Table 3 Clinical characters of systemic IgG4-related lymphadenopathy

Clinical presentation	(i)	Patients are middle-aged–elderly with marked male predominance
	(ii)	Systemic lymphadenopathy
	(iii)	Lymph node are not very large (usually up to 2 cm)
	(iv)	Exocrine or extranodal lesions may precede, follow, or present together with the lymph node swelling
	(iv)	Absence of fever
Abnormal laboratory findings	(i)	Polyclonal hyperimmunoglobulinemia
	(ii)	Raised serum IgG and IgE levels
	(iii)	Elevation of serum soluble interleukin-2 receptor
	(iv)	Presence of autoantibodies
Normal laboratory findings	(i)	Interleukin-6 level
	(ii)	Negativity of C-reactive protein
	(iii)	Lactate dehydrogenase level

may precede, follow, or present together with the lymph node swelling; and (v) despite the systemic nature of the disease, there is no fever or other B symptoms. The diagnostic laboratory clues to diagnosis are polyclonal hyperimmunoglobulinemia, raised serum IgG and IgE levels, elevation of serum soluble interleukin-2 (IL-2) receptor and presence of autoantibodies, whereas the IL-6, CRP and lactate dehydrogenase level were within normal limits in the majority of cases.

Differential diagnostic problems of IgG4-related lymphadenopathy

The present review demonstrates the histological variety of IgG4-related lymphadenopathy. Clinically, this disease frequently affected middle-aged and elderly patients, producing systemic lymphadenopathy associated with various immunological abnormalities.[53,54]

IgG4-related lymphadenopathy should be differentiated from various atypical and malignant LPD containing numerous and plasma cells.

Type I lesions had similar clinicopathological findings to multicentric Castleman's disease (MCD), including idiopathic plasmacytic lymphadenopathy with polyclonal hyperimmunoglobulinemia (IPL).[67,70] In Japan, HHV-8 appears to be unrelated to the etiology of MCD except for HIV type-1 infection as well as IgG4-related lymphadenopathy.[70,71] We (YS and MK) have seen numerous IgG4-positive plasma cells in the lymph nodal lesion of IPL, although the serum IL-6 level was within normal limits in the majority of type I lesions.[54,66] The abnormal clinical findings, such as general fatigue, anemia and polyclonal hypergammaglobulinemia, elevated CRP and thrombocytosis may be related to a high level of IL-6 in the MCD,[72–74] but there were no clinical characteristics of MCD in any of the IgG4-related lymphadenopathies.

Type I lesions also should be differentiated from lymph node lesions of autoimmune disease-associated lymphadenopathy, in particular rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).[75,76] The characteristic histological finding of lymph nodal lesion of RA is both reactive follicular hyperplasia and interfollicular plasmacytosis.[75] The lymph nodal lesion of SLE occasionally has similar histological findings to Castleman's disease,[76] but there is no evidence of definite autoimmune disease in any of the IgG4-related lymphadenopathies.

One of the most important differential diagnostic problems is atypical lymphoplasmacytic and immunoblastic proliferation (autoimmune-disease-associated lymphadenopathy).[77] Koo *et al.* reported an unusual lymph node lesion, namely 'ALPIB',[77] which is associated with various autoimmune disease including RA and SLE.[77,78] Histologically, the lesion is characterized by prominent polyclonal lymphoplasmacytic infiltration with various numbers of immunoblasts.[77] There is no evidence, however, of definite autoimmune disease in any of the IgG4-related lymphadenopathies.

When AITL contains a few tumor cells (clear cells) with numerous plasma cells and B-immunoblasts, it can be confused with type III lesions. In contrast to AITL, there are no cytologically atypical CD10+ T-cells and there is no extrafollicular follicular dendritic proliferation in type III lesions.[79] Moreover, AITL usually involves systemic symptoms such as fever.[79]

Type IV lesion has histological findings of early stage PTGC.[68] A portion of PTGC containing numerous plasma cells in the germinal center may be an IgG4-related lymphadenopathy.

Type V lesions have similar histological findings to those of the IPT of the lymph node. IPT of the lymph node, however, mainly affects the lymph node framework such as hilum, trabeculae and capsule,[68,69] whereas lesions of IgG4-related disease are usually located in the lymph node parenchyma.

The importance of recognition of this entity lies in the remarkable response to steroid therapy. The diagnosis requires awareness and a high index of suspicion for this entity, which could present as unexplained lymphadenopathy with numerous plasma cells and scattered eosinophils, or lymphadenopathy in patients with known pancreatitis, lacrimal gland lesion or salivary gland lesion.

OCULAR ADNEXAL IgG4-RELATED DISEASE

Clinical and pathological findings of ocular adnexal IgG4-related disease

IgG4-related diseases frequently involve the ocular adnexal region.[80,81] Ocular adnexal IgG4-related disease is also

called Mikulicz's disease or chronic sclerosing dacryoadenitis.[82–86] Clinically, the lacrimal glands are involved, and bilateral lacrimal gland swelling is frequently observed.[80] Though some patients do not show obvious lacrimal gland involvement clinically, lacrimal gland component was frequently detected histologically. This suggests that accessory lacrimal glands may be involved.

Mikulicz's disease is a unique condition that refers to bilateral, painless and symmetrical swelling of the lacrimal, parotid and submandibular glands. Although Mikulicz's disease has been considered a subtype of Sjögren syndrome, there are several differences between the two diseases. Patients with Mikulicz's disease lack anti-SS-A and anti-SS-B antibodies, but frequently have elevated serum IgG4 levels.[34,82–84] Infiltration of many IgG4-positive plasma cells into the lacrimal and salivary glands has been detected in Mikulicz's disease. Additionally, Mikulicz's disease has good responsiveness to steroids, and reversible of lacrimal and salivary gland function. Thus, it is important to distinguish Mikulicz's disease from Sjögren syndrome.[34,82–84]

The ocular adnexal IgG4-related disease is histologically uniform: marked lymphoplasmacytic infiltration and lymphoid follicles, admixed with dense fibrosis, and infiltration of many IgG4-positive plasma cells.[80] These findings are similar to those of previous reports of IgG4-related disease of other organs. The ocular adnexal IgG4-related diseases often are associated with ones of the salivary glands.[80]

As referred to here, obliterative phlebitis has been identified as a histological feature of IgG4-related diseases since Kawaguchi *et al.* reported on the histopathology of sclerosing pancreatitis in 1991,[9] and it has been easily and characteristically found in sclerosing pancreatitis and sclerosing sialadenitis. But obliterative phlebitis is usually not detected in ocular adnexal IgG4-related disease.[80] Therefore, we suggest that obliterative phlebitis may be organ specific, but not a common feature of IgG4-related diseases.

Interestingly, although serum IgG4 levels are often evaluated after treatment, it remains elevated even in remission.[80] This may be due to residual IgG4-secreting plasma cells located subclinically elsewhere.

Ocular adnexal IgG4-related disease and mucosa-associated lymphoid tissue lymphoma

Little is known about lymphomagenesis in the context of IgG4-related disease.[80,85,87] We recently first reported ocular adnexal mucosa-associated lymphoid tissue (MALT) lymphomas arising from IgG4-related disease, occurring in the same organ.[80]

MALT lymphoma is an extranodal lymphoma consisting of morphologically heterogeneous small B-cells including marginal zone cells.[88,89] The infiltrate is in the marginal zone of reactive B-cell follicles and extends into the interfollicular region. In epithelial tissues, the neoplastic cells typically infiltrate the epithelium, forming lymphoepithelial lesion. The presence of lymphoepithelial lesion is important when making a diagnosis of MALT lymphoma.[88,89]

In many cases of MALT lymphoma, there is a history of chronic inflammatory, often autoimmune disorders that result in accumulation of extranodal lymphoid tissue. These include *Helicobacter pylori*-associated chronic gastritis, Sjögren syndrome or Hashimoto thyroiditis.[88,89] Thus, we considered that patients with ocular adnexal IgG4-related disease may be at an increased risk of developing ocular adnexal MALT lymphoma. Another study has also described ocular adnexal lymphomas arising from IgG4-related disease.[85] Takahashi *et al.* reported that three patients with IgG4-related disease with or without autoimmune pancreatitis later developed B-cell non-Hodgkin lymphoma (two of whom developed diffuse large B-cell lymphoma).[87] In addition, Ochoa *et al.* reported on marginal zone B-cell lymphoma of the salivary gland arising in Küttner tumor.[90] It has previously been noted that autoimmune pancreatitis and Küttner tumor were considered to be IgG4-related disease. Therefore, these reports suggest that IgG4-related disease may be a risk factor for malignant lymphoma.

We experienced seven patients with the ocular adnexal MALT lymphomas arising from IgG4-related disease (IgG4-related ocular adnexal MALT lymphoma), occurring in the same organ. Six patients had localized disease (clinical stage IE or IIE; unpubl. data, 2009). Histologically, in this series of patients there was dense fibrosis subdividing the lacrimal gland, and marked lymphoid cell infiltration with lymphoid follicles. These histological findings were consistent with previous reports of IgG4-related disease. However, some infiltrated lymphoid cells showed centrocyte-like features, and Dutcher bodies were found in some of the cases (Fig. 6) in addition to histological finding of IgG4-related disease. All cases had immunoglobulin light chain restriction, and immunoglobulin heavy chain gene rearrangement on polymerase chain reaction and/or Southern blot hybridization. Interestingly, lymphoepithelial lesion was not found in any cases. Lymphoepithelial lesions usually are not found in ocular adnexal MALT lymphomas (especially in the lacrimal gland region).[90] Another report also noted that lymphoepithelial lesion was not found in ocular adnexal IgG4-related MALT lymphoma. It remains unclear whether the absence of lymphoepithelial lesion indicates biological differences in the lacrimal gland, or whether the epithelium may have been destroyed due to IgG4-related chronic inflammation.

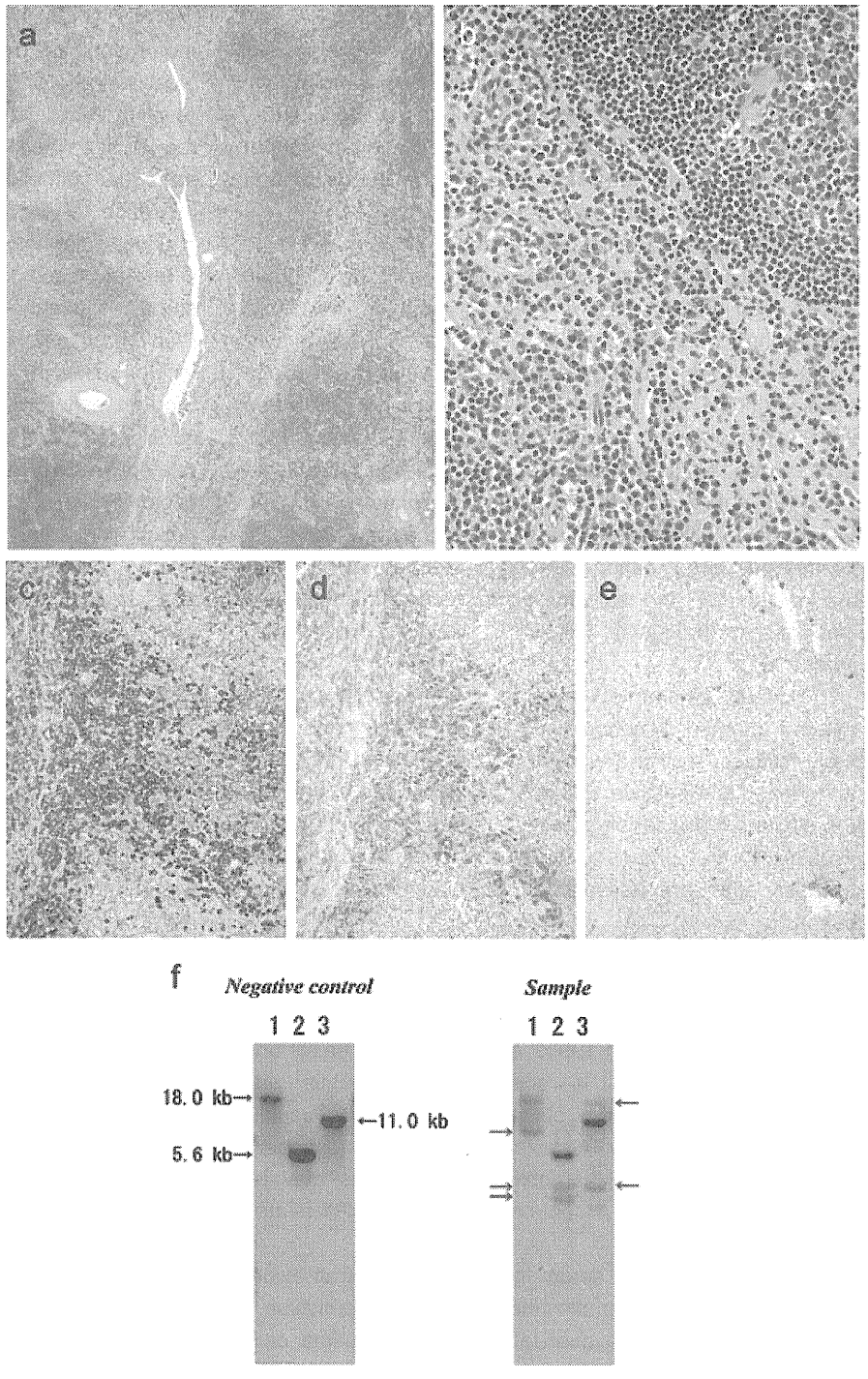

Figure 7 Ocular adnexal IgG4-producing mucosa-associated lymphoid tissue lymphoma. (**a**,**b**) Dense fibrosis and marked lymphoplasmacytic infiltration in the lacrimal gland. Histologically, this is compatible with previous reports of IgG4-related sclerosing disease. Immunostain for (**c**) IgG4, (**d**) kappa-light chain and (**e**) lambda-light chain. Most of the IgG4-positive cells exhibit kappa-light chain restriction. (**f**) Immunoglobulin heavy chain gene rearrangement was detected on Southern blot hybridization.

There have been many reports on ocular adnexal IgG4-related lymphomas at the annual meetings of the Japanese Society, but in IgG4-related disease of other sites, there is rare or absent IgG4-related MALT lymphoma. In the orbital region, the most common tumor is malignant lymphoma, especially MALT lymphoma.[91] In contrast, submandibular gland and pancreas have a low incidence of MALT lymphoma. Therefore IgG4-related MALT lymphoma may occur more easily in the ocular adnexa.

IgG4-PRODUCING LYMPHOMA

Little is known about IgG4-producing lymphoma.[85,92] We recently reported the first case of IgG4-producing marginal zone B-cell lymphoma of the lymph node.[92] The IgG4-positive tumor cells were lambda light-chain-restricted and CD138 partially positive, although the expression was fainter than that of the non-neoplastic cells. Additionally, the tumor cells were partially positive for CD20, which is normally

negative in non-neoplastic plasma cells, and had elevation of serum IgG4 level.[92] Therefore that case indicates that not only can malignant lymphomas occur in the setting of IgG4-related diseases, but that IgG4-producing cells can also be neoplastic.

Moreover, we encountered a case of ocular adnexal IgG4-producing MALT lymphoma (Fig. 7). The histology was compatible with ocular adnexal IgG4-related disease (Fig. 7a,b), and there was elevation of serum IgG4 level, serum IgG4/IgG ratio, and IgG4/IgG-positive cell ratio (≥50%). The lesion exhibited immunoglobulin light chain restriction of IgG4-positive cells (Fig. 7c–e) and immunoglobulin heavy chain gene rearrangement (Fig. 7f). Previously, Cheuk *et al.* also reported on ocular adnexal IgG4-producing lymphoma.[85] They concluded that it remains unclear whether ocular adnexal IgG4-producing MALT lymphoma arises from pre-existing IgG4-related disease, or de novo IgG4-postive MALT lymphoma. We suggest that it may clonal expansion of IgG4-positive cells occurring against a background of IgG4-related chronic inflammation. This is because the case showed marked lymphoplasmacytic infiltration and lymphoid follicles, admixed with dense fibrosis, and also detected elevation of serum IgG4 level. These findings are compatible with IgG4-related disease.

Clinicopathological features of IgG4-producing lymphoma should be clarified in the future by accumulation and evaluation of such cases.

CONCLUSION

IgG4-related diseases are a new clinicopathological systemic entity, but the pathogenesis and etiology remain unclear. IgG4-related diseases have a good response to steroids. Accordingly, accurate pathological diagnosis is very important.

ACKNOWLEDGMENTS

This work was supported in part by grants from Intractable Diseases, the Health and Labour Sciences Research Grants from Ministry of Health, Labor and Welfare (H21-112).

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J Gastroenterol (2011) 46:277–288
DOI 10.1007/s00535-011-0386-x

REVIEW

Recent advances in the concept and diagnosis of autoimmune pancreatitis and IgG4-related disease

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Received: 6 February 2011 / Accepted: 7 February 2011 / Published online: 11 March 2011

Abstract Recent studies have suggested the existence of two subtypes of autoimmune pancreatitis (AIP): type 1 AIP, related to IgG4 (lymphoplasmacytic sclerosing pancreatitis); and type 2 AIP, related to a granulocytic epithelial lesion (idiopathic duct-centric chronic pancreatitis). Compared with type 2 AIP, the clinicopathological features of type 1 AIP, with increased serum IgG4/IgE levels, abundant infiltration of IgG4 + plasmacytes and lymphocytes, autoantibodies, and steroid responsiveness, are more suggestive of abnormal immunity such as allergy or autoimmunity. Moreover, patients with type 1 AIP often have extrapancreatic lesions, such as sclerosing cholangitis, sclerosing sialadenitis, or retroperitoneal fibrosis, showing pathological features similar to those of the pancreatic lesions. Based on these findings, an international concept of and diagnostic criteria for AIP have been proposed recently. Of interest, many synonyms have been proposed for the conditions of AIP and extrapancreatic lesions associated with IgG4, such as "multifocal idiopathic fibrosclerosis," "IgG4-related autoimmune disease," "IgG4-related sclerosing disease," "systemic IgG4-related plasmacytic syndrome (SIPS)," 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 fibrosclerosing disease, in 2009, in which the term "IgG4-related disease" was agreed upon as a minimal consensus to cover 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 diseases. The concept and diagnostic criteria of "IgG4-related disease" will be changed in accordance with future studies.

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	Systemic IgG4 plasmacytic syndrome
SLE	Systemic lupus erythematosus
Treg	Regulatory T cell
UBR2	Ubiquitin-protein ligase E3 component n-recognin 2

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Introduction

In 1961, Sarles et al. [1] first observed a case of particular pancreatitis with hypergammaglobulinemia. Yoshida et al. [2] first proposed the concept of autoimmune pancreatitis (AIP). Hamano et al. [3] reported increased serum levels of IgG4 in Japanese patients with AIP. Thereafter, many studies of AIP were reported, mainly by 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, 11]. 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 regarded as a specific pancreatic disease with a granulocytic epithelial lesion (GEL) [12, 13] and occasional coexistence with ulcerative colitis [10, 11].

Of note, patients with Mikulicz's disease (MD) –originally classified as an atypical type of Sjögren's syndrome– who usually have bilateral, painless, and symmetrical swelling of the lachrymal, parotid, and submandibular glands [14], show elevated serum levels of IgG4, infiltration of IgG4-positive plasma cells into the glands, and recovery of secretion with steroid treatment. Similar to patients with AIP, these patients often show other organ involvement (OOI) such as AIP, sclerosing cholangitis, retroperitoneal fibrosis, enlarged celiac and hilar lymph nodes, chronic thyroiditis, and interstitial nephritis [4–6, 14–16]. Recently, however, MD has been considered to be completely different from Sjögren's syndrome because of the lack of anti-SS-A/Ro or anti-SS-B/La antibodies, and the showing of steroid responsiveness [2–6]. The steroid responses and the prognoses of AIP patients with sclerosing cholangitis differ from these features in 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, 17], systemic IgG4-related plasmacytic syndrome (SIPS) [18], and IgG4-positive multiorgan lymphoproliferative syndrome (IgG4-MOLPS) [19]. Although the pathogenesis and pathophysiology of AIP remain unclear, we will discuss the most recent advances in the concept of AIP, especially IgG4-related type 1 AIP, and the advances in the novel concept of "IgG4-related disease."

Subtypes of autoimmune pancreatitis: type 1 and type 2 AIP

Recent studies have suggested that "AIP" manifests as two distinct subtypes, type 1 and type 2 AIP (Table 1) [10, 11]. Hypergammaglobulinemia, the deposition of immunoglobulins, the presence of autoantibodies, and steroid efficacy in Type 1 AIP confirm the definition of autoimmune disease proposed by Mackay [20]. Different from type 1 AIP, patients with type 2 AIP have no serological markers of autoimmunity. Therefore, it is still in debate as to whether or not type 2 AIP should be classified as a clinical entity of AIP, but the deposition of C3c and IgG in the basement membrane of pancreatic ducts and acini suggests an immune complex-mediated destruction of ducts and acini in type 2 AIP as well as type 1 AIP [21]. The nomenclature of the two subtypes of AIP and international consensus diagnostic criteria were proposed at the meeting of the International Association of Pancreatology held at Fukuoka in 2010 (Tables 2, 3) [22].

Type 1 AIP

In type 1 AIP, whose histological description is called LPSP, the pancreatic histopathology shows the following characteristic features: (1) abundant infiltration of plasma cells ($IgG4^+$ cells; high-power field [hpf], IgG4/IgG cells; >40%) and lymphocytes, (2) peculiar storiform or swirling fibrosis, and (3) 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 lesions (e.g., sclerosing cholangitis, sclerosing sialadenitis, and retroperitoneal fibrosis) associated with the infiltration of abundant IgG4 + plasma cells. Patients with type 1 AIP are often elderly males who have obstructive jaundice, and the pancreatic and extrapancreatic manifestations respond to steroid therapy.

Extrapancreatic lesions in type 1 AIP

A variety of extrapancreatic lesions have been noted in patients with AIP, including lachrymal and salivary gland lesions [23], pulmonary lesions including hilar lymphadenopathy [24], sclerosing cholangitis [25, 26], retroperitoneal fibrosis [27], and tubulointerstitial nephritis (TIN) [15, 28, 29]. Associations were also reported with hypophysitis

Table 1 Subtypes of autoimmune pancreatitis (AIP)

Subtype of AIP	Type 1	Type 2
Other nomenclatures	AIP without GEL	AIP with GEL
	IgG4-related LPSP	IgG4-unrelated IDCP
Prevalence	Asia > USA, Europe	Europe > USA > Asia
Age	High age	Younger
Gender	Male ≫ female	Male = female (NS)
Symptoms	Often 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 (−)
Other organ involvement (OOI)	Sclerosing cholangitis	Unrelated to OOI
	Sclerosing sialadenitis	
	Retroperitoneal fibrosis	
	Others	
Ulcerative colitis	Rare	Often
Steroid	Responsive	Responsive
Relapse	High rate	Rare

GEL granulocytic epithelial lesion, *LPSP* lymphoplasmacytic sclerosing pancreatitis, *IDCP* idiopathic duct-centric chronic pancreatitis, *Abs* antibodies, *NS* not significant

[30], chronic thyroiditis [16, 31], and prostatitis [32]. Other extrapancreatic involvements have been reported in a few cases [33–36]. Though it is not certain that all of these involvements have a relation with AIP, extrapancreatic lesions are prevalent systemically in various organs (Table 4) [29–41], 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 responses to steroid therapy; these characteristics suggest a common pathophysiological background. The lesions are usually detected by imaging and blood tests (computed tomography [CT], magnetic resonance imaging [MRI], gallium scintigraphy, fluorodeoxyglucose positron emission tomography [FDG-PET], and IgG4); however, such findings 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. Patients with IgG4-related sialodacryoadenitis, synonymous with IgG4-related MD [13, 41], usually have symmetrical enlargement of the salivary and lacrimal glands. The IgG4-related central nervous system lesions include infundibulohypophysitis, hypertrophic pachymeningitis, intracranial inflammatory pseudotumor, and orbital pseudotumor [21–41].

Type 2 AIP

Type 2 AIP was proposed from histological examination of pancreases resected from patients with chronic non-alcoholic pancreatitis by American and European pathologists, who reported another histopathological pattern, named idiopathic duct-centric pancreatitis (IDCP) or AIP with GEL [11–13]. The most characteristic feature of type 2 AIP is the GEL, often with destruction and obliteration of the pancreatic duct. 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 from that of type 1 AIP, with no associated serum IgG4, IgG elevation, presence of autoantibodies, or other organ involvement, except for inflammatory bowel disease (approximately 30%).

The concept of IgG4-related disease and proposal of the clinical diagnostic criteria

Patients with IgG4-related disease show diffuse/focal organ enlargement, with mass-forming or nodular/thickened lesions in various organs, occurring synchronously or metachronously, due to the prominent infiltration of lymphocytes and plasmacytes with fibrosis [21–41]; however, the causes of the disease are still not clear. The organs known to be affected include the pancreas, biliary duct, lacrimal/salivary glands, retroperitoneum, central nervous system, thyroid gland, lungs, liver, gastrointestinal tract, kidneys, prostate gland, and lymph nodes [21–41]. Clinical

Table 2 Diagnosis of definitive and probable type 1 AIP using international consensus diagnostic criteria (ICDC) [22]

Diagnosis	Primary basis for diagnosis	Imaging evidence	Collateral evidence
Definitive type 1 AIP	Histology	Typical/indeterminate	Histologically confirmed LPSP (level 1 H)
	Imaging	Typical	Any non-D level 1/level 2
		Indeterminate	Two or more from level 1 (+level 2 D*)
	Response to steroid	Indeterminate	Level 1 S/OOI + Rt or level 1 D + level 2 S/OOI/H + Rt
Probable type 1 AIP		Indeterminate	Level 2 S/OOI/H + Rt

	Criterion	Level 1	Level 2
P	Parenchymal imaging	*Typical*	*Indeterminate (including atypical*)*:
		Diffuse enlargement with delayed enhancement	Segmental/focal enlargement with delayed enhancement
		(sometimes associated with rim-like enhancement)	
D	Ductal imaging (ERP)	Long (>1/3 length of the MPD) or multiple strictures	Segmental/focal narrowing without marked upstream dilatation (duct size <5 mm)
		without marked upstream dilatation	
S	Serology	IgG4 > 2× upper limit of normal value	IgG4 1–2× upper limit of normal value
OOI	Other organ involvement	a or b	a or b
		a. Histology of extrapancreatic organs	**a. Histology of extrapancreatic organs including endoscopic biopsies of bile duct****
		Any three of the following	*Both of the following*
		Marked lymphoplasmacytic infiltration with fibrosis and without granulocytic infiltration	Marked lymphoplasmacytic infiltration without granulocytic infiltration
		Storiform fibrosis	Abundant (>10 cells/hpf) IgG4-positive cells
		Obliterative phlebitis	**b. Physical or radiological evidence**
		Abundant (>10 cells/hpf) IgG4 positive cells	*At least one*
		b. Typical radiological evidence	Symmetrically enlarged salivary/lacrimal glands
		At least one	Radiological evidence of renal involvement described in association with AIP
		Segmental/multiple proximal (hilar/intra hepatic) or proximal and distal bile duct stricture	
		Retroperitoneal fibrosis	
H	Histology of the pancreas	LPSP (core biopsy/resection)	LPSP (core biopsy)
		At least 3 of the following	*Any 2 of the following*
		Periductal lymphoplasmacytic infiltrate without granulocytic infiltration	Periductal lymphoplasmacytic infiltrate without granulocytic infiltration
		Obliterative phlebitis	Obliterative phlebitis
		Storiform fibrosis	Storiform fibrosis
		Abundant (>10 cells/hpf) IgG4-positive cells	Abundant (>10 cells/hpf) IgG4positive cells
Diagnostic steroid trial			
Response to steroid (Rt)[#]		Rapid (≤2 weeks) radiologically demonstrable resolution or marked improvement in pancreatic/extrapancreatic manifestations	

hpf high-power field, *MPD* main pancreatic duct, *D* ductal imaging, *Rt* response to steroid treatment, *S* serology, *H* histology, *ERP* endoscopic retrograde pancreatography

Table 3 Diagnosis of definitive and probable type 2 AIP using international consensus diagnostic criteria (ICDC) [22]

Diagnosis	Imaging evidence	Collateral evidence
Definitive type 2 AIP	Typical/ indeterminate	Histologically confirmed IDCP (level 1 H) or clinical IBD + level 2 H + Rt
Probable type 2 AIP	Typical/ indeterminate	Level 2 H/clinical IBD + Rt

	Criterion	Level 1	Level 2
P	Parenchymal imaging	*Typical* Diffuse enlargement with delayed enhancement (sometimes associated with rim-like enhancement)	*Indeterminate (including atypical*)* Segmental/focal enlargement with delayed enhancement
D	Ductal imaging (ERP)	Long (>1/3 length of the MPD) or multiple strictures without marked upstream dilatation	Segmental/focal narrowing without marked upstream dilatation (duct size <5 mm)
OOI	Other organ involvement		Clinically diagnosed inflammatory bowel disease
H	Histology of the pancreas (core biopsy/resection)	IDCP: *Both of the following* Granulocytic infiltration of duct wall (GEL) with or without granulocytic acinar inflammation Absent or scant (0–10 cells/hpf) IgG4-positive cells	*Both of the following* Granulocytic and lymphoplasmacytic acinar infiltrate Absent or scant (0–10 cells/hpf) IgG4-positive cells
Diagnostic steroid trial			
Response to steroid (Rt)[#]		Rapid (<2 weeks) resolution or marked improvement in manifestations	

IBD inflammatory bowel disease, *D* ductal imaging, *Rt* response to steroid treatment, *H* histology, *ERP* endoscopic retrograde pancreatography

symptoms vary depending on the organ in which the lesions are located, but many cases are treated effectively by steroid therapy [21–41]. 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" [17–19, 26–29]. Although the infiltration of IgG4-positive cells and increased serum levels of IgG4 are characteristic of 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" [17], SIPS [18], and "IgG4 + MOLPS" [19], all of which may refer to the same conditions. It has been debated which term is the most appropriate. 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., these features are very seldom found in lachrymal/salivary gland lesions or lymph node lesions. The term "IgG4-related sclerosing disease" is mainly based on fibrous swollen organs, whereas the terms "IgG4-SIPS" and "IgG4 + MOLPS" are based on lymphoplasmacytic proliferation and swollen lymph nodes without fibrosis.

Although most patients with type 1 AIP have multiorgan lesions that occur synchronously or metachronously, about 10–20% of the patients show a solitary organ involved without confirmation of other organ involvement. Therefore, it is unclear whether or not the pathogenetic mechanism is the same in individual organs. Based on these findings, the members of the Japanese Research Committees for "Systemic IgG4-Related Sclerosing Disease" (chaired by Professor K. Okazaki) [40] and "IgG4-MOLPS" (chaired by Professor H. Umehara) [41], both of which Committees 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" be regarded as minimally accepting these conditions at present. To study these conditions, the Japanese Research Committee for "Systemic IgG4-Related Sclerosing Disease" (chaired by Professor K. Okazaki) proposed a disease concept and clinical diagnostic criteria of "systemic IgG4-related sclerosing disease" in 2009 (Table 5) [40]. However, the concept and diagnostic criteria should be changed in accordance with the findings of the future studies.

Table 4 Extrapancreatic lesions complicated with autoimmune pancreatitis. (from Ref. [38])

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

Immunological approaches to the pathophysiology of AIP and IgG4-related disease

The pathogenesis and pathophysiology of AIP have been studied mainly from immunological approaches and

Table 5 Clinical diagnostic criteria 2009 for IgG4-related disease (proposed by the Japanese Research Committee for "Systemic IgG4-related Sclerosing Disease") [40]

(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 of lymphocytes and plasmacytes with fibrosis, but no neutrophilic infiltration

② Abundant infiltration of IgG4-positive plasmacytes (>10/hpf) and/or a ratio of IgG4/IgG-positive cells of >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

studies have focused mainly on IgG4-related type 1 AIP, because little evidence of abnormal immunity has been reported in type 2 AIP.

Humoral immunity

IgG4 and its possible role in IgG4-related diseases

In healthy subjects, IgG1 usually accounts for most of the total IgG [42]. 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 [42]. The ratios for each IgG subclass are 65% of IgG1, 25% of IgG2, 6% of IgG3, and 4% of IgG4 [42]. In IgG4-related diseases, total IgG, IgG1, IgG2, IgG4, and IgE ratios are usually increased compared with healthy subjects, while IgM, IgA, and the ratios of IgG to IgM or IgA, are decreased compared with findings in healthy subjects or those with other diseases [3, 15, 19, 43]. Ratios of IgG subclasses other than IgG4 are somewhat different among individual diseases; in AIP, all subclasses (IgG1-G4) of IgG are increased compared with findings in other types of pancreatitis. In contrast, IgG_1 and IgG_3 in MD show significantly lower negative correlations with IgG4 than those shown in typical Sjögren's syndrome.

Although the association of IgE-mediated allergy and IgG4 antibodies is well known [44], IgG4 characteristics are still poorly understood. Basically, IgG4 has non-active characteristics for immune responses involved in a continuous process referred to as 'Fab-arm exchange', which occurs by the swapping of a heavy chain and attached light chain (half-molecule) with a heavy-light chain pair from another molecule [45], which usually results in asymmetric antibodies with two different antigen-combining sites. While these modified antibodies are hetero-bivalent, they behave as monovalent antibodies [45]. Another aspect of IgG4 mimics IgG rheumatoid factor (RF) activity by interacting with IgG on a solid support [46]. In contrast to conventional RF, which binds via its variable domains, the activity of IgG4 is located in its constant domains, but is inefficient in activating potentially dangerous effector systems due to its low affinity for C1q and the classical Fcγ-receptors.

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 [47]. IgG4 Fc–Fc binding may have a pathological role within the inflammatory process, or may even induce inflammation through the aggregation of immunoglobulins, as occurs in a mouse lupus model [48]. Although some earlier reports of AIP suggested the presence of autoantibodies against the systemically distributed antigens described above, it remains unclear whether or not IgG4-type autoantibodies have a direct role in the pathogenesis of IgG4-related diseases. To date, there have been few reports indicating IgG4 deposition in IgG4-related renal diseases [15, 29]. Therefore, in some IgG4-related diseases, the infiltration of IgG4 + plasma cells might have an association with pathological roles, similar to those in pemphigoid diseases, through IgG4 Fc–IgG Fc binding.

On the other hand, although IgG4 is associated with several clinical conditions, it is generally considered to be a benign, non-pathogenic antibody [49]. Some of these associations suggest a protective effect, such as in allergen-specific immunotherapy, tolerance induction after food avoidance [50], and protection from allergic effects during parasitosis [51, 52]. Recent data on the regulation of IgG4 showed that IgG4-related diseases may reflect an excessive production of anti-inflammatory cytokines such as interleukin (IL)-10, triggering an overwhelming expansion of IgG4-producing plasma cells. In AIP, increased peripheral inducible-memory regulatory T cells (Tregs) are positively correlated with serum levels of IgG4 [53]. In addition, prominent infiltration of Tregs upregulated IL-10 in the livers of patients with IgG4-related sclerosing cholangitis [54]. These findings suggest that IgG4 or IgG4-immune complexes do not act as a pathogenetic factor, but act as an anti-inflammatory factor in IgG4-related diseases [46]. Further studies are necessary to clarify the role of IgG4 in IgG4-related diseases.

The complement system

Patients in active stages of AIP occasionally show decreased complement (C3, C4) with elevated circulating immune complex as well as elevated serum levels of IgG4 and the IgG4 subclass of immune complexes [3, 55]. 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 [56]. Moreover, IgG4 is bound to other isotypes such as IgG1, 2, and 3 with an Fc–Fc interaction immune complex in patients with AIP [46], and thus IgG4 may contribute to the clearance of immune complexes or termination of the inflammatory process by preventing the formation of large immune complexes by blocking the Fc-mediated effector functions of IgG1. Compared with findings in systemic lupus erythematosus (SLE), TIN is more often observed in the renal lesions of IgG4-related disease. But, in acute TIN associated with AIP, the deposition of immune complex (IgG and C3) was observed in the glomerular basement membrane but not in the tubular basement membrane, which suggests that membranous glomerulonephritis is also associated with severe TIN associated with IgG4-related disease [15, 29]. Recently, the deposition of C3 and IgG in the basement membrane of pancreatic ducts have been identified in both type 1 and 2 AIP [21].

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 anti-nuclear antibody (ANA), the association of IgG4-related disease and well-known autoimmune diseases such as Sjögren's syndrome and SLE is rare. From the viewpoint 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, and renal tubules. Although disease-specific antibodies have not been identified at present, several disease-related antibodies such as anti-lactoferrin (LF) [57, 58], anti-carbonic anhydrase (CA)-II [57–60], anti-CA-IV [61], anti-pancreatic secretory trypsin inhibitor (PSTI) [62], anti-amylase-alpha [63], anti-heat-shock protein (HSP)-10 [64], and anti-plasminogen-binding protein (PBP) peptide autoantibodies [65] have been reported. Although the patients show increased serum levels of IgG4, the major subclass of these autoantibodies is not necessarily IgG4, but is often IgG1 [62]. CA-II [59], CA-IV [61], LF [58], and PSTI [62] are distributed in the ductal cells of several exocrine organs, including the pancreas, salivary glands, biliary duct, lungs, and renal tubules. 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 mouse models similar to human IgG4-related diseases [66, 67]. The high prevalence of the above antibodies suggests that they may be candidates for the target antigens in AIP [58].

Molecular mimicry among microbes and target antigens may be a possible mechanism for breaking down immune tolerance. This 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 [68–70]. 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 [70]. Moreover, the homologous segments contained the binding motif of DRB1*0405 [71], which confers a risk for AIP development [70]. The PBP peptide newly identified in European patients with AIP shows homology with an amino acid sequence of PBP of *H. pylori* and with the ubiquitin-protein ligase E3 component n-recognin 2 (UBR2), 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 [68–70].

Diabetes mellitus (DM) complications exist in 43–68% of AIP patients, but autoantibodies against glutamic acid decarboxylase, beta-cell, or tyrosine phosphatase-like protein [67] associated with 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 rare association of IgG4-related disease and well-known autoimmune diseases such as Sjögren's syndrome and SLE must be discussed.

Cellular immunity

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 [57] suggest that, as well as the infiltration of plasmacytes and B cells, an immunological mechanism may be involved in the development of AIP. $CD4^+$ T cells differentiate from naïve T cells (Th0) to Th1, Th2, Th17, and Treg cells [72]. IL-12 induces Thl cells, which produce IL-2, tumor necrosis factor (TNF)-alpha, and interferon (IFN)-gamma, and mediate cellular immunity, macrophage activation, and cytotoxicity, as well as helping 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)-beta, IL-6, IL-21, and IL-23 induce Th17 cells, which secrete IL-17, and may be involved in inflammation in mice [73].

In some patients with AIP, Th1 cells are predominant over Th2 type cells in the periphery [58, 74]. On the other hand, a Th2-type immune reaction, as well as the Th1 responses, is induced in the livers of patients with IgG4-related sclerosing cholangitis [54]. This discrepancy may be explained by the shift of Th2 cells from the periphery to local tissues, or by different disease stages. Mouse models with depletion of Tregs induced by neonatal thymectomy (nTx) support the hypothesis that Th1 cells act mainly as effectors in the initial early stage [75]. In Sjögren's syndrome [76] and PSC [77], the major infiltrating cells in the tissue are $CD4^+$HLA-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-beta can induce $CD4^+CD25^+$ Tregs, which have a potent inhibitory function, via the transcription factor Foxp3, to show $CD4^+$ T cell-mediated immune responses such as Th1, Th2, and Th17 [73]. 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-beta and IL-10, and/or cell-to-cell contact via ligation of cytotoxic T lymphocyte antigen-4 (CTLA-4). Recent studies have clarified several subtypes of Tregs [78]. Tregs originating in the thymus are naturally occurring $CD4^+CD25^+$ Tregs (nTregs), which are different from adaptive Tregs (aTregs) induced in the periphery by different antigens [78]. As Tregs expressing Foxp3 are critical in the transfer of immune tolerance, Treg deficiency has been shown to induce various autoimmune diseases in animal experimental models [73]. 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's syndrome, and inflammatory bowel disease, compared with healthy controls [79]. 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 the major population of memory (CD45RA−)-Tregs is significantly increased [53]. In addition, prominent infiltration of Tregs with upregulation of IL-10 is observed in the livers of patients with IgG4-related sclerosing cholangitis [58]. These findings suggest that increased memory-Tregs in the periphery and local tissues may be inhibitory immune responses against inflammation in patients with AIP, although decreased naïve Tregs may be pathogenetic.

Our hypothesis for the pathogenesis of AIP as IgG4-related disease

In nTx-BALB/c mouse models immunized with CA-II or LF, $CD4^+$ T cells, rather than B cells, are the predominant infiltrates in pancreatitis, sialoadenitis, and cholangitis, which is similar to human AIP [75]. These findings suggest

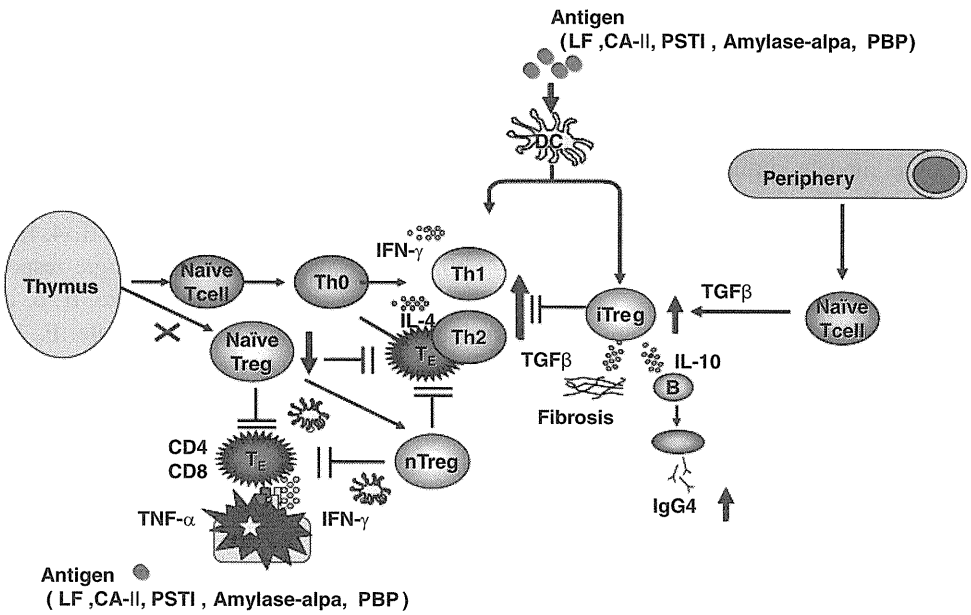

Fig. 1 Hypothesis for the pathogenesis of autoimmune pancreatitis (AIP) in IgG4-related disease. In regard to central tolerance, naïve and natural regulatory T cells (*Tregs*) derived from the thymus suppress autoreactive CD4 or CD8 cells in the normal state. In IgG4-related disease, the basic concept is a biphasic mechanism of "induction" and "progression". Initial response to self-antigens (e.g., lactoferrin [*LF*], carbonic anhydrase II [*CA-II*], CA-IV, pancreatic secretory trypsin inhibitor [*PSTI*], amylase-alpha, and plasminogen-binding protein [*PBP*] peptide of *Helicobacter pylori*) might be induced by decreased naïve Tregs. Th2 immune responses are followed by a Th1-type immune response with the release of proinflammatory cytokines (interferon-γ [*IFN-*γ], interleukin [*IL*]-1beta, IL-2, tumor necrosis factor-α [*TNF-*α]). Th2-type immune responses, producing IgG, IgG4, and autoantibodies may be involved in the pathophysiology of progression. IgG4 and fibrosis may be regulated by increased IL-10 and transforming growth factor-β (*TGF-*β), respectively, secreted from inducible memory Tregs. *DC* ductal cell, *iTreg* inducible Treg, *TE* effector T cell, *nTreg* natural Treg

that the depletion of naïve Tregs in the periphery [80] and major histocompatibility complex (MHC)-class II restricted-autoreactive $CD4^+$ T cells that escape from positive selection in the thymus, may play 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 cytotoxic effects through Fas ligand expression [81]. On the other hand, $CD8^+$ T cells may play roles as effector cells in the MHC class II-deficient mouse [82] and in WBN/Kob rat models [83]. WBN/Kob rats with congenitally decreased peripheral Tregs spontaneously develop sialadenitis, thyroiditis, sclerotic cholangitis, and TIN. Although the target antigens remain unclear, $CD8^+$ cells also seem to be effectors. Although rodents lack the IgG4 subclass, 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 [83]. These animal models suggest that although $CD8^+$ T cells may be partially involved, $CD4^+$ T cells play major roles in the development of experimental systemic lesions, which are similar to the lesions in human IgG4-related diseases [4, 58], although the counterpart of IgG4 in mouse IgG subclasses has not been identified. As TGF-beta is an important regulatory factor in maintaining immune homeostasis [84], TGF-beta-dominant negative mutant mice suggest that the loss of TGF-beta signaling may contribute to AIP [85].

From the above findings, we propose a hypothesis for the pathogenesis of AIP (Fig. 1). The basic concept is a biphasic mechanism of "induction" and "progression." An initial response to self-antigens (e.g., LF, CA-II, CA-IV, PSTI, amylase-alpha, and PBP peptide of *H. pylori*) might be induced by decreased naïve Tregs, followed by a Th1-type immune response with the release of proinflammatory cytokines (IFN-gamma, IL-1-beta, IL-2, TNF-alpha). Then Th2-type immune responses producing IgG, IgG4, and autoantibodies may be involved in the pathophysiology of progression. IgG4 and fibrosis may be regulated by increased IL-10 and TGF-beta, respectively, secreted from inducible memoryTregs. 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 play important roles in the progression as well as the induction of the disease, further studies are necessary to clarify the pathogenesis, including studies of genetic backgrounds, disease-specific antigens, and the role of IgG4.

Acknowledgments This study was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Culture and Science of Japan (20590810), and a Grant-in-Aid for the "Research for Intractable Disease" Program from the Ministry of Health, Labor and Welfare of Japan.

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Involvement of Inducible Costimulator– and Interleukin 10–Positive Regulatory T Cells in the Development of IgG4-Related Autoimmune Pancreatitis

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Objectives: Immunoglobulin G4 (IgG4)–related autoimmune pancreatitis (AIP) is a new clinical entity of pancreatic disorder. There are immunologic and histological abnormalities, including increased serum IgG4 levels and the infiltration of IgG4-positive plasmacytes. However, the role of IgG4 is unclear. Recently, regulatory T cells (Tregs) were reported to contribute to the development of various autoimmune diseases as well as in B-cell shifting to IgG4-producing plasmacytes. We studied Tregs in the pancreas and peripheral blood.

Methods: We recruited 44 patients with IgG4-related AIP. For comparison, we recruited 37 patients with other pancreatic diseases and 27 healthy subjects as controls. We studied infiltrating cells in the pancreas by immunohistochemistry and analyzed inducible costimulator–positive Tregs and interleukin 10–positive Tregs in the peripheral blood by flow cytometry.

Results: The ratio of Foxp3-positive cells to infiltrated mononuclear cells (Foxp3/Mono) in AIP patients was significantly higher than in patients with alcoholic chronic pancreatitis. In AIP, Foxp3/Mono and IgG4/Mono were positively correlated. Inducible costimulator–positive Tregs were significantly higher in AIP patients than in the patients with other pancreatic diseases and the healthy control group. Interleukin 10–positive Tregs were significantly higher in AIP patients than in the healthy control group.

Conclusions: Increased quantities of inducible costimulator–positive Tregs may influence IgG4 production in IgG4-related AIP.

Key Words: IgG4-related disease, autoimmune pancreatitis (AIP), regulatory T cells (Tregs), IgG4, IL-10, inducible costimulator (ICOS)

(*Pancreas* 2011;40: 1120–1130)

In 1961, Sarles et al[1] observed the first case of idiopathic chronic pancreatitis (CP) with hypergammaglobulinemia, in which an autoimmune mechanism was supposedly involved. In 1991, Kawaguchi et al[2] reported 2 cases of an unusual lymphoplasmacytic sclerosing inflammatory disease involving the total pancreas, common bile duct, gallbladder, and, in 1 patient, the lip. In addition, 2 patients presented mass-like enlargement of the pancreatic head. Histopathologic characteristics included diffuse lymphoplasmacytic infiltration, marked interstitial fibrosis, acinar atrophy, and obliterative phlebitis of the pancreatic and portal veins, which was termed as lymphoplasmacytic sclerosing pancreatitis (LPSP). In 1995, Yoshida et al[3] first proposed the concept of "autoimmune pancreatitis (AIP)," in which patients showed a diffusely enlarged pancreas, a narrowing pancreatogram, increased serum immunoglobulin G (IgG), the presence of autoantibodies, fibrotic changes with lymphocytic infiltration, and steroidal efficacy. In 2001, Hamano et al[4] reported that elevated serum IgG4 levels were highly specific and sensitive for the diagnosis of AIP. In 2003, Kamisawa et al[5] suggested that AIP is a systemic disease, based on the findings that the pancreas and other involved organs have abundant infiltration of IgG4-positive plasma cells. Thereafter, many AIP cases have been reported by Japanese investigators, and AIP has been accepted as a new clinical entity.[6–11]

On the other hand, reports from Europe[12] and the United States[13] described unique histological patterns in the resected pancreata of patients with mass-forming, chronic, nonalcoholic pancreatitis with epithelial destruction by granulocytes, which is now supposed to be distinguishable from IgG4-related AIP (or type 1 AIP) and called idiopathic duct centric pancreatitis (IDCP) or AIP with granulocyte epithelial lesions (AIP with GELs) or type 2 AIP.[14] Most of the Japanese AIP cases are LPSP, whereas those concerning IDCP are very few. Although we recently reported the first case of IDCP in Japan with full radiological and histopathologic findings,[15] it still remains unclear whether the clinical manifestations of the Japanese patients with IDCP are similar to those of Western countries. Therefore, Japanese consensus clinical guidelines have focused on IgG4-related AIP (LPSP).[9–11] An overlap in the histological features of the 2 patterns may exist in some patients. Although the pathogenesis is still unclear, the most important issue in managing AIP is to differentiate it from pancreas and biliary malignancy.

Great attention has been focused on the relation between various autoimmune diseases and regulatory T cells (Tregs), which are present in human peripheral blood,[16–21] intestinal lamina propria,[22] and the thymus.[20,21] Recent reports have shown that Tregs can be classified into 2 groups: (1) naturally occurring Tregs expressing $CD4^+CD25^{high}$ and (2) naive Tregs expressing the naive T-cell marker CD45RA in addition to CD4 and CD25. Naive Tregs also expressed very high levels of mRNA for Foxp3 and manifested equivalent suppressive activity in vitro.[23,24] We have previously reported that increased quantities of $CD4^+CD25^{high}$ Tregs may influence IgG4 production, and naive Tregs may be involved in the development of AIP.[25] Patients with IgG4-related AIP often show high serum levels of IgG4, but the pathologic significance and mechanism are unknown. Immunoglobulin 4 is well known as a T-helper 2 (T_H2)–dependent isotype. Interleukin (IL) 4 directs naive human B cells to switch to IgG4 and IgE production.[26] Interleukin 10 promotes isotype switching from IgM to IgG1, IgG3, IgG4, and/or IgE. Interleukin 10 also stimulates IgG4 production.[27] The association with the IL-10 response still holds and has strengthened, with the

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Received for publication September 21, 2010; accepted April 14, 2011.

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This study was partially supported by (1) Grant-in-Aid for Scientific Research (C) of the Ministry of Culture and Science of Japan (20590810, 23591017-0001), (2) Intractable Diseases, the Health and Labor Sciences Research Grants (KO) from the Minister of Labor and Welfare of Japan, and (3) grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan, from CREST Japan Science and Technology Agency.

TABLE 1. Profiles of Subjects in the Present Study

	n	Sex, n (Female/Male)	Age, Mean (SD), y
Healthy control	27	10/17	61 (13)
Alcoholic CP	20	0/20	59 (11)
Idiopathic CP	17	10/7	55 (15)
AIP	44	14/30	65 (9)
Without steroid	21	9/12	64 (14)
With steroid	23	5/18	67 (8)

development of IL-10–producing Tregs in vivo being found to be inducible costimulator (ICOS) dependent.[28,29] Of note, a recent report revealed the existence of 2 functional subsets of human Foxp3$^+$ Tregs, including ICOS$^+$ cells, which produced high amounts of IL-10.[30] However, the relationship between Tregs in the peripheral blood and the pancreas remains unclear. In this study, we attempt to clarify that relationship by comparing Tregs in the peripheral blood and the pancreatic tissues.

MATERIALS AND METHODS

Subjects

We examined 44 patients with AIP at Kansai Medical University and its affiliated hospitals (21 untreated patients and 23 patients treated with corticosteroids; 14 women and 30 men; mean age, 65 years; range, 42–81 years). There were 20 patients with alcoholic pancreatitis (0 woman, 20 men; mean age, 59 years; range, 38–75 years), 17 patients with idiopathic pancreatitis (10 women, 7 men; mean age, 55 years; range, 20–72 years), and 27 healthy volunteers (10 women, 17 men; mean age, 61 years; range, 33–82 years), who served as control subjects. Nine AIP cases (6 women and 3 men; mean age, 54 years; range, 56–73 years) and 9 alcoholic pancreatitis cases (9 men; mean age, 53 years; range, 39–75 years) were surgery cases in that. All cases were collected between 1992 and 2009 (Table 1). All the AIP patients were diagnosed according to the clinical diagnostic criteria for AIP proposed by the Research Committee of Intractable Diseases of the Pancreas supported by the Japanese Ministry of Health, Labor, and Welfare,[31] and the Asian criteria.[32] Thirty-five AIP patients fulfilled Japanese diagnostic criteria 1 and 2. In brief, they exhibited diffuse enlargement of the pancreas and diffuse narrowing of the main pancreatic duct with an irregular wall. They also showed high levels of serum γ-globulin (>2 g/dL), IgG (>1800 mg/dL), and IgG4 (>135 mg/dL). Because 9 cases showed LPSP with a pancreatectomy specimen, AIP was diagnosed. The diagnosis of CP was made by imaging studies (ultrasound, computed tomography, endoscopic retrograde cholangiopancreatography, and magnetic resonance cholangiopancreatography), a pancreatic function test (secretin stimulation test), or histopathologic examination according to the clinical diagnosis criteria of the Japan Pancreatic Society.[33] Alcoholic CP was considered to be alcoholic in origin if the patient had a daily alcohol intake of more than 80 g/d for more than 10 years. Patients had a diagnosis of idiopathic CP when no apparent causes such as alcoholism, gallstones, or autoimmunity could be identified. This study was approved by the Kansai Medical University's ethics committee, and all patients and healthy volunteers gave informed consents.

Histopathology and Immunohistochemistry

Formalin-fixed and paraffin-embedded specimens were prepared and used for histopathologic and immunohistochemical studies. Sections measuring 4 μm were cut from each paraffin block and stained with hematoxylin and eosin, periodic acid-Schiff after diastase digestion, Azan-Mallory, reticulin, or orcein. The remaining material was used for immunohistochemical analysis. Immunoglobulin G4 immunostaining was performed using a monoclonal antibody for human IgG4 (ZYMED Laboratories, San Francisco, Calif). Immunoglobulin 1 and Foxp3 immunostaining was performed by avidin-biotin complex method with reagents provided by Vector Laboratories (Burlingame, Calif). The antibodies used to identify the inflammatory cells in the pancreas were IgG1 antibodies (Binding Site, Birmingham, UK) and Foxp3 (eBioscience, San Diego, Calif). The deparaffinized sections were pretreated in an EDTA buffer (pH 8.0) in a pressure cooker at 100°C for 5 minutes. After incubation with the first antibody at 4°C overnight, biotinylated rabbit anti–sheep serum IgG (Vector) was used as the secondary antibody (sections for IgG1), and immunoreactive deposits were visualized with 3,3-diaminobenzidine tetrahydrochloride. Cells positive for IgG1, IgG4, and Foxp3 were counted under 5 different high-power fields with intense inflammation. The ratios between IgG1, IgG4, Foxp3-positive cells and infiltrated mononuclear cells were calculated in each case by 2 pathologists.[34]

Flow Cytometric Analysis of Tregs in Peripheral Blood Cells

Flow cytometric analysis was performed according to the method described by Okazaki et al.[35] Briefly, cells were stained with fluorescein isothiocyanate–conjugated anti-CD4 (BD Bioscience, Franklin Lakes, NJ), PE-Cy7–conjugated anti-ICOS (CD278) (eBioscience), and allophycocyanin-conjugated anti-CD25 (BD Bioscience), for Tregs at 4°C for 30 minutes. After staining, the cells were fixed in 1% paraformaldehyde, and 3 color flow cytometric analyses were performed using FACS-LSR (BD Biosciences) and FlowJo (FlowJo LLC, Ashland, Ore).

Flow Cytometric Analysis of Intracellular IL-10

Intracellular staining of IL-10 was performed according to the manufacturer's instruction. In brief, cells magnetically labeled with CD4 and CD25 microbeads (CD4$^+$CD25^{high} Treg Isolation Kit; Miltenyi Biotec, Bergisch Gladbach, Germany) were loaded onto auto MACS (Miltenyi Biotec). The cells were then washed with phosphate-buffered saline before analysis. For intracellular staining, CD4$^+$CD25^{high} Tregs were stimulated with phorbol 12-myristate 13-acetate, ionomycin for 2 to 4 hours before adding Golgi stop/Golgi plug solution (BD Biosciences). The cells were then stained with fluorescein isothiocyanate–conjugated anti-CD4, allophycocyanin-conjugated anti-CD25, and PE-Cy7–conjugated anti-ICOS (CD278) (eBioscience). After fixation with a fixation buffer (eBioscience) for 20 minutes at 4°C, the cells were permeabilized with a permeabilization buffer (eBioscience) and counterstained with PE-conjugated anti–IL-10 for 30 minutes. Stained cells were analyzed using FACS-LSR (BD Biosciences) and FlowJo (FlowJo LLC).

Statistical Analysis

For all studies, data are expressed as mean (SD); differences were analyzed using the nonparametric Mann-Whitney rank test and Fisher exact test, where $P < 0.05$ was considered significant.

RESULTS

Immunohistochemical Findings of IgG1 and IgG4

The ratio of IgG4-positive plasma cells to infiltrated mononuclear cells (IgG4/Mono) was significantly higher in AIP (n = 9, 0.122 [SD, 0.049]) than in alcoholic CP (n = 9, 0.013 [SD, 0.007]; $P < 0.05$; Figs. 1–3). The ratio of IgG1-positive plasma cells to infiltrated mononuclear cells (IgG1/Mono) was significantly lower in AIP (n = 9, 0.041 [SD, 0.024]) than in