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

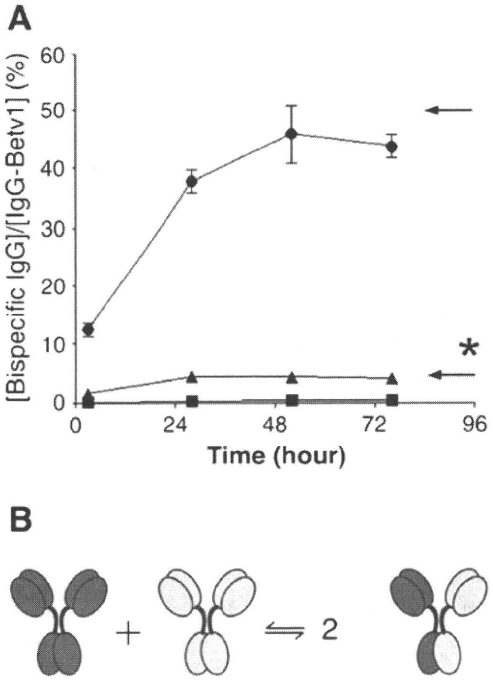

Fig. 4 Characteristic forms of IgG4. **a** Schematic representation of the generation of bispecific IgG4 antibodies by the exchange of half-molecules ("Fab-arm exchange"; cited from [46]). IgG4 Fab arm exchange occurs by the exchange of a heavy chain-light chain pair (half-molecule) of one IgG4 molecule 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.

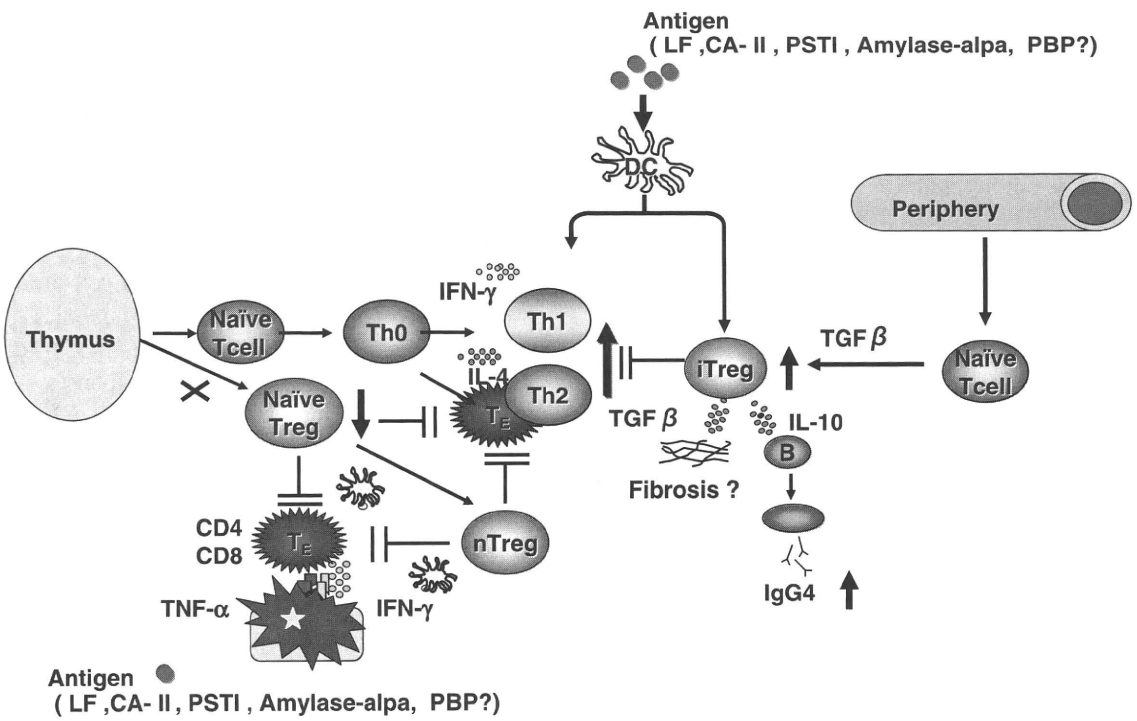

Fig. 5 Hypothesis for the pathogenesis of AIP and IgG4-related disease. In the central tolerance, naïve, and natural regulatory T cells (*Tregs*) derived from the thymus suppress autoreactive CD4 or CD8 cells in the normal state. In the IgG4-related disease, the basic concept is the biphasic mechanism 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.

Acknowledgment 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 "Research for Intractable Disease" Program from the Ministry of Health, Labor and Welfare of Japan.

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Langenbecks Arch Surg
DOI 10.1007/s00423-010-0714-2

NEW SURGICAL HORIZONS

Autoimmune pancreatitis—a new evolving pancreatic disease?

Kazuichi Okazaki · Kazushige Uchida · Toshiro Fukui · Makoto Takaoka · Akiyoshi Nishio

Received: 7 January 2010 / Accepted: 25 January 2010

Abstract
Introduction Recent advances support the concept of autoimmune pancreatitis as a unique systemic disease because occasional extrapancreatic lesions such as sclerosing cholangitis, sclerosing sialoadenitis, and retroperitoneal fibrosis show similar pathological features with fibrosis and abundant infiltration of IgG4-positive plasma cells, and are steroid responsive. Based on these findings, several diagnostic criteria have been proposed.
Materials and methods Although AIP is accepted worldwide as a unique clinical entity, pathogeneic mechanism still remains unclear. To clarify it, genetic background, humoral immunity, candidates of target antigens including self-antigens and molecular mimicry from microbes, cellular immunity including regulatory T cells, complement system, and experimental models are reviewed.
Results Based on these findings, we have proposed a hypothesis for the pathogenesis of AIP in the biphasic mechanism of "induction" and "progression." In the early stage, initial response to self-antigens (LF, CA-II, CA-IV, PSTI, or α-fodrin) or molecular mimicry (*Helicobacter pylori*) is induced by decreased naive regulatory T cells (Tregs), and Th1 cells release proinflammatory cytokines (IFN-γ, IL-1b, IL-2, and TNF-α).
Discussion In the chronic stage, progression is supported by increased memory Tregs and Th2 immune responses. The classical pathway of complement system may be activated by IgG1 immune complex.
Conclusion As Tregs seem to take important roles in progression as well as induction of the disease, further studies are necessary to clarify the pathogenesis.

Keywords Autoimmune pancreatitis · IgG4 · Diagnostic criteria · IgG4-related diseases · LPSP

Abbreviations

Anti-CA-II	Carbonic anhydrase II
AIP	Autoimmune pancreatitis
LF	Lactoferrin
AMA	Anti-mitochondrial antibody
ANA	Antinuclear antibody
CA-II	Carbonic anhydrase II
ERCP	Endoscopic retrograde cholangio-pancreatography
IFN-γ	Interferon-γ
IL-4	Interleukin-4
LF	Lactoferrin
PBC	Primary biliary cirrhosis
PSC	Primary sclerosing cholangitis
RF	Rheumatoid factor
SjS	Sjögren's syndrome

Introduction

In 1961, Sarles et al. firstly observed a case of particular pancreatitis with hypergammaglobulinemia [1], which appears to be identical to autoimmune pancreatitis (AIP). In 1992, Toki et al. [2] have reported four cases with unusual diffuse irregular narrowing of the main pancreatic duct and diffuse enlargement of the entire pancreas with lymphocyte infiltration. Yoshida et al. firstly reported such a case as AIP [3]. The histopathological findings of AIP are

K. Okazaki (✉) · K. Uchida · T. Fukui · M. Takaoka · A. Nishio
The Third Department of Internal Medicine,
Division of Gastroenterology and Hepatology,
Kansai Medical University,
Shinmachi,
Hirakata, Osaka 573-1197, Japan
e-mail: okazaki@hirakata.kmu.ac.jp

Published online: 24 September 2010

characterized by the periductal localization of predominantly CD4-positive T cells, IgG4-positive plasma cells, storiform fibrosis with acinar cell atrophy frequently resulting in the stenosis of the main pancreatic, and obliterative fibrosis [4, 5], which is called as lymphoplasmacytic sclerosing pancreatitis (LPSP) [6]. During the last decade, autoimmune pancreatitis has been intensively studied and is now accepted as a new clinical entity [7]. Moreover, several diagnostic criteria for AIP have been proposed from Japan [8, 9], Korea [10, 11], Asian consensus [12], Mayo clinic [13, 14], and Verona [15, 16]. However, pathogenesis still remains unclear, although some investigators have paid great attentions to the pathogenesis and pathophysiology AIP, especially in genetic analysis [17, 18], IgG4 [19], disease-associated autoantibodies [20–23], complement system [24], and extrapancreatic lesions [4, 5, 10–13, 25–31].

Here, we discuss the recent advances in the concept, diagnosis, pathophysiology, and treatment of AIP.

Recent advances in the concept of AIP

Autoimmune pancreatitis is a concept of disease originally proposed in Japan [3]. Because its characteristics are associated with evidence of possible involvement of autoimmune mechanisms such as hypergammaglobulinemia, increased serum levels of IgG or IgG4, presence of autoantibodies, and effective response to steroid therapy, the disease is defined as pancreatitis in which pathogenesis could possibly involve autoimmune mechanisms. It is commonly observed in elderly males and is comparable with LPSP, which is characterized by histopathological findings of abundant infiltration of lymphocytes and plasmacytes, infiltration of IgG4-positive plasmacytes, storiform fibrosis, and obstructive phlebitis [6]. The characteristic clinical findings in most cases of AIP can be summarized as follows [5] (Table 1): (1) mild abdominal symptoms, usually without acute attacks of pancreatitis; (2) occasional existence of obstructive jaundice; (3) increased levels of serum gammaglobulin, IgG or IgG4; (4) presence of autoantibodies; (5) diffuse enlargement of the pancreas with capsule-like low-density rim; (6) irregular narrowing of the pancreatic duct (sclerosing pancreatitis) on endoscopic retrograde cholangiopancreatographic (ERCP) images; (7) histological LPSP; (8) occasional association with extrapancreatic lesions, such as sclerosing cholangitis similar to primary sclerosing cholangitis (PSC), sclerosing cholecystitis, sclerosing sialadenitis, retroperitoneal fibrosis, interstitial renal tubular disorders, enlarged celiac and hilar lymph nodes, chronic thyroiditis, and pseudotumor of the liver; and (9) effective steroid therapy [5]. These suggest that it may be a systemic disorder. Therefore, the following concepts have been proposed: IgG4-related systemic sclerosing disease [32], systemic IgG4-related plasmacytic syndrome [33], and IgG4-positive multi-organ lymphoproliferative syndrome (IgG4 MOLPS.) [34]. Because sialadenitis, in most cases, is found negative for both the anti-SSA antibody and anti-SSB antibody that are distinctive from Sjögren's syndrome [5], and the histopathological images show pronounced infiltration of IgG4-positive plasmacytes seen in Mikulicz's disease and Kűttner's tumor, AIP is considered to be different from typical Sjögren's syndrome. Since sclerosing cholangitis-like lesions seen in patients with AIP show different responses to steroids and different prognosis from those with PSC, and AIP is characterized by the infiltration of IgG4-producing plasmacytes, the two diseases are considered to be different pathological conditions.

Cases in young patients associated with ulcerative colitis, commonly reported in Europe and the USA, show

Table 1 Clinical characteristics of autoimmune pancreatitis

Age and genders
More common in elderly males
Clinical symptoms
Mild abdominal symptoms, usually without acute attacks of pancreatitis
Occasional existence of obstructive jaundice
Laboratory data
Increased levels of serum gammaglobulin, IgG, or IgG4
Presence of autoantibodies
Increased hepatobiliary or pancreatic enzymes
Impaired exocrine and endocrine function
Imaging of the pancreatobiliary system
Enlargement of the pancreas
Irregular narrowing of the pancreatic duct
Stenosis of intra-pancreatic bile duct
Sclerosing cholangitis similar to PSC
Histopathologic findings of the pancreas
Interlobular fibrosis
Atrophic pancreatic lobule
Infiltration of lymphocyte and IgG4-positive plasmacyte
Obliterative thrombo-phlebitis
Occasional extrapancreatic lesions
Sclerosing cholangitis similar to PSC
Sclerosing sialoadenitis
Retroperitoneal fibrosis
Interstitial nephritis
Chronic thyroiditis
Interstitial pneumonia
Lymphadenopathy (mediastinum/periponeum)
Occasional association with other autoimmune diseases
Effective steroid therapy
Prognosis
Unclear long-term prognosis
Pancreatic stone formation in some cases

pathological neutrophilic lesions and are called idiopathic duct-centric chronic pancreatitis (IDCP) [35] or granulocyte epithelial lesions (GEL) [36]. Although their image findings show resemblance to those of AIP, there are not enough serological findings so it is highly possible that their pathological conditions are different from LPSP [37]. Therefore, it still remains debatable whether LPSP and IDCP can be classified as the same clinical entity of AIP or not. Since most cases of AIP show a diffusely enlarged pancreas and narrowing of the main pancreatic duct, it is believed that typical AIP lesions spread to over one third of the pancreas; however, there are also cases of localized lesions or mass-forming type [13]. Although the long-term prognosis of AIP is not clear, the formation of pancreatic stones has been reported.

Further studies are necessary to clarify whether pathogenetic mechanism of GEL is different from LPSP or not.

Diagnosis and differential diagnosis of AIP

The biggest problem in diagnosing AIP is how to distinguish it from pancreatic or biliary cancer [9, 37]. Although histological findings of LPSP can suggest AIP, it is usually difficult to obtain enough specimens from the pancreas. In pancreatic images, low-echoic swelling on US, sausage-like swelling with capsule-like rim and homogeneously delayed enhancement on CT, low-intensity on T1-weighted MR image, and diffuse narrowing of the main pancreatic duct on ERCP images are characteristic [5]. Increased serum levels of gammaglobulin, IgG, especially IgG4, IgG4 subclass of immune complexes, or autoantibodies such as ANF, ALF, ACA-II, and rheumatoid factor (RF) are useful for the diagnosis of AIP [4, 5, 21, 22]. Among them, serum IgG4 is the best marker for diagnosing AIP at this moment, although it is not necessarily specific for it [7]. Although the majority of AIP can be distinguished from other diseases with clinical features, radiological imaging, and immunological markers, some cases are difficult to be differed from pancreas or bile duct cancer [5]. To diagnose AIP, several diagnostic criteria have been proposed from Japan (Table 2) [8, 9], Korea (Table 3) [10, 11], Asian consensus (Table 4) [12], USA (Table 5) [13, 14], and Verona (Table 6) [15, 16]. Each criterion is fundamentally based on the Japanese original diagnostic criteria proposed by Japan Pancreas Society in 2002 [8], although there are several differences (Table 7). Mayo criteria are more based on histopathological findings, whereas Japanese and Korean criteria are on pancreatic images for practical use. The most difference among them is whether IDCP/GEL and LPSP are classified as the same clinical entity of AIP or not. In Asian consensus, and Japanese and Korean criteria, only LPSP but not IDCP/GEL is defined as AIP [8–12] because IDCP/GLE is quite rare in these countries. On the other hand, LPSP is defined as one AIP in Italian criteria [16] because IDCP/GEL cases are more often observed than LPSP in Europe. LPSP and IDCP cases are similarly observed in USA and classified as type 1 and type 2 in revised Mayo's criteria, respectively [14]. Even if any of them are used, it is noted that pancreatic cancer may accompany with AIP [38, 39]. The diagnostic algorithm based on the Asian consensus proposed by the Japanese guidelines [37] has been shown (Fig. 1)

Pathogenesis and pathophysiology of AIP

Genetic backgrounds

Immuno-genetic backgrounds have been studied in a few series of AIP and then not conclusive. Susceptibility to AIP may be associated with genetic factors such as the class II antigen of the major histocompatibility complex (MHC), polymorphism of nuclear factor (NF)-kB, and Fc-receptor-like (FCRL) 3 genes expressed on B cells [17, 18]. Two studies of HLA association with AIP have been reported from the Japanese [17] and Korean group [18]. In the Japanese patients with AIP, HLA haplotype DRB1*0405-DQB1*0401 (class II) and ABCF1 proximal to C3-2-11, telomeric HLA-E (class I) is susceptible to AIP [17], but not in the Korean patients [18]. However, substitution of aspartic acid to nonaspartic acid at DQβ1 may be a

Table 2 Japanese clinical diagnostic criteria [9]

1. Diffuse or segmental narrowing of the main pancreatic duct with irregular wall and diffuse or localized enlargement of the pancreas by imaging studies, such as abdominal ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI)
2. High serum γ-globulin, IgG or IgG4, or the presence of autoantibodies, such as antinuclear antibodies and rheumatoid factor
3. Marked inter-lobular fibrosis and prominent infiltration of lymphocytes and plasma cells in the periductal area, occasionally with lymphoid follicles in the pancreas

For diagnosis, criterion 1 must be present, together with criterions 2 and/or 3

Diagnosis of autoimmune pancreatitis is established when criterion 1, together with criterion 2 and/or 3, is fulfilled

However, it is necessary to exclude malignant diseases such as pancreatic or biliary cancers

Table 3 Korean diagnostic criteria [11]

Definite diagnosis: criterion I together with any of criteria II to IV
Criterion I. Imaging (both required)
Imaging (CT or MRI) of pancreatic parenchyma; diffusely/segmentally/focally enlarged gland, occasionally with mass and/or hypoattenuation rim
Imaging (ERCP or MRCP) of pancreaticobiliary ducts; diffuse/segmental/focal pancreatic ductal narrowing, often with the stensosis of bile duct
Criterion II. Serology (one required)
Elevated level of serum IgG or IgG4
Detected autoantibodies
Criterion III. Histopathology of pancreatic/extrapancreatic lesions (one required)
Lymphoplasmacytic infiltration & fibrosis, often with obliterative phlebitis
Presence of abundant (>10 cells/HPF) IgG4-positive plasma cells
Criterion IV. Response to steroids
Resolution/marked improvement of pancreatic/extrapancreatic lesion with steroid therapy
Probable diagnosis: criterion V or VI
Criterion V
Unexplained pancreatic disease but only with characteristic pancreatic histology
Criterion VI. (Both required)
Other organ involvement and/or serologic abnormalities
Various atypical pancreatic imaging suggesting chronic pancreatitis with negative workup for known etiologies

predictive factor for relapse of AIP in Korean patients [18]. FCRL3 polymorphisms are linked to various autoimmune diseases, such as rheumatoid arthritis, autoimmune thyroid disease, and systemic lupus erythematosus in the Japanese population [40, 41]. 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 distinct underlying mechanisms of disease development [41].

A few immuno-genetic 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 TLR4 gene have been linked with several autoimmune and allergic diseases, it does not seem to play an important role in the development of AIP [42]. 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 [43] and Japanese population [44]. 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 [45]. Uemura et al. [44] reported that the 3′ untranslated region of *CTLA-4* +6230 SNP plays a pivotal role for both susceptibility (+6230G/G genotype) to and protection (haplotype of the +6230A allele) from AIP, while exon 1+49 SNP 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 TNF-a promoter 863A was significantly associated with higher risk of AIP. Racial and geographical differences may be associated with SNPs of the different locus of CTLA-4 [43]. Soluble isoform of CTLA4 (sCTLA4) is reported to be elevated in patients with autoimmune diseases, such as autoimmune thyroid disease, systemic lupus erythematosus, and myasthenia gravis [44]. 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 [46]. 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 [46]. The

Table 4 Asian criteria [12]

Criterion I. Imaging (both required)
Imaging of pancreatic parenchyma; diffusely/segmentally/focally enlarged gland, occasionally with mass and/or hypoattenuation rim
Imaging of pancreatic parenchyma; diffusely/segmentally/focally enlarged gland, occasionally with mass and/or hypoattenuation rimImaging of pancreaticobiliary ducts; diffuse/segmental/focal pancreatic ductal narrowing, often with the stenosis of bile duct
Criterion II. Serology (one required)
Elevated level of serum IgG or IgG4
Detected autoantibodies
Criterion III. Histopathology of pancreatic biopsy lesion
Lymphoplasmacytic infiltration in fibrosis, common with abundant IgG4-positive cell infiltration
Option: response to steroids
Diagnostic trial of steroid therapy could be done carefully in patients fulfilling criterion 1 alone with negative workup for pancreatobiliary cancer by experts
Diagnosis of AIP is made when any two criteria including criterion I are satisfied or histology of lymphoplasmacytic sclerosing pancreatitis is present in the resected pancreas

Table 5 Revised HISORt criteria: definitions [14]

Category	1: Highly suggestive/diagnostic of AIP	2: Indeterminate/supportive of AIP	3: Highly suggestive/diagnostic of PaC
H, Histology (pancreatic core biopsy or resection specimen)	Any of these: 1 (A) Lymphoplasmacytic sclerosing pancreatitis or (B) Abundant (>10 cells/hpf) IgG4-positive cells with ≥2 of the following: periductal lymphoplasmacytic infiltrate obliterative fibrosis 2 (A) Idiopathetic duct-centric pancreatitis or (B) Granulocyte epithelial lesion in pancreatic duct with minimal IgG4-positive cells in pancreatic parenchyma [20–23]	Storiform fibrosis with lymphoplasmacytic infiltrate	Positive for cancer on cytology/resection specimen of pancreas or other organ
I, Imaging of pancreas	Diffusely enlarged gland with featureless borders and delayed enhancement with or without capsule-like rim No features highly suggestive of cancer (I3, S3, or O3)	Focally enlarged gland without features highly suggestive of cancer (I3 or O3)	Any of these: Low density mass Pancreatic ductal dilatation Pancreatic duct cutoff Upstream parenchymal atrophy
S, Serology	Serum IgG4 ≥twice the upper limit of normal	Elevated serum IgG4 (<twice the upper limit or normal)	Elevated Ca 19-9 >150 IU/ml after biliary decompression
O, Other organ involvement	Typical histology (e.g., bile duct resection specimen, salivary gland) or Typical radiologic features+positive IgG4 immunostaining in affected organ	Radiologic evidence of Hilar/intrahepatic biliary strictures Renal involvement Retroperitoneal fibrosis Parotid/lacriminal gland enlargement Positive IgG4 immunostaining in organs not noted above (e.g., gallbladder, ampulla) Inflammatory bowel disease[a]	Liver lesions suggestive of or biopsy-proven metastases
Rt, Response to steroid treatment	Resolution/marked improvement in pancreatic/extrapancreatic manifestation		No response or increase in size of pancreatic mass No improvement in biliary stricture Rising CA 19-9

[a] Seen especially in association with idiopathic duct-centric pancreatitis (up to 30%); only 6% of lymphoplasmacytic sclerosing pancreatitis has inflammatory bowel disease in which it is generally not included as other organ involvement

Table 6 Verona diagnostic criteria [16]

Suggestive radiological features (CT or MR)	Diffuse or focal involvement of the pancreas
	Delayed enhancement in the involved parenchyma
	No dilation of the main pancreatic duct in diffuse form
	No extrapancreatic or vascular involvement
Association with autoimmune diseases	Ulcerative colitis, Crohn's disease, Sjögren's syndrome, primary biliary cirrhosis, primary sclerosing cholangitis, retroperitoneal fibrosis, autoimmune thyroiditis, tubulointerstitial nephritis, uveitis, and Mikulicz's disease
Consistent cytological or histological features	Periductal lymphoplasmacytic infiltration
	Presence of granulocytic epithelial lesions
	Negative for epithelial atypia
Response to steroid therapy	Clinical: resolution of symptoms/signs of AIP
	Radiological (CT or MR): disappearance/significant reduction in the size of the involved pancreas, normalization of the main pancreatic duct

ratios for each IgG subclass were 65% of IgG1, 25% of IgG2, 6% of IgG3, and 4% of IgG4 [46]. In AIP as one of IgG4-related diseases, total IgG, IgG1, IgG2, IgG4, and IgE were 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, 47].

Although the association with IgE-mediated allergy and IgG4 antibodies has been well known [48], IgG4 has still poorly understood characteristics. 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 attaching a light chain (half-molecule) with a heavy-light chain pair from another molecule [49], which results usually in asymmetric antibodies with two different antigen-combining sites. While these modified antibodies are hetero-bivalent, they behave as monovalent antibodies [49]. Another aspect of IgG4 mimics IgG RF activity by interacting with IgG on a solid support [50]. 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 with active stage of AIP occasionally show decreased complement (C3 and C4) with elevated circulating immune complex as well as serum levels of IgG4 and the IgG4 subclass of immune complexes [3, 51]. Deposition of immune complex has been identified in some patients of renal lesions associated with AIP [52]. However, the 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 [24]. Moreover, IgG4 bound to other isotype such as IgG1, 2, and 3 with an Fc–Fc interaction immune complex in patients with AIP [53], 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, blocking Fc-mediated effector functions of IgG1.

Autoantibodies

Patients with IgG4-related diseases generally show several autoantibodies in addition to increased IgG and IgG4 [4, 5].

Table 7 Comparison of diagnostic criteria for AIP

	JPS criteria 2006	Revised Korean criteria	Asian criteria	Revised Mayo (HISORt)	Verona criteria
ERCP with CT/MRI	Mandatory ERCP	ERCP/MRCP	Mandatory ERCP	ERCP/MRCP	ERCP/MRCP (no dilated PD)
Serology	g-glb/IgG/IgG4/autoAb	IgG/IgG4/autoAb	IgG/IgG4/autoAb	IgG/IgG4/Abs (type 1)	IgG/IgG4/autoAb
Histology	LPSP	LPSP	LPSP (resected)	LPSP/IDCP (type 1) (type 2)	LPSP/IDCP
Steroid response	No	Yes	Yes (option)	Yes	Yes
Extrapancreatic	Exclude (supportive)	Include	Exclude (supportive)	Include IgG4-relate/UC	

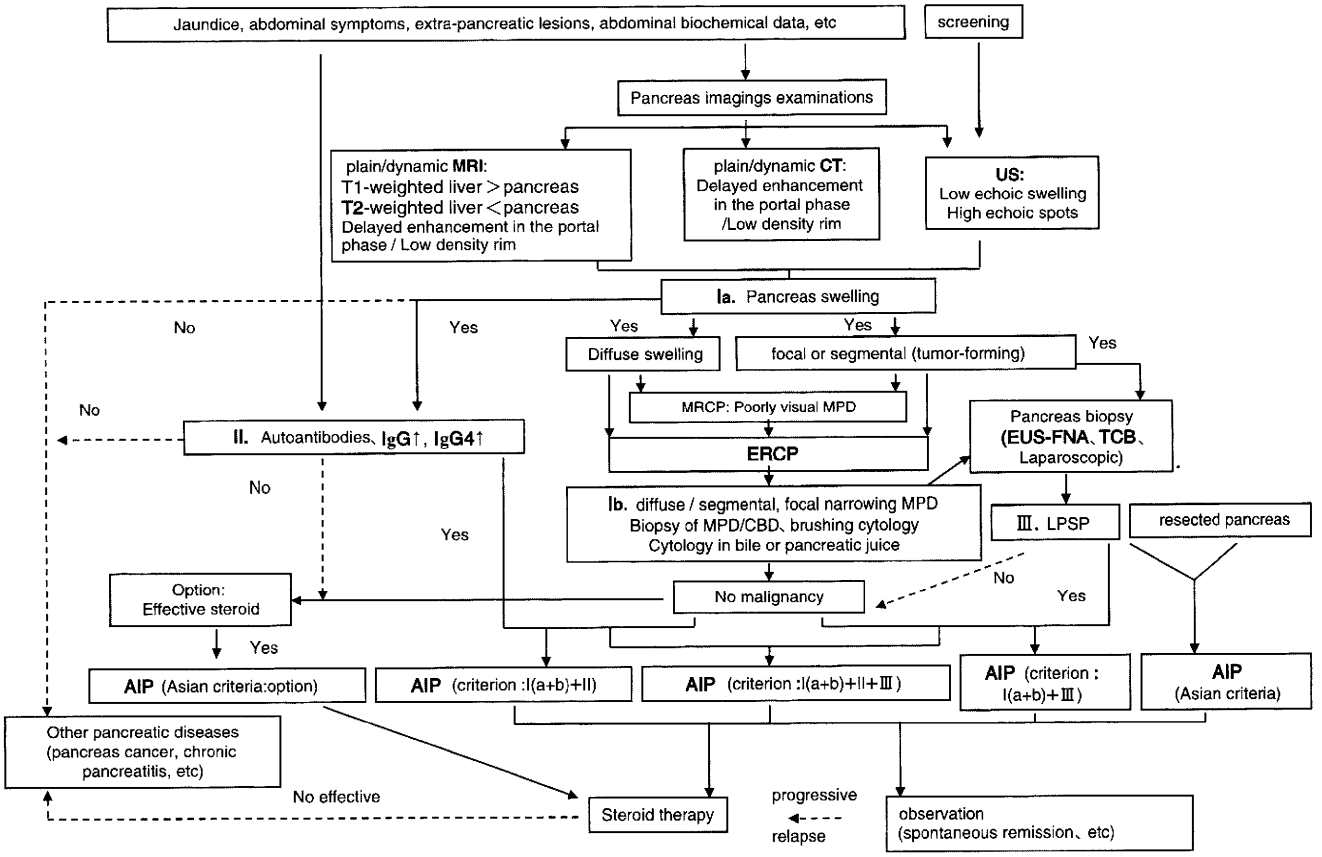

Fig. 1 Algorithm of diagnosis and management of AIP based on Asian diagnostic criteria proposed by the Japanese guidelines. A comprehensive diagnosis is performed based on pancreatic imaging, and serological and histopathological findings. In Japan, as defined by the Clinical Diagnostic Criteria 2006, the diagnosis of AIP requires specific image findings, along with serological and/or histopathological evidences. The presence of extrapancreatic lesions may suggest the possibility of AIP

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 gland, biliary tract, lung and renal tubules, and so on. Although the disease-specific antibodies have not been identified at this moment, several disease-related antibodies such as anti-lactoferrin (LF) [20, 21], anti-carbonic anhydrase (CA)-II [20, 21, 54, 55], anti-CA-IV [56], anti-pancreatic secretory trypsin inhibitor (PSTI) [22], anti-amylase-alpha [57], anti-HSP-10 [58], and anti-plasminogen-binding protein (PBP) peptide autoantibodies [59] have been reported. Although the patients show increased serum levels of IgG4, the major subclass of these autoantibody is not necessarily IgG4, but often IgG1 [22]. CA-II [21], CA-IV [56], LF [21], and PSTI [54] are distributed in the ductal cells of several exocrine organs, including the pancreas, salivary gland, biliary duct, lung, renal tubules, and so on [20, 21]. Although all peptides have not been studied, immunization with CA-II or LF induced systemic lesions such as pancreatitis, sialadenitis, cholangitis, and interstitial nephritis in the mouse models similar to human IgG4-related diseases [60, 61]. The high prevalence of these antibodies suggests that these may be the candidates for the target antigens in AIP [21].

Molecular mimicry among microbes and target antigens may be a possible mechanism to break 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 [62–64]. Guarneri and colleagues showed a significant homology between human CA-II and alpha-CA of *Helicobacter pylori*, a fundamental enzyme for bacterial survival and proliferation in the stomach [64]. Moreover, the homologous segments contain the binding motif of DRB1*0405, which confers a risk for AIP development [64]. 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 (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 in IgG4-related diseases [64]. These findings suggest that gastric *H. pylori* infection might trigger AIP in genetically predisposed subjects.

Diabetes mellitus is complicated with 43~68% of the patients with AIP, but autoantibodies against glutamic acid decarboxylase, beta-cell, or tyrosine phosphatase-like protein [65] 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.

Th1 and Th2 immune balance

The effecter cells in IgG4-related diseases have been poorly understood. Presence of autoantibodies, predominant infiltration of $CD4^+$ and $CD8^+$ T cells, and expression of HLA-DR antigens in the pancreas [20] suggest that an immunological mechanism may be involved in development of AIP as well as infiltration of plasmacytes and B cells. $CD4^+$ T cells differentiate from naive T cells (Th0) to Th1, Th2, Th17, and Tregs [66]. IL-12 induces Th1 cells, which produce IL-2, tumor necrosis factor (TNF)-a, and IFN-g; mediate cellular immunity, macrophage activation, and cytotoxicity; and help B cell production of opsonizing and complement fixing antibodies [4]. IL-4 induces Th2 cells, which produce IL-4, 5, 6, and 10, promote humoral and allergic responses [4]. TGF-b, IL-6, IL-21, and IL-23 induce Th17 cells, which secrete IL-17, which may be involved in inflammation in mice [67].

In some patients with AIP, Th1 cells but not Th17 cells are predominant over Th2-type cells in the periphery [21, 68]. On the other hand, Th2-type immune reaction is induced in the liver of IgG4-related sclerosing cholangitis as well as Th1 responses [69]. The discrepancy may be explained by the shift of Th2 cells from the periphery to local tissues, or different disease stages. Mouse models with depletion of Tregs by neonatally thymectomy (nTx) support the hypothesis that Th1 cells mainly act as effectors in the initial early stage [70]. In Sicca 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. Similarly the Sicca 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 maturation and proliferation of local B cells and plasmacytes [4].

Regulatory T cells

From naive Th0 cells, TGF-b can induce $CD4^+$ $CD25^+$ regulatory T cells (Tregs), which have 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 transforming growth factor β (TGFβ) and IL-10, and/or cell-to-cell contact via ligation of CTLA-4. Recent studies clarified several subtypes of Treg [73]. 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 [73]. As Tregs expressing Foxp3 are critical in the transfer of immune tolerance, deficient Tregs induce various autoimmune diseases in animal studies [67]. However, in human, 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 [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 still remains unclear. In AIP, in addition to increased soluble *CTLA4*, circulatory naive ($CD45RA^+$) 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 [69]. 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 naive Tregs may be pathogenetic.

Possible role of IgG4 in IgG4-related diseases

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 systemic distributed antigens described above, it still remains unclear whether IgG4 type of autoantibodies have a direct role in the pathogenesis of IgG4-related diseases or not. To date, there have been a few reports indicating IgG4 deposition in IgG4-related renal diseases [52]. Therefore, in some IgG4-related diseases, 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 of 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 liver of patients with IgG4-related sclerosing cholangitis [69]. These findings suggest that IgG4 or IgG4–immune complexes unlikely act as a pathogenetic factor but not anti-inflammatory factor in IgG4-related diseases [53]. Further studies for clarifying the role of IgG4 in IgG4-related diseases are necessary.

Our hypothesis for the pathogenesis of AIP

In nTx-BALB/c mouse 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 naive 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 the activation of macrophage 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, similarly electrophoretic position 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, 21], although a counterpart of IgG4 in mice IgG subclasses has not been identified. As tumor growth factor (TGF)-b is an important regulating factor in maintaining immune homeostasis [86], TGF-b dominant negative mutant mice suggested that loss of TGF-b signaling may contribute to autoimmune pancreatitis [87].

From the above findings, we propose a hypothesis for the pathogenesis of AIP. 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 naive Tregs Th2 immune responses followed by Th1-type immune response with release of proinflammatory cytokines (IFN-γ, IL-1b, IL-2, and TNF-α). In progression, Th2-type immune responses producing IgG, IgG4, and autoantibodies may be involved in the pathophysiology. IgG4 and fibrosis may be regulated by increased IL-10 and TGF-beta secreted from inducible-memory Tregs, respectively (Fig. 2). The classical pathway of complement system may be activated by IgG1 immune complex.

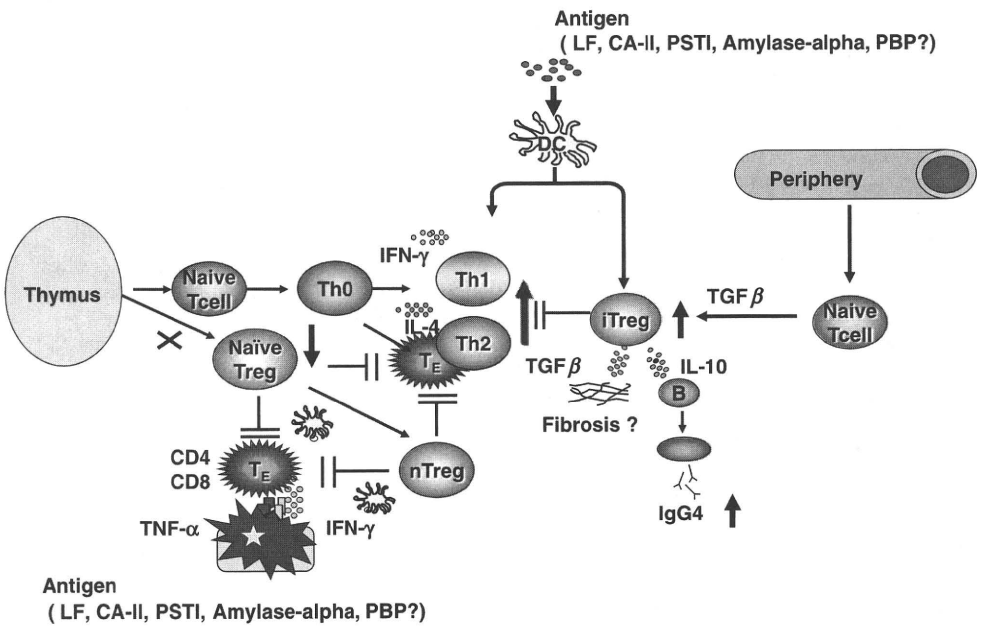

Fig. 2 Hypothesis for the pathogenesis of AIP. In the early stage, initial response to self-antigens (LF, CA-II, CA-IV, PSTI, or α-fodrin) or molecular mimicry (*H. pylori*) is induced by decreased naive Tregs, and Th1 cells release proinflammatory cytokines (IFN-γ, IL-1b, IL-2, and TNF-α). In the chronic stage, progression is supported by increased memory Tregs and Th2 immune responses. The classical pathway of complement system may be activated by IgG1 immune complex

Conclusion

In conclusion, recent advances support the concept of IgG4-related diseases, 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 background, disease specific antigens, and the role of IgG4.

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

Conflicts of interest None.

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The Role of Innate Immunity in the Pathogenesis of Experimental Autoimmune Pancreatitis in Mice

Akiyoshi Nishio, MD, PhD, Masanori Asada, MD, PhD,† Kazushige Uchida, MD, PhD,* Toshiro Fukui, MD, PhD,* Tsutomu Chiba, MD, PhD,‡ and Kazuichi Okazaki, MD, PhD**

Objective: To determine the role of innate immunity in the development of autoimmune pancreatitis in mice induced by toll-like receptor (TLR) stimulation.

Methods: Six-week-old female MRL/Mp mice were injected intraperitoneally with polyinosinic polycytidylic acid (poly I:C) or lipopolysaccharide (LPS) at doses of 5 mg/kg body weight twice weekly for 12 weeks. The mice were killed, and the severity of pancreatitis was graded using a histological scoring system. Serum cytokine levels of mice with pancreatitis and mice that were given a single injection of TLR ligands were measured using enzyme-linked immunosorbent assays. The effect of TLR stimulation on the development of pancreatitis was also examined using C57BL/6 interleukin (IL)-10-deficient mice.

Results: Administration of poly I:C accelerated the development of pancreatitis in MRL/Mp mice, but LPS did not. Serum levels of IL-10 and IL-12 were significantly elevated in mice with autoimmune pancreatitis. A single injection of LPS markedly increased serum levels of interferon-γ, tumor necrosis factor-α, IL-10, and IL-12 compared with those of poly I:C-treated mice. Treatment with not only poly I:C but also LPS induced pancreatitis in IL-10-deficient mice but not in wild-type mice.

Conclusion: Repeated stimulation of innate immunity induces autoimmunity in the pancreas of mice via an imbalance between proinflammatory and anti-inflammatory cytokines.

Key Words: autoimmune pancreatitis, innate immunity, toll-like receptor, cytokine imbalance

(*Pancreas* 2011;40: 95–102)

Autoimmune pancreatitis (AIP) is an increasingly recognized entity of pancreatitis that is characterized by a steroid-responsive, fibroinflammatory condition that often involves multiple organs. Since the first case was reported in 1961 by Sarles et al,[1] subsequent studies have revealed that the disease has clinical, radiological, and histopathological features distinct from those of forms of chronic pancreatitis.[2,3]

The morphological characteristics of AIP include diffuse or localized enlargement of the pancreas and irregular narrowing of the main pancreatic duct. Histologically, the disease is associated with progressive lymphoplasmacytic infiltration, predominantly localized to the ductal structures, and varying degrees of parenchymal and acinar destruction. A high serum IgG4 level is considered a serological hallmark of the disease, and increased infiltration of IgG4-positive cells in the affected organs is pathognomonic for AIP.[4] Autoantibodies against carbonic anhydrase, lactoferrin, and other antigens are present in the sera of patients with AIP.[5–8] Based on a combination of findings obtained from patients with AIP, several diagnostic criteria have been proposed for differentiating AIP from other pancreatic diseases, especially pancreatic cancer.[9–11]

However, little is known about the precise pathogenesis of AIP, and the natural course of the disease is unclear. The disease may progress asymptomatically for prolonged periods, and symptoms often develop in the later stages of the disease. Autoimmune mechanisms are thought to be involved in the pathogenesis of AIP. Zen et al[12] reported that T helper type 2 (Th2) cells and T regulatory cells predominantly mediate the immune reaction in AIP and IgG4-associated cholangitis. Kawa et al[13] showed that the engagement between IgG4 and IgG Fc does not occur through Fab but as an Fc-Fc interaction. However, the early immune response underlying the pathogenesis of AIP is difficult to study in patients with this disease.

Several animal models have been used to avoid difficulties inherent in the study of the autoimmune mechanism of AIP in human patients.[14–20] MRL/Mp mice develop pancreatitis similar to that of human AIP: they exhibit selective destruction of pancreatic exocrine tissues coupled with infiltration of lymphocytes and plasmacytes, and various autoantibodies are produced.[14,21] Induction of the disease in MRL/Mp mice is cell mediated, and destruction of pancreatic tissue is induced by Fas/Fas ligand–mediated cytotoxicity.[18,22] The development of the disease is accelerated by administration of polyinosinic polycytidylic acid (poly I:C), a synthetic double-stranded RNA and toll-like receptor (TLR) 3 ligand.[18] Toll-like receptors play important roles in innate immunity and initiate intracellular signaling to macrophages and dendritic cells after stimulation with various antigens.[23] The majority of known TLRs mediate the development of Th1 cell-promoting dendritic cells, possibly causing an autoimmune response.[24,25]

In this study, we investigated the role of innate immunity in the development of murine AIP induced by repeated stimulation with various TLR ligands, with a specific focus on inflammatory cytokine production.

MATERIALS AND METHODS

Mice

Female MRL/Mp mice and C57BL/6 interleukin 10–deficient (IL-10KO) mice were purchased from the Jackson Laboratory (Bar Harbor, Me). Female C57BL/6 wild-type (WT) mice were purchased from Japan SLC (Shizuoka, Japan). All mice were bred at the animal facility of Kyoto University under specific pathogen-free conditions.

From the *Third Department of Internal Medicine, Kansai Medical University, Moriguchi; †Digestive Disease Center, Kitano Hospital, Tazuke Kofukai Medical Research Institute, Osaka; and ‡Department of Gastroenterology and Hepatology, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Received for publication December 11, 2009; accepted June 16, 2010.

Reprints: Akiyoshi Nishio, MD, PhD, Third Department of Internal Medicine, Kansai Medical University, 10-15 Fumizono-cho, Moriguchi 570-8507, Japan (e-mail: nishioa@takii.kmu.ac.jp).

This study was supported in part by Grants-in-Aid for Scientific Research C21590878 and C20590810 from the Japan Society for the Promotion of Science and by a Health and Labor Science Research Grant for Intractable Diseases from the Japanese Ministry of Health, Labor and Welfare.