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

1. Clinically, diffuse/focal enlargement, or mass forming, nodular/thickened lesions in one or more organs
2. Elevated levels of serum IgG4 (>135 mg/dl)
3. Histopathological findings
   ① Prominent infiltration and fibrosis of lymphocytes and plasmacytes, but no neutrophilic infiltration
   ② Abundant infiltration of IgG4-positive plasmacytes (>10/hpf) and/or the ratio of IgG4/IgG-positive cells (>40%)
   ③ Storiform/swirling fibrosis
   ④ Obliterative phlebitis

Diagnosis of IgG4-related disease: 1+2, 1+3 ①②, 2+3 ①②, or 3 ①②③④

The following cases must be excluded from the diagnosis: malignant tumors developed in organs (e.g., cancers, malignant lymphomas) or similar diseases (e.g., Sjögren's syndrome, primary sclerosing cholangitis), bronchial asthma, and Castleman's disease

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

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

Immunoglobulin Subclasses and IgG4

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

Table 4 Immunoglobulin subclasses in IgG4-related disease

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

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

[a] [45]

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

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

The Complement System

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

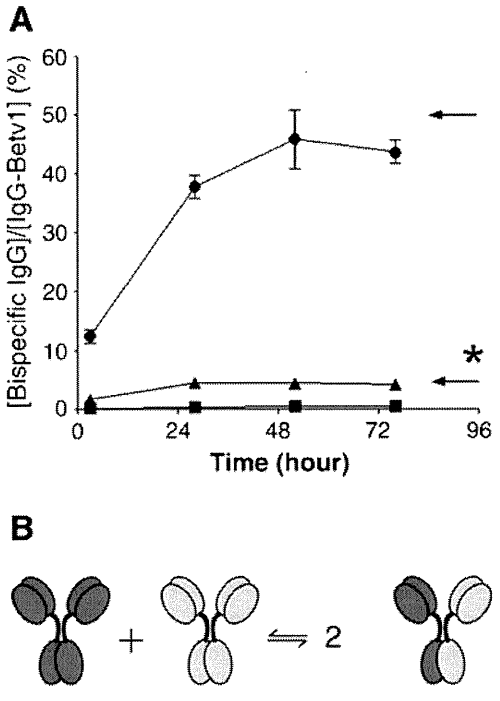

Fig. 4 Characteristic forms of IgG4. **a** Schematic representation of the generation of bispecific IgG4 antibodies by the exchange of half-molecules ("Fab-arm exchange"; cited from [46]). IgG4 Fab arm exchange occurs by the exchange of a heavy chain-light chain pair (half-molecule) of one IgG4 molecule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

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

Autoantibodies

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

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

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

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

Th1 and Th2 Immune Balance

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

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

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

Regulatory T Cells

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

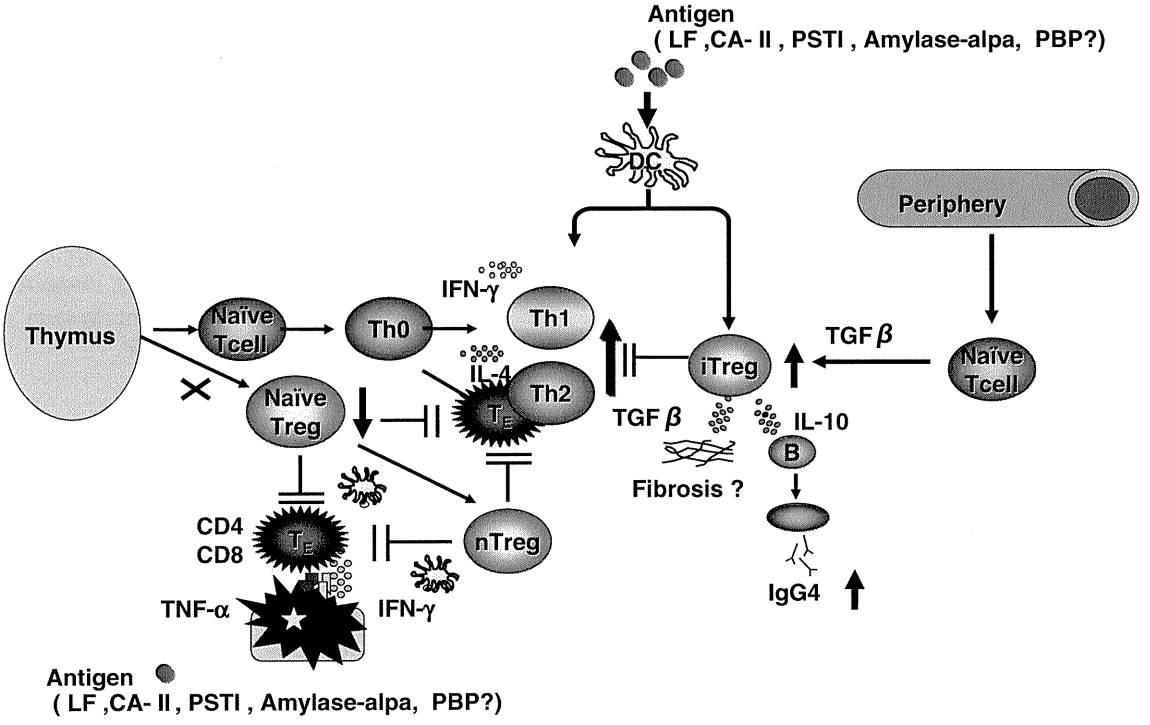

Fig. 5 Hypothesis for the pathogenesis of AIP and IgG4-related disease. In the central tolerance, naïve, and natural regulatory T cells (*Tregs*) derived from the thymus suppress autoreactive CD4 or CD8 cells in the normal state. In the IgG4-related disease, the basic concept is the biphasic mechanism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

Possible Role of IgG4 in "IgG4-Related Disease"

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

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

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

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

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

Conclusion

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

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Immunological Aspects of IgG4-Related Disease

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Abstract: Recent advances support the concept of IgG4-related disease as a unique systemic disease, because autoimmune pancreatitis, sclerosing cholangitis, sclerosing sialadenitis, retroperitoneal fibrosis show similar pathological features with an abundant infiltration of IgG4 positive plasma cells and fibrosis, and steroid responsive. Based on these findings, a novel concept of IgG4-related disease such as IgG4-related systemic sclerosing disease, IgG4-systemic plasmacytic syndrome (SIPS), and IgG4-related multiorgan lymphoproliferative disease (IgG4-MOLPS) have been proposed. However, pathogenetic mechanisms still remain unclear. For clarifying it, genetic background, humoral immunity, complement system, disease-related antibodies, cellular immunity, and regulatory T cells were reviewed. Although the significance of IgG4 in the development of IgG4-related disease still remains unclear, we have proposed a hypothesis for the pathogenesis in AIP, one of IgG4-related diseases. In induction of lesions, the initial response to self-antigens or molecular mimicry for components of *H. pylori* may be induced by decreased naïve-Tregs, and Th1 cells release proinflammatory cytokines. In progression, increased memory-Tregs and Th2 immune responses regulate IgG4 production. Further studies are necessary to clarify the pathogenesis.

Keywords: IgG, IgG4-related disease, autoimmune pancreatitis, Mikulicz's disease, regulatory T cell (Treg).

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). Thereafter, Yoshida *et al.* firstly reported such a case as AIP [2]. Hamano *et al.* reported increased serum levels of IgG4 in AIP [3]. 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 the stenosis of the main pancreatic duct, and obliterative fibrosis [4-6], which is so called as lymphoplasmacytic sclerosing pancreatitis (LPSP) [7]. On the other hand, Mikulicz disease (MD) with bilateral, painless, and symmetrical swelling of the lachrymal, parotid, and submandibular glands [8] shows elevated concentrations of serum IgG4, infiltration of IgG4-bearing plasma cells into the glands, and recovery of secretion by steroid treatment, which are similar to those of autoimmune pancreatitis. These patients often show other systemic organ involvements such as sclerosing cholangitis, retroperitoneal fibrosis, enlarged celiac and hilar lymph nodes, chronic thyroiditis, interstitial nephritis and so on [4-6, 9]. Recently, MD is considered to be different from typical Sjögren's syndrome because most cases show negative results for both anti-SS-A/Ro and anti-SS-B/La antibodies distinctive to Sjögren's syndrome [2-6]. Sclerosing cholangitis in patients with AIP shows different responses to steroids and prognoses from 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 [10, 11], systemic IgG4-related plasmacytic syndrome (SIPS) [12], or IgG4-positive multi-organ lymphoproliferative syndrome (IgG4-MOLPS) [13].

Although the infiltration of IgG4-positive cells and increased serum levels of IgG4 are characteristics of IgG4-related disease, severity of fibrosis seems to be different among the involved individual organs. Although pathogenesis or pathophysiology still remains unclear, we discuss the recent advances in the immunological aspects of IgG4-related disease.

IMMUNOGENETIC FACTORS

Immunogenetic factors have been in a few series of AIP, and yet not conclusive. Susceptibility to AIP may be associated with immunogenetic factors such as the class II antigen of the major histocompatibility complex (MHC), polymorphism of nuclear factor (NF)-κB and Fc-receptor-like (FCRL) 3 genes expressed on B cells [14, 15]. Two studies of HLA in association with AIP have been reported from the Japanese [14] and Korean group [15]. In the Japanese patients with AIP, HLA haplotype DRB1*0405-DQB1*0401 (class II) and ABCF1 proximal to C3-2-11, telomeric of HLA-E (class I) are susceptibility to AIP [14], but not in the Korean patients [15]. However, substitution of aspartic acid with nonaspartic acid at DQβ1 may be a predictive factor for the relapse of AIP in Korean patients [15]. FCRL3 polymorphisms are linked to various autoimmune diseases, such as rheumatoid arthritis, autoimmune thyroid disease, and systemic lupus erythematosus (SLE) in the Japanese population [16, 17]. However, Fc-receptor like 3 gene polymorphisms is 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 [16, 17].

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A few immunogenetic studies for innate or acquired immunity have been reported. Innate immunity is important in the development of acquired immunity or autoimmune diseases. Although polymorphisms in toll-like receptor (TLR)-4 gene have been linked with several autoimmune and allergic diseases, it does not seem to play an important role in the development of AIP [18]. 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 [19] and Japanese population [20]. 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 the antigen presenting cells [21]. Umemura T *et al.* [20] reported 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 is not associated with AIP in the Japanese patients. They also found that +49A/A or +6230A/A genotypes may be associated with recurrence of the disease, which is observed in Graves' disease, type 1 diabetes, and clearance of hepatitis B virus [20]. On the other hand, Chan MC *et al.* [19] have reported that *CTLA-4* SNPs have shown significantly a 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-α promoter 863A was significantly associated with a higher risk of AIP. Racial and geographical differences may be associated with SNPs of the different locus of CTLA-4 [19]. Soluble isoform of CTLA4 (sCTLA4) is reported to be elevated in patients with autoimmune diseases, such as autoimmune thyroid disease, SLE, and myasthenia gravis [20]. Therefore, the sCTLA4 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 [22]. 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 [22]. The ratios for each IgG subclass were 65% of IgG1, 25% of IgG2, 6% of IgG3, and 4% of IgG4 [22]. In 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, were decreased as compared with normal or other control diseases [3, 12, 13, 23] (Table **1**). Ratios of other subclasses of IgG other than IgG4 are somewhat different among individual diseases; In AIP, all subclasses (IgG1-G4) of IgG increased compared with other pancreatitis. In contrast, IgG_1 and IgG_3 in MD are significantly lower with negative correlations with IgG4 than in typical SS.

Although the association with IgE-mediated allergy and IgG4 antibodies has been well-known [24], IgG4 have 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 an attached light chain (half-molecule) with a heavy-light chain pair from another molecule [25], which results usually in asymmetric antibodies with two different antigen-combining sites. While these modified antibodies are hetero- bivalent, they behave as monovalent antibodies [25] (Fig. **1A**). Another aspect of IgG4 mimics is IgG rheumatoid factor (RF) activity by interacting with IgG on a solid support [26] (Fig. **1B**). 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, C4) with an elevated circulating immune complex as well as serum levels of IgG4 and the IgG4 subclass of immune complexes [3, 27]. 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 [28]. Moreover, IgG4

Table 1.

	Year		N	IgG	IgG1	IgG2	IgG3	IgG4/IgG	IgM	IgA	IgE	IC(μg/ml)
Hamano *et al.*	2001	AIP		2389	NT	NT	NT	742 (28 %)	NT	NT	NT	30
		Control										
Yamamoto *et al.*	2006	MD	16	3226.9	1256.4 (41.5%)		NT	1111 (28.6%)				
		SS	16	2398	1624.9(73.0%)			NT	88.8 (2.8%)			
		Normal	#		65%	25%	6%	4%				
Masaki *et al.*	2008	MD	64	2960.1	1153.3	786.5	57.6	697.7	63	194.7	307.4	
		SS	31	2473.1	1437.1	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	

[22].
AIP: autoimmune pancreatitis. MD: Mikulicz disease. SS: Sjögren's syndrome. CP: chronic pancreatitis. IC: immune complex.

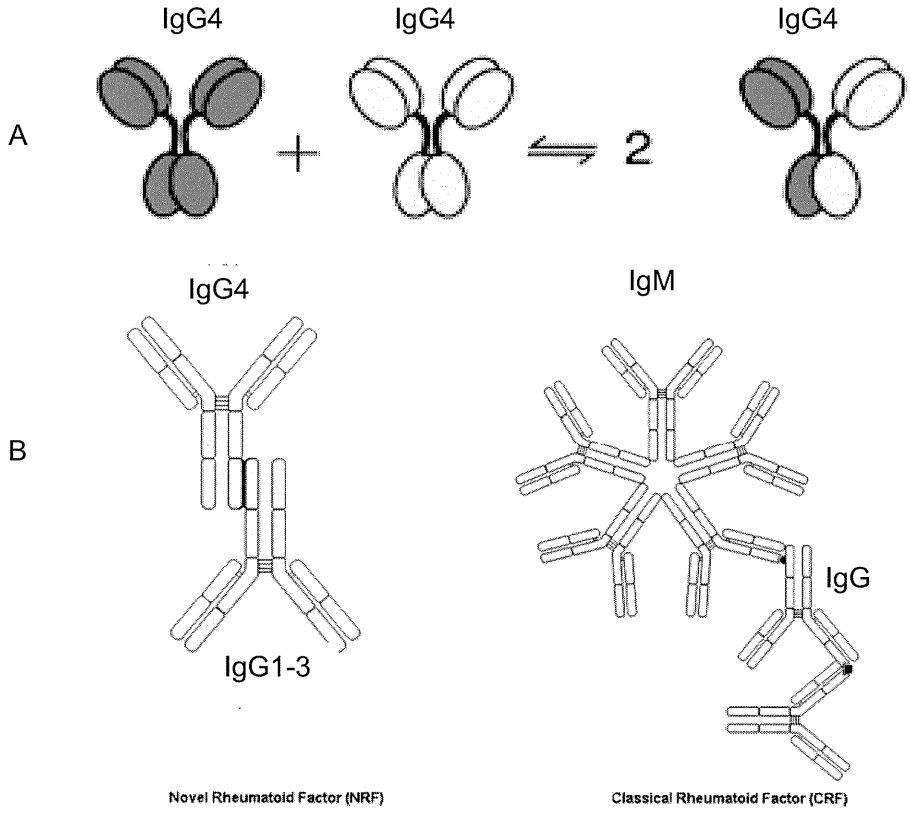

Fig. (1). Characteristic forms of IgG4. (**A**) Schematic representation of the generation of bispecific IgG4 antibodies by the exchange of half-molecules ('Fab-arm exchange') [25]. IgG4 Fab arm exchange occurs by the exchange of a heavy chain–light chain pair (half-molecule) of one IgG4 molecule with 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 [25]. (**B**) On the left: IgG4 Fc interacts with Ig Fc. On the right: IgM RF recognizes IgG in a ''classical'' Fab-Fc recognition [26].

bound to other isotypes such as IgG1, 2, and 3 with a Fc-Fc interaction immune complex in patients with AIP [26], 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 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 that is associated with IgG4-related disease [29].

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), there is scarcely any association of IgG4-related disease and well-known autoimmune diseases such as Sjögren's syndrome and SLE. From the view of IgG4 function, a big mystery whether IgG4-related disease is autoimmune or allergic disease is addressed. However, occasional coexistence of other organ involvement lead us to the concept that there may be some 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) [30, 31], anti-carbonic anhydrase (CA)-II [30-33], anti-CA-IV [34], anti-pancreatic secretory trypsin inhibitor (PSTI) [35], anti-amylase-alpha [36], anti-HSP-10 [37], and anti-plasminogen-binding protein (PBP) peptide autoantibodies [38] 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 [35]. CA-II [31], CA-IV [34], LF [31] and PSTI [32] are distributed in the ductal cells of several exocrine organs, including the pancreas, salivary gland, biliary duct, lung, renal tubules and so on [30, 31]. 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 mice models are similar to the human IgG4-related diseases [39, 40]. The high prevalence of these antibodies suggests that these may be the candidates for the target antigens in AIP [31].

Molecular mimicry among microbes and target antigens may be a possible mechanism to break down the 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 the autoaggressive potential [41-43]. Guarneri and colleagues showed a significant homology between human CA-II and alpha-CA of *H. pylori*, a fundamental enzyme for bacterial survival and proliferation in the stomach [43]. Moreover, the homologous segments contain the binding motif of DRB1*0405, which confers a risk for the AIP development [43]. 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 [43]. These findings suggest that gastric *H. pylori* infection might trigger AIP in genetically predisposed subjects [41-43].

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 [44] associated with type1A 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 the well-known autoimmune diseases such as Sjögren's syndrome and SLE must be discussed.

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 [30] suggest that an immunological mechanism may be involved in the development of AIP as well as infiltration of plasmacytes and B cells. CD4+T-cells differentiate from naïve T-cells (Th0) to Th1, Th2, Th17, and regulatory T (Treg) cells [45]. IL-12 induces Thl cells, which produce IL-2, tumor necrosis factor (TNF)-α and IFN-γ, 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, 5, 6 and 10, promote humoral and allergic responses [4]. TGF- β, IL-6 IL-21 and IL-23 induce Th17 cells, which secrete IL-17, that may be involved in inflammation in mice [46].

In some patients with AIP, Th1-cells but not Th17 cells are predominant over Th2 type cells in the periphery [31, 47]. On the other hand, Th2 type immune reaction is induced in the liver of IgG4-related sclerosing cholangitis as well as Th1 responses [48]. The discrepancy may be explained by the shift of Th2-cells from the periphery to local tissues, or different disease stages. Mice models with depletion of Tregs by neonatally thymectomy (nTx) support the hypothesis that Th1 cells mainly act as effectors in the initial early stage [49]. In Sjögren's syndrome [50] and PSC [51], the major infiltrating cells in the tissue are $CD4^+HLA\text{-}DR^+$ Th1 cells, although $CD8^+$ and B-cells are also present. Similarly 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 maturation and proliferation of local B cells and plasmacytes [4].

REGULATORY T CELLS

From naïve Th0 cells, TGF- β 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 [46]. 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 (TGF-β) and IL-10, and/or cell-to-cell contact *via* ligation of CTLA-4. Recent studies clarified several subtypes of Treg [52]. 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 [52]. As Tregs expressing Foxp3 are critical in the transfer of immune tolerance, deficient Tregs induce various autoimmune diseases in animal studies [46]. 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 [53]. 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 naïve (CD45RA+) Tregs are significantly decreased in the peripheral blood of patients with AIP, whereas memory (CD45RA-)-Tregs in major population are significantly increased [54]. In addition, prominent infiltration of Tregs with an upregulation of IL-10 is observed in the liver of IgG4-related sclerosing cholangitis [48]. 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 pahtogenetic.

POSSIBLE ROLE OF IgG4 IN IgG4-RELATED DISEASES

IgG4 seems to be associated with a pathogenic effect in few situations. In pemphigus, recognition of skin autoantigens (desmogleins) by IgG4 is at the origin of the disease process [55]. 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 [56]. Although some preliminary reports for AIP suggested 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 the IgG4 deposition in IgG4-related renal diseases [28]. 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 is generally considered to be a benign, non-pathogenic antibody [57]. Some of these associations suggest a protective effect, such as in allergen-specific immunotherapy, tolerance induction after food avoidance [58] and protection from allergic effects during parasitosis [59, 60]. Recent data of regulating IgG4 showed 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 [54]. In addition, the prominent infiltration of Tregs upregulated IL-10 in the liver of patients with IgG4-related sclerosing cholangitis [48]. These findings suggest that IgG4 or IgG4-immune complexes unlikely act as a pathogenetic factor but not as an anti-inflammatory factor in IgG4-related diseases [26]. 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 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 [49]. These findings suggested that depletion of naïve Tregs in the periphery [61] 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 [62]. On the other hand, CD8+T cells may play role as effector cells in the major histocompatibility complex (MHC) class II-deficient mouse [63] or WBN/Kob rat models [64]. 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 the effectors. Although rodents lack IgG4 subclass, the deposits of tissue-specific IgG2b, similar electrophoretic position to human IgG4, were observed in the injured pancreas and lachrymal glands in WBN/Kob rats [64]. These animal models suggest that although CD8+ T-cells may be partially involved, CD4+ T-cells take major role in the development of experimental systemic lesions, which is similar to human IgG4-related diseases [4, 31], although the counterpart of IgG4 in mice IgG subclasses has not been identified. As tumor growth factor (TGF)- β is an important regulating factor in maintaining immune homeostasis [65], TGF-β dominant negative mutant mice suggested that loss of TGF-b signaling may contribute to autoimmune pancreatitis [66].

From the above findings, we propose a hypothesis for the pathogenesis of AIP (Fig. **2A**, **B**). 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 the release of proinflammatory cytokines (IFN-γ, IL-1β, IL-2, TNF-α). In progression, the Th2 type immune responses producing IgG, IgG4 and autoantibodies may be involved in pathophysiology. IgG4 and fibrosis may be regulated by increased IL-10 and TGF-beta secreted from inducible memory-Tregs, respectively (Fig. **2B**). The classical pathway of complement system may be activated by 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 background, disease specific antigens and the role of IgG4.

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ABBREVIATIONS

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

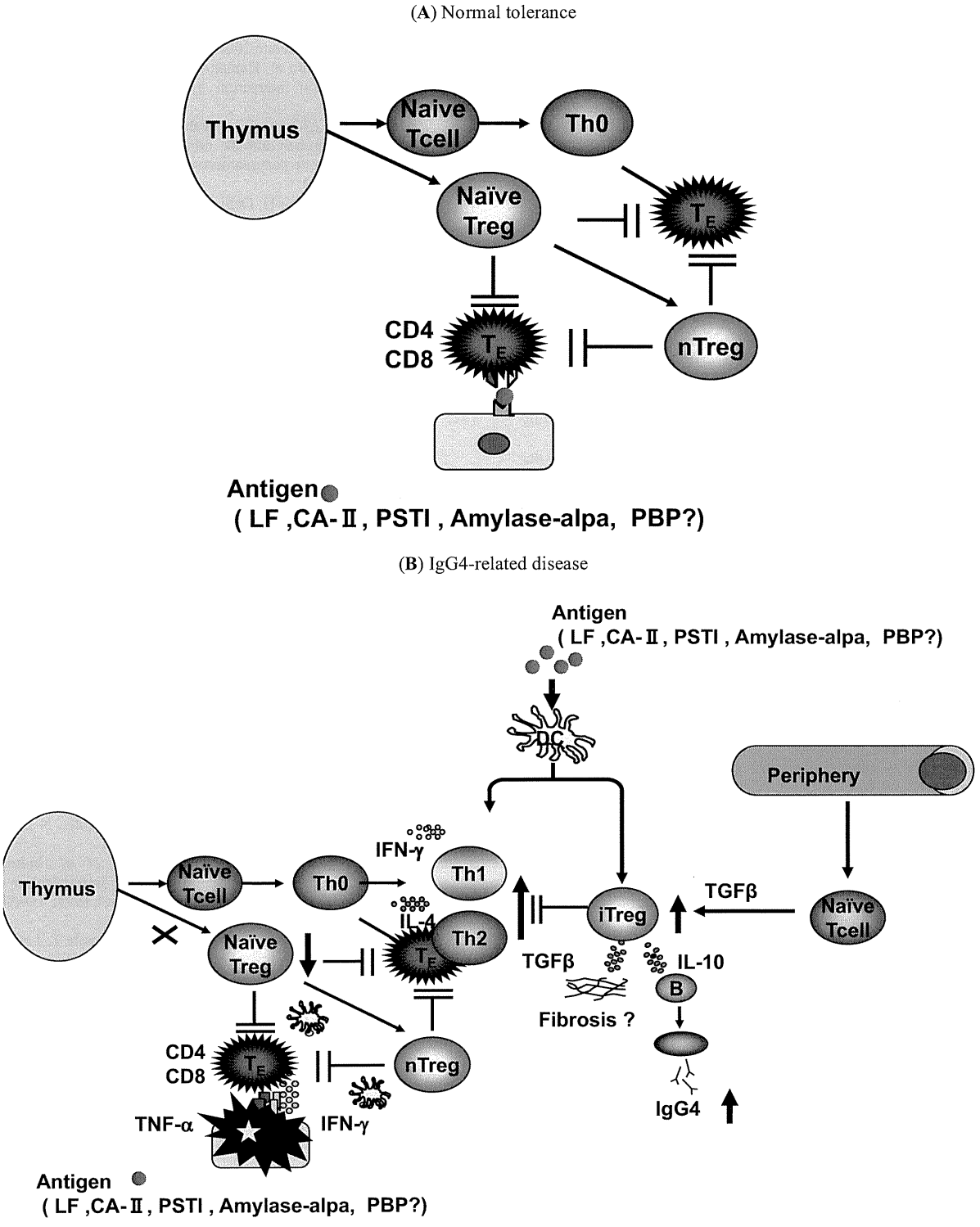

Fig. (2). Hypothesis for the pathogenesis of AIP. (**A**) Normal: In the central tolerance, naïve and natural regulatory T cells (Tregs) derived from the thymus suppress autoreactive CD4 or CD8 cells. TE; effector T cell. nTreg;natural Treg. (**B**) IgG4-related disease: The basic concept is the biphasic 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-1b, 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-beta secreted from inducible memory-Tregs, respectively. iTreg; inducible Treg.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Comparison of steroid pulse therapy and conventional oral steroid therapy as initial treatment for autoimmune pancreatitis

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Abstract
Background The efficacy of oral steroid therapy for autoimmune pancreatitis (AIP) is well known, and oral prednisolone treatment is most usually commenced at 30–40 mg/day, but there have been few reports about comparative studies of oral steroid therapy and steroid pulse therapy as the initial treatment for AIP. We studied the clinical course and image findings to estimate the utility of steroid pulse therapy for AIP, comparing it with oral steroid therapy.
Methods Laboratory and image findings were assessed retrospectively in 11 patients who received steroid pulse therapy, and the findings were compared to those in 10 patients who received conventional oral steroid therapy.
Results Change in pancreatic size showed no significant difference between the therapies after 2 weeks of treatment. Significant improvement of lower bile duct strictures after 2 weeks of treatment and that of immunoglobulin values within 6 months were shown with both therapies. However, steroid pulse therapy showed significant improvement of γ-guanosine triphosphate (GTP) in 2 weeks and of alanine aminotransferase (ALT) in 2 and 8 weeks, compared with oral steroid therapy. Moreover, there was one patient in whom the lower bile duct stricture was not improved by oral steroid therapy, but it did show improvement with steroid pulse therapy.
Conclusions Initial steroid pulse therapy is a beneficial alternative to oral steroid therapy for the improvement of bile duct lesions. In future, the accumulation of a larger number of patients receiving steroid pulse therapy is needed, and prospective studies will be required.

Keywords Autoimmune pancreatitis (AIP) · Steroid pulse therapy · Bile duct stricture · Diabetes mellitus · Pancreatic cancer

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Introduction

Sarles et al. [1] reported a case of chronic pancreatitis with hypergammaglobulinemia, but the clinical entity was not confirmed thereafter. Autoimmune pancreatitis (AIP), which was first proposed as a clinical entity by Yoshida et al. [2] from Japan, is now generally accepted as a distinctive type of pancreatitis [1]. AIP is characterized by diffuse irregular narrowing of the main pancreatic duct, sausage-like diffuse swelling of the pancreas, high serum levels of IgG or IgG4, and steroid responsiveness [3–6]. Since the fibroinflammatory process of AIP responds well to steroids, autoimmune mechanisms are thought to be involved in the development of AIP. A recent large Japanese study of AIP and guidelines for treatment recommend standard oral steroid therapy with an initial dose of 0.5–0.6 mg/kg/day [7, 8].

While steroid responsiveness as a diagnostic component is not included in the revised Japanese criteria, it is included in the Korean criteria, Mayo Clinic HISORt (Histology, Imaging, Serology, Other organ involvement, and Response to steroids) criteria, and recently proposed Asian criteria [8–11]. The most important issue in AIP management is making the diagnosis of AIP, especially the

mass-forming type, differentiating it from pancreatic or biliary cancers [12–15], although some cases of pancreatic cancer are accompanied by AIP [16–19]. In some tumor-forming AIP cases, the efficacy of a steroid trial has been reported as useful in diagnosing AIP by criteria other than the Japanese diagnostic criteria [20]. Moreover, Korean investigators have reported the usefulness of a 2 weeks' trial of oral steroids in differentiating AIP from malignancy, with continuing administration if AIP is diagnosed [20]. However, it has not yet been established whether or not withdrawal of steroids in reconsidering malignancy presents a risk of postoperative adrenal insufficiency [21–23]. Therefore, it is desirable to have an alternative to the discontinuation of steroid administration immediately after surgery.

Steroid pulse therapy is widely used to initiate treatment in patients with rapidly progressive and immunologically mediated disorders such as acute graft rejection, Graves ophthalmopathy, pemphigus, and severe systemic lupus erythematosus [24–27]. Moreover, high doses of systemic steroid can be given with comparative safety within a period of 1 week [28]. We therefore evaluated the efficacy of short-term steroid pulse therapy, in comparison with oral steroid therapy, in patients with AIP.

Methods

Patients and treatment

For this study, we retrospectively examined the records of all 21 AIP patients treated in our hospitals from November 2004 to May 2009. All patients were diagnosed with AIP 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, and the Japan Pancreas Society. Following diagnosis, 20 patients with AIP were randomly distributed to two treatment groups by their attending physicians. One patient (case 10) was referred to our hospital after the withdrawal of oral steroid for AIP because his bile duct lesion had not responded to the treatment. Eleven patients (cases 1–11; 5 male and 6 female; aged 47–80 years, with a mean age of 66 years, named the "pulse group") (Table 1) received steroid pulse therapy, and ten patients (cases 12–21; 8 male and 2 female, aged 49–72 years, with a mean age of 69 years, named the "oral group") (Table 2) received oral steroid therapy. For the pulse group, the initial dose of methylprednisolone was 500 mg/day for 3 days each week as 1 course, and we treated them with 2 weekly courses. Then oral prednisolone at 20 mg/day was prescribed as maintenance therapy and the dose was tapered off. For the oral group, ten patients commenced oral prednisolone at 30–40 mg/day. Two weeks after the start of the treatment, oral prednisolone at 20 mg/day was prescribed, and the dose was tapered off. This study was approved by the Kansai Medical University ethics committee.

Serological study

We analyzed immunological findings for the following: IgG, IgG4, antinuclear antibodies (ANA), rheumatoid factor (RF), antimitochondrial antibodies (AMA), myeloperoxidase-antineutrophil cytoplasmic antibodies (MPO-ANCA), anti-Sjögren's syndrome A antibodies (SS-A), anti-Sjögren's syndrome B antibodies (SS-B), anti-thyroid peroxidase antibodies (TPOAb), and anti-thyroglobulin antibodies (TgAb). To compare liver and endocrine function in both groups, we evaluated the serum levels of γ-guanosine triphosphate (GTP) and alanine aminotransferase (ALT) on day 0 (data just before the treatment), and at weeks 2 and 8 after therapy, and checked glycosylated hemoglobin values (HbA1c) at months 1, 3, and 7 after therapy, which closely reflected glucose tolerance at months 0, 2, and 6, respectively. In each evaluation, patients who did not show abnormal values during the clinical course were excluded in order to evaluate the therapeutic effect strictly.

Radiological study

All the patients were examined by contrast-enhanced helical computed tomography (CT), magnetic resonance imaging (MRI), and endoscopic retrograde cholangiopancreatography (ERCP) and underwent liver function tests, combined with bile duct drainage and pathological tests as necessary.

For morphological changes, CT, MRI, and ERCP were studied. The width of the pancreas along its longest axis was measured on CT or MRI images and compared with the transverse diameter of the vertebral body according to the method of Heuck et al. [29]. The pancreas size on the first image was defined as 100%.

Cases showing lower bile duct stricture were classified as follows: 0 = absent, 1 = <0–25%, 2 = <25–50%, 3 = <50–75%, and 4 = <75–100%, according to the method of Craig et al. [30]. Using the method described above, pancreas size was evaluated after 2 weeks on steroids, and stricture of the distal third of the common bile duct was measured after 2 weeks and after 8 weeks.

Statistical analysis

Statistical analysis was performed using the Mann–Whitney *U*-test, Wilcoxon signed-ranks test, paired *t*-test, and

Table 1 Background of AIP patients who received steroid pulse therapy

Patient ID	Age (years)/ sex	Symptoms	IgG <1,700 (mg/dl)	IgG 4 <135 (mg/dl)	Amy <130 (IU/L)	T-Bil <0.9 (mg/dl)	ALT <30 (IU/L)	γ-GTP <35 (IU/L)	ANA	PFD <73.4 (%)	DM	Extrapancreatic lesion	Stenosis on ERCP	Morphological change of the pancreas
1	80/F	Jaundice	2,604	1,230	52	0.9	43	260	−	52.1	−	Sialoadenitis	Head, CBD	FS in head
2	63/M	Epigastralgia	1,714	354	66	0.9	56	222	−	29.7	−	Warthin tumor	Head, CBD	DS
3	54/F	Epigastralgia	1,828	324	561	1.0	100	403	−	NT	−	Hypothyroidism	Body, CBD	DS
4	71/F	None	1,916	295	78	0.6	60	101	−	97.9	−	Sialoadenitis, mediastinum LNS	Head	FS in head
5	66/F	Nausea	1,535	235	77	2.2	118	1,311	−	NT	−	Hypothyroidism, retroperitoneal fibrosis	Head to tail, CBD	FS in head
6	66/M	Epigastralgia, jaundice	2,695	1,790	164	12.5	98	137	−	58.1	+	None	Body, CBD	DS
7	47/F	Jaundice	2,453	629	15	1.0	190	65	−	NT	−	None	Head, body to tail, CBD	DS
8	72/M	Epigastralgia	1,692	452	66	1.3	721	1,352	+	30.9	+	None	Head to tail CBD	FS in head
9	72/M	Epigastralgia, jaundice	1,513	411	32	14.1	114	352	−	NT	+	None	Head, CBD	DS
10	63/M	Malaise	1,514	394	76	0.5	20	82	−	NT	−	None	Head to tail, CBD	FS in head
11	73/F	Epigastralgia	1,598	373	55	0.6	33	588	−	59.7	+	None	Head to tail, CBD	FS in head

AIP autoimmune pancreatitis, *T Bil* total bilirubin, *ALT* alanine aminotransferase, *ID* identification, *γ-GTP* γ-guanosine triphosphate, *Amy* amylase, *ANA* antinuclear antibody, *PFD* pancreatic functional diagnostic test, *DM* diabetes mellitus, *ERCP* endoscopic retrograde cholangiopancreatography, *CBD* common bile duct, *FS* focal swelling, *DS* diffuse swelling, *NT* not tested, *LNS* lymph node swelling

Table 2 Background of AIP patients treated with oral prednisolone

Patient ID	Age (years)/sex	Symptoms	IgG <1,700 (mg/dl)	IgG4 <135 (mg/dl)	Amy <130 (IU/L)	T-Bil <0.9 (mg/dl)	ALT <30 (IU/L)	γ-GTP <35 (IU/L)	ANA	PFD <73.4 (%)	DM	Extrapancreatic lesion	Stenosis on ERCP	Morphological change of the pancreas
12	71/M	Jaundice	3,274	1,870	58	1.0	24	76	−	32.7	−	Mediastinum LNS	Head to tail, CBD	DS
13	66/M	Thirst	4,060	1,170	64	0.4	18	116	−	65.8	−	Sialoadenitis, mediastinum LNS	Tail, CBD	DS
14	58/F	Epigastralgia	2,754	1,110	95	0.9	13	10	−	NT	−	Thyroiditis, mediastinum LNS	Body to tail	FS in head
15	52/F	None	2,190	661	435	0.6	15	15	−	69.7	−	Interstitial pneumonia, Mikulicz tumor	Tail	FS in tail
16	68/M	Fever	1,622	407	34	0.6	30	30	−	73.4	−	Thyroiditis	Head, body, CBD	DS
17	72/M	Vomiting	2,010	773	51	0.3	34	67	+	NT	−	None	Head	FS in head
18	55/M	Epigastralgia	1,461	659	53	0.6	178	240	−	17.9	+	None	Head to tail, CBD	DS
19	63/M	Jaundice	2,073	487	41	1.1	52	149	−	NT	+	None	Head, CBD	FS in head
20	49/M	Jaundice	2,065	479	62	0.9	52	503	−	35.7	−	None	Body, tail, CBD	DS
21	66/M	Malaise	1,607	200	47	1.0	41	189	−	77.9	+	None	Head, CBD	FS in head

Amy amylase, *ANA* antinuclear antibody, *PFD* pancreatic functional diagnostic test, *DM* diabetes mellitus, *ERCP* endoscopic retrograde cholangiopancreatography, *LNS* lymph node swelling, *CBD* common bile duct, *DS* diffuse swelling, *NT* not tested, *FS* focal swelling