recently led to immunoglobulin G4-related sclerosing cholangitis (IgG4-SC) being recognized as a disease entity characterized by sclerosing inflammation with abundant IgG4-positive plasma cells; most cases reported to date have been associated with AIP [11]. The cholangiographic findings on radiological images of patients with IgG4-SC and primary sclerosing cholangitis (PSC) are similar [8, 12]. However, while IgG4-SC responds dramatically to steroid therapy, PSC, even in patients receiving medication, remains a progressive disease that involves the intra- and extra-hepatic bile ducts and leads to biliary cirrhosis. Therefore, it is important that the clinician is able to distinguish between IgG4-SC and PSC.

Attention has recently focused on the relationship between various autoimmune diseases and regulatory T cells (Tregs), which are present in human peripheral blood [13–15], intestinal lamina propria [16], and the thymus [15]. $CD4^{+}CD25^{high}$ Tregs, which are characterized by the expression of a specific transcription factor, forkhead box P3 (Foxp3), play a key role in the autoimmune diseases. We have previously reported that increased numbers of $CD4^{+}CD25^{high}$ Tregs may influence IgG4 production and that naive Tregs may be involved in the pathogenesis of AIP [17]. However, the relationship between Tregs in the peripheral blood and the liver remains unclear. The aim of the study reported here was to clarify the role of IgG4 in IgG4-SC by comparing the immunohistochemical features of the liver in patients with IgG4-SC, PSC, autoimmune hepatitis (AIH), and primary biliary cirrhosis (PBC).

Patients and methods

Subjects

The patient cohort consisted of 16 IgG4-SC patients of the Kansai Medical University and its affiliated hospitals (16 patients untreated with corticosteroids; 15 men and one woman; mean age 63 years; range 31–81 years). Of these 16 patients, 14 had SC with AIP based on the Japanese clinical diagnostic criteria for AIP proposed by the Research Committee of Intractable Diseases of the Pancreas and accepted by both the Japanese Ministry of Health, Labor, and Welfare and the Japan Pancreas Society in 2006 [5] and on Asian criteria [18]. Two patients were diagnosed according to the Mayo criteria for IgG4-associated cholangitis [19].

We classified the IgG4-SC patients into two groups, intra-/extra-hepatic (intra-IgG4-SC) and extra-hepatic (extra-IgG4-SC), based on the nature of the biliary strictures as characterized by endoscopic retrograde cholangiopancreatography (ERCP) (Fig. 1). In those patients with intra-IgG4-SC ($n = 8$), strictures were present in the intra-hepatic or in both the intra-hepatic and extra-hepatic bile ducts. In those patients with extra-IgG4-SC ($n = 8$), the strictures were present only in the extra-hepatic bile duct. In our study, we analyzed only the eight intra-IgG4-SC patients. Prior to biliary drainage, we performed an intraductal ultrasonography (IDUS) in all patients to evaluate thickening of the bile duct wall. The IDUS demonstrated

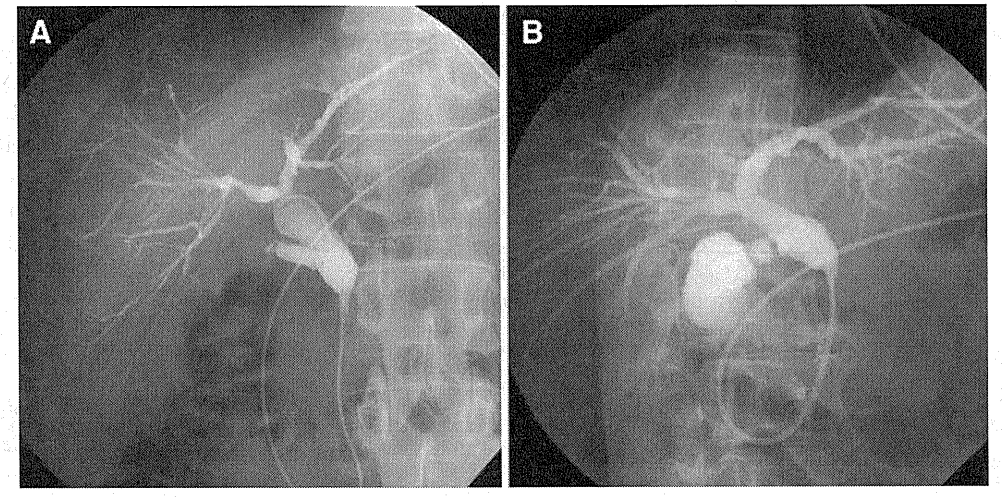

Fig. 1 Endoscopic nasobiliary drain (ENBD) cholangiogram of immunoglobulin G4-related sclerosing cholangitis (IgG4-SC). The IgG4-SC patients were classified into two groups (intra- and extra-IgG4-SC, respectively) based on the nature of the biliary strictures. **a** Stricture of the intra-hepatic and extra-hepatic bile duct (intra). **b** Stricture of the extra-hepatic bile duct (extra)