that it was the thickening of the bile-duct wall itself that caused the biliary strictures in all patients—and not extrinsic compression from inflammatory pancreatic tissue. We consequently diagnosed all patients with IgG4-SC.

The control group consisted of patients with PSC (n = 26; all patients treated with ursodeoxycholic acid; 15 men and 11 women; mean age 36 years; range 6–77 years) (Ludwig's stage 2, n = 4; stage 3, n = 8; stage 4, n = 14), AIH (n = 9; five patients untreated and four patients treated with corticosteroids; nine women; mean age 53 years; range 33–70 years), and PBC (n = 9; all patients untreated with ursodeoxycholic acid; nine women; mean age 53 years; range 35–67 years) (Table 1). All patients with AIH were diagnosed as definitely having AIH based on the scoring system established by the International Autoimmune Hepatitis Group and classified as having type 1 AIH [20, 21]. The diagnosis of PBC was based on internationally accepted criteria, and the antimitochondrial antibody status of each patient was verified [22]. Patients with overlap syndrome were excluded from this study. The diagnosis of PSC was based on typical cholangiographic and liver biopsy criteria [23]. Liver biopsy was performed in all IgG4-SC, AIH, and PBC patients and in three of the 26 PSC patients. In the remaining 23 PSC patients, a liver specimen was obtained during liver transplantation surgery. This study was approved by the Kansai Medical University's Ethics Committee.

Histopathology and immunohistochemistry

Formalin-fixed and paraffin-embedded specimens were prepared and used for histopathological and immunohistochemical studies. Sections measuring 4 μm were cut from each paraffin block and stained with hematoxylin and eosin, periodic acid–Schiff after diastase digestion, Azan–Mallory, reticulin, or orcein. The remaining material was used for immunohistochemical analysis. The immunostaining of IgG4 was performed using a monoclonal antibody for human IgG4 (ZYMED Laboratories, San Francisco, CA) and that of IgG1 and Foxp3 using the avidin–biotin complex (ABC) method with reagents obtained from Vector Laboratories (Burlingame, CA). The antibodies used to identify the inflammatory cells in the liver were the IgG1 antibody (Binding Site, Birmingham, UK) and Foxp3 (eBioscience, San Diego, CA). The deparaffinized sections were pretreated in ethylenediaminetetraacetic acid buffer (pH 8.0) in a pressure cooker at 100°C for 5 min. Following incubation with the first antibody at 4°C overnight, biotinylated rabbit anti-sheep serum IgG (Vector Laboratories) was used as the secondary antibody (sections for IgG1), and immunoreactive deposits were visualized with 3,3′-diaminobenzidine tetrahydrochloride. To correct for differences in the sizes of the portal tracts, we counted the numbers of immunohistochemically identifiable IgG1-, IgG4-, and Foxp3-positive cells and mononuclear cells contained within the portal tracts selected in each specimen under five different high power fields (hpf); two pathologists subsequently calculated the ratio between IgG1-, IgG4-, Foxp3-positive cells and infiltrated mononuclear cells in each case.

Table 1 Clinical profile and characteristics of the patient and control groups

Clinical profile	n	Sex (male/female)	Age (years)[a]
IgG4-SC	16	15/1	63 ± 3 (31–81)
Intra	8	7/1	59 ± 6 (31–75)
Extra	8	8/0	67 ± 3 (54–81)
PSC	26	15/11	36 ± 4 (6–77)*, **
AIH	9	0/9	53 ± 4 (33–70)
PBC	9	0/9	53 ± 4 (35–67)

IgG4-SC Immunoglobulin G4-related sclerosing cholangitis, *Intra* intra-hepatic or both intra-hepatic and extra-hepatic biliary strictures, *Extra* extra-hepatic biliary strictures, *PSC* primary sclerosing cholangitis, *AIH* autoimmune hepatitis, *PBC* primary biliary cirrhosis

* $p < 0.01$ vs. AIH and PBC; ** $p < 0.001$ vs. IgG4-SC, intra- and extra-hepatic

[a] Values are given as the mean ± standard error of the mean (SEM), with the range in parenthesis

Statistical analysis

For all studies, data are expressed as mean ± standard error of the mean (SEM). Differences were analyzed using the nonparametric Mann–Whitney rank test and Fisher's exact test, where p values <0.05 were considered to be significant.

Results

Patients profile

Patient age was significantly lower in the PSC group than in the other groups. There was one peak in the age distribution of the IgG4-SC patients between 60 and 70 years and two peaks in the age distribution of the PSC patients (one between 20 and 30 years, and the other between 40 and 50 years). Of the eight intra-IgG4-SC patients, seven were male; all AIH and PBC patients were female (Table 1).

Laboratory findings

The serum asparate aminotransferase and alanine aminotransferase values were elevated in all groups, with no difference in laboratory values among the groups. The

serum alkaline phosphatase level was significantly higher in the PSC group than in the AIH group, and the serum γ-glutamyl transpeptidase level was significantly higher in the PBC group than in the AIH group. The serum total bilirubin was significantly higher in the PSC group. The eosinophil level was significantly higher in the PBC and intra-IgG4-SC groups than in the PSC and AIH groups. There were no significant differences in the serum levels of IgA. The serum IgG4 values were elevated in patients with intra-IgG4-SC. Antinuclear antibodies were positive in 38% of the intra-IgG4-SC patients, 48% of the PSC patients, 78% of the AIH patients, and 22% of the PBC patients. Antimitochondrial antibodies were positive in 0% of the intra-IgG4-SC and PSC patients, 22% of the AIH patients, and 78% of the PBC patients (Table 2).

Immunohistochemical findings of IgG1 and IgG4

As shown in Figs. 2 and 3, the ratio of IgG4-positive plasma cells to infiltrated mononuclear cells (IgG4/Mono) was significantly higher in patients with intra-IgG4-SC (0.121 ± 0.069) than in those with PSC (0.02 ± 0.003; $p = 0.002$), AIH (0.013 ± 0.004; $p = 0.0052$), and PBC (0.013 ± 0.002; $p = 0.0052$; Fig. 3a). The ratio of IgG1-positive plasma cells to infiltrated mononuclear cells (IgG1/Mono) was significantly lower in patients with intra-IgG4-SC (0.041 ± 0.009) than in those with AIH (0.084 ± 0.014; $p = 0.0161$; Fig. 3b). The ratio of IgG4/Mono to IgG1/Mono (IgG4/G1) was significantly higher in patients with intra-IgG4-SC (3.084 ± 1.824) than in those with PSC (0.424 ± 0.068; $p = 0.0018$), AIH (0.169 ± 0.042; $p = 0.004$), and PBC (0.196 ± 0.02; $p = 0.0044$; Fig. 3c). The intra-IgG4-SC patients were found to have an IgG4/G1 ratio >1.

Immunohistochemical findings of Foxp3

As shown in Figs. 4 and 5, patients with PBC has a significantly higher ratio of Foxp3-positive cells to infiltrated mononuclear cells (Foxp3/Mono) (0.042 ± 0.008) than those with intra-IgG4-SC (0.013 ± 0.006; $p = 0.0007$) and PSC (0.006 ± 0.001; $p < 0.0001$). Patients with AIH (0.027 ± 0.009) had a significantly higher Foxp3/Mono ratio than those with PSC ($p = 0.0016$). The Foxp3/Mono ratio was significantly higher in patients with intra-IgG4-SC than in those with PSC ($p = 0.0314$; Fig. 5, dotted line).

Correlation between the Foxp3/Mono and IgG4/Mono ratios in patients with IgG4-SC

The Foxp3/Mono and IgG4/Mono ratios were found to be positively correlated in the group of patients with intra-IgG4-SC ($R = 0.75$), but there was no correlation found in the other patient groups (PSC, $R = 0.05$; AIH, $R = 0.07$; PBC, $R = 0.11$; Fig. 6).

Table 2 Laboratory findings of AIP-SC, PSC, AIH, and PBC patients

Laboratory parameters[a]	Intra-IgG4-SC ($n = 8$)	PSC ($n = 26$)	AIH ($n = 9$)	PBC ($n = 9$)	Statistical significance[b]
AST (13–35 U/l)	57 ± 15	135 ± 14	152 ± 69	144 ± 96	NS
ALT (5–35 U/l)	52 ± 17	93 ± 16	185 ± 77	132 ± 91	NS
ALP (107–340 U/l)	837 ± 313	1284 ± 228*	360 ± 57	626 ± 66	$p < 0.05$
γ-GTP (11–64 U/l)	242 ± 59	211 ± 34	136 ± 31	330 ± 99*	$p < 0.05$
T-Bil (0.2–1.2 mg/dl)	2.7 ± 1.4	15.8 ± 2.5***	2.5 ± 1.1	1.0 ± 0.3	$p < 0.001$
ALB (3.8–5.0 g/dl)	3.3 ± 0.2	3.2 ± 0.1	3.6 ± 0.2‡	3.8 ± 0.1**, †	$p < 0.05$, 0.01
Eosinophils (%)	6.2 ± 0.9*	2.7 ± 0.7	1.0 ± 0.3	9.3 ± 2.5***	$p < 0.05$, 0.001
IgM (33–190 mg/dl)	97 ± 22 (6)	154 ± 24 (17)	316 ± 126	558 ± 132**, ***	$p < 0.01$, 0.001
IgA (110–410 mg/dl)	307 ± 85 (6)	509 ± 112 (16)	281 ± 44 (8)	280 ± 44 (8)	NS
IgG (870–1700 mg/dl)	2585 ± 345 (7)*	1780 ± 156 (20)	2323 ± 457	1685 ± 168	$p < 0.05$
IgG4 (4.8–105 mg/dl)	556 ± 224	–	–	–	–
ANA-positive	38% (3/8)	48% (10/21)	78% (7/9)*	22% (2/9)	$p < 0.05$
AMA-positive	0% (0/6)	0% (0/14)	22% (2/9)	78% (7/9)***	$p < 0.001$

Values are given as the mean ± SEM

[a] *AST* Aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *γ-GTP* γ-glutamyl transpeptidase, *T-Bil* total bilirubin, *ALB* albumin, *ANA* antinuclear antibody, *AMA* antimitochondrial antibody

[b] Significance: *NS* not significant; ALP, *$p < 0.05$ vs. AIH; γ-GTP, *$p < 0.05$ vs. AIH; T-Bil, ***$p < 0.001$ vs. intra-IgG4-SC, AIH, and PBC; ALB, **$p < 0.01$ vs. PSC, †$p < 0.05$ vs. intra-IgG4-SC, ‡$p < 0.05$ vs. PSC; eosinophils, ***$p < 0.001$ vs. PSC and AIH, *$p < 0.05$ vs. AIH and PSC; IgM, **$p < 0.01$ vs. intra-IgG4-SC, ***$p < 0.001$ vs. PSC; IgG, *$p < 0.05$ vs. PSC and PBC; ANA, *$p < 0.05$ vs. PBC; AMA, ***$p < 0.001$ vs. intra-IgG4-SC, PSC, and AIH

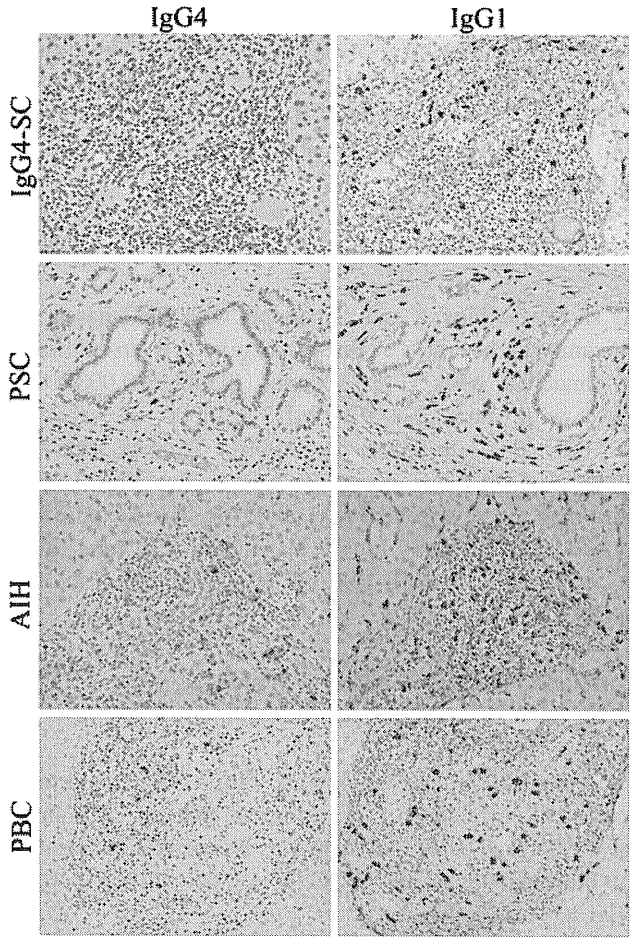

Fig. 2 Immunostaining of formalin-fixed, paraffin-embedded liver sections obtained from patients with IgG4-SC, primary sclerosing cholangitis (*PSC*), autoimmune hepatitis (*AIH*), and primary biliary cirrhosis (*PBC*). Representative liver sections of IgG4-SC, PSC, AIH, and PBC patients show immunostaining of IgG4 and IgG1. The density of IgG4-positive cells is higher than that of IgG1-positive cells in the IgG4-SC liver sections. In the liver sections of PSC, AIH and PBC patients, the density of IgG1-positive cells is higher than that of IgG4-positive cells (×200)

Comparison between immunohistochemical findings in the liver specimens of AIH patients with and without steroid therapy

In terms of the Foxp3/Mono, IgG4/Mono, IgG1/Mono, and IgG4/G1 ratios, AIH patients not receiving steroid therapy showed an increasing tendency relative to those receiving steroid therapy, but the differences were not significant.

Discussion

In general, AIP is currently accepted to be a unique distinctive disease in which histopathological findings show an abundant infiltration of IgG4-positive plasma cells and fibrosis, a condition denoted as lymphoplasmacytic

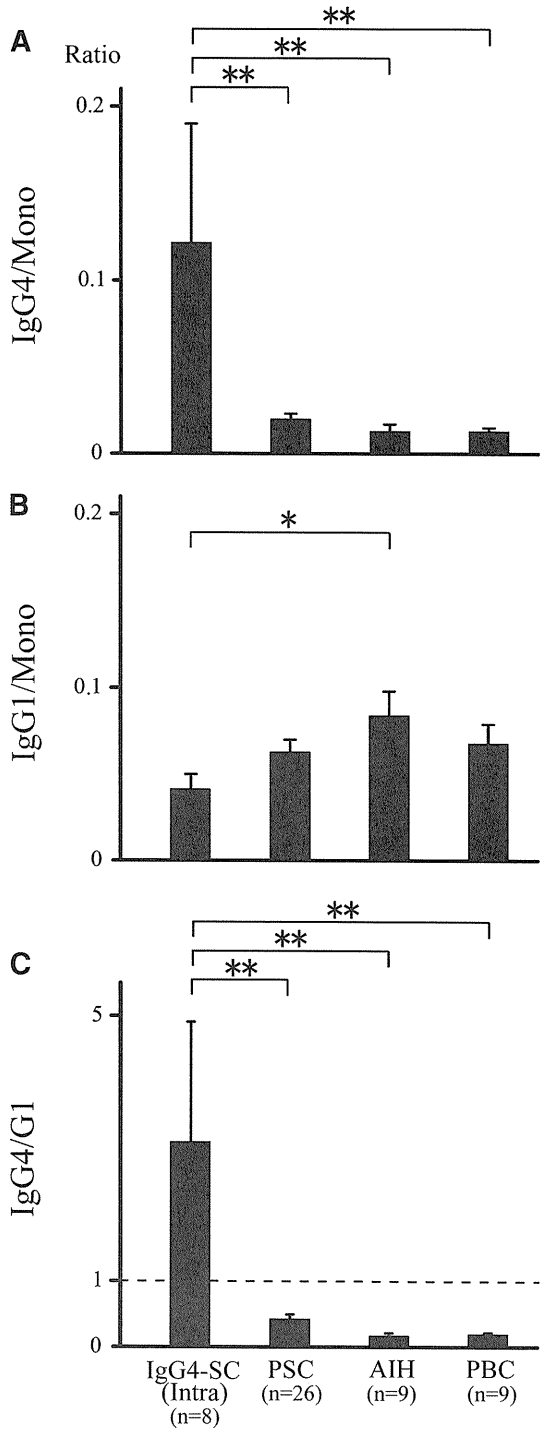

Fig. 3 Ratios of IgG4-positive plasma cells/infiltrated mononuclear cells (*IgG4/Mono*), IgG1-positive plasma cells/infiltrated mononuclear cells (*IgG1/Mono*), and IgG4/Mono to IgG1/Mono (*IgG4/G1*) in the liver of patients with intra-IgG4-SC in comparison with those with other liver diseases. **a** The IgG4/Mono ratio was significantly higher in patients with intra-IgG4-SC than in those with other liver diseases. **b** The IgG1/Mono ratio was significantly lower in patients with intra-IgG4-SC than in those with AIH. **c** The IgG4/G1 was significantly higher in patients with intra-IgG4-SC than in those with other liver diseases. Data are expressed as the mean ± standard error of the mean (SEM). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, *NS* not significant

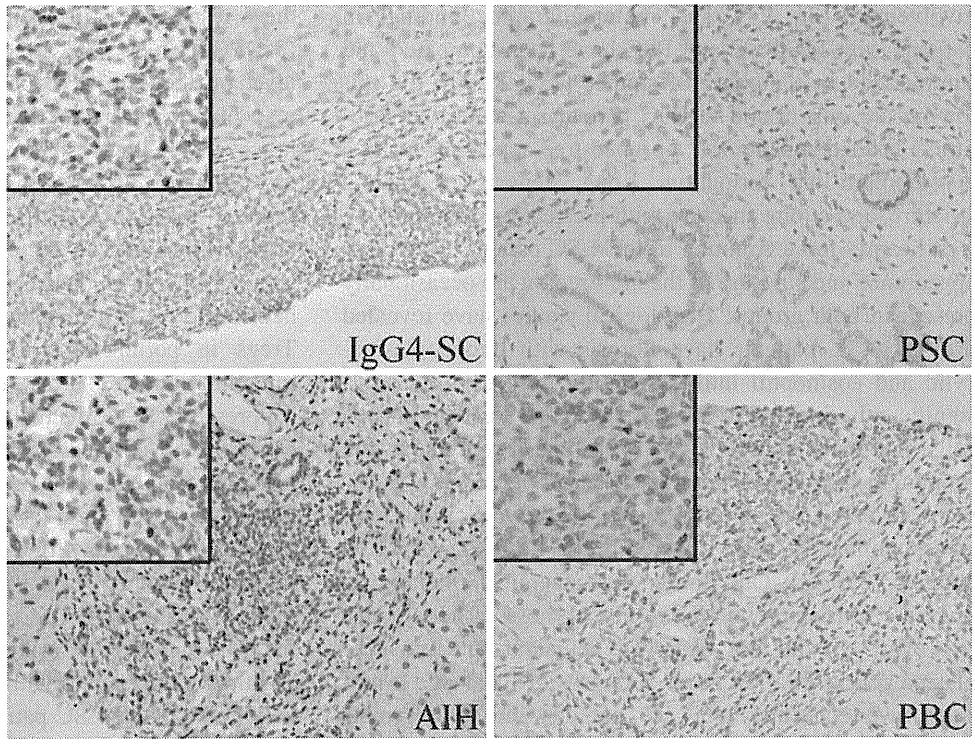

Fig. 4 Immunostaining of forkhead box P3 (Foxp3) in liver sections from IgG4-SC, PSC, AIH, and PBC patients. Foxp3-positive cells were found to be scattered among the lymphoid infiltrates in the portal tracts of the IgG4-SC, AIH, and PBC sections but among those of the PSC sections (×200)

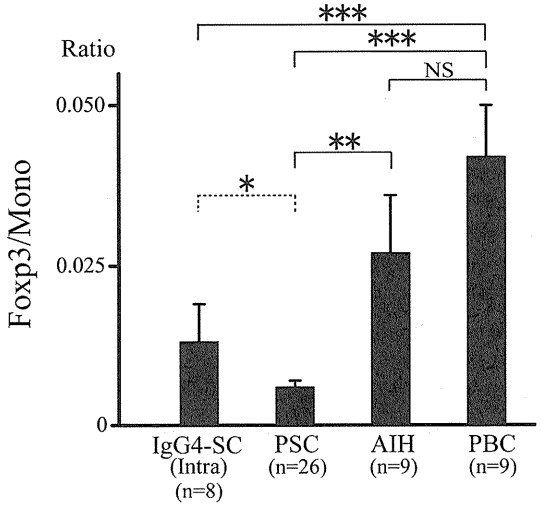

Fig. 5 Ratios of Foxp3 to infiltrated mononuclear cells (*Foxp3/Mono*) in the liver sections of patients with intra-IgG4-SC in comparison with those with other liver diseases. Patients with PBC had a significantly larger Foxp3/Mono ratio than those with intra-IgG4-SC and PSC. The Foxp3/Mono ratio was significantly decreased in PSC patients compared with those with intra-IgG4-SC, AIH, and PBC. *Dotted line* Comparison between intra-IgG4-SC and PSC patients. Data are expressed as the mean ± SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, *NS* not significant

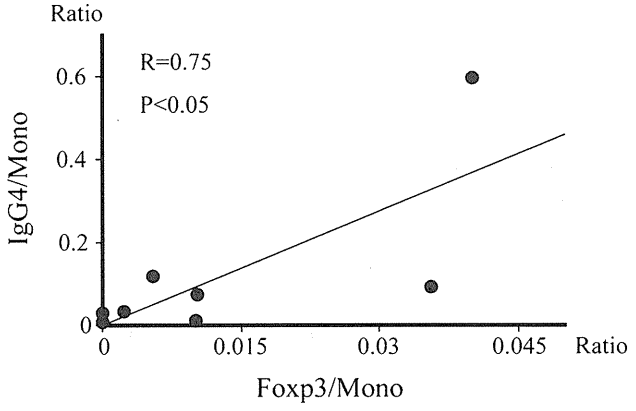

Fig. 6 Correlation between the Foxp3/Mono and IgG4/Mono ratio in patients with intra-IgG4-SC. The Foxp3/Mono and IgG4/Mono ratios are positively correlated in the intra-IgG4-SC patients ($R = 0.75$), but there is no correlation in the other patient groups

sclerosing pancreatitis (LPSP), and the clinical manifestations dramatically respond to steroid treatment. In addition to pancreatic lesions, patients with AIP have occasional extrapancreatic lesions, such as SC, sclerosing sialoadenitis, and retroperitoneal fibrosis (all of which are similar to LPSP). The novel features of this systemic disease have been proposed to be IgG4-related sclerosing disease [10], systemic IgG4-related plasmacytic disease [24], and IgG4-positive multi-organ lymphoproliferative syndrome [25]. Among these, IgG4-SC is the most common and serious extrapancreatic lesion resulting in obstructive jaundice. Prior to the concept of AIP being established, IgG4-SC was often misdiagnosed as PSC complicating chronic pancreatitis. Therefore, differential diagnosis between IgG4-SC and PSC is important. Although IgG4-SC is usually associated with pancreatic lesions, some patients with IgG4-SC have no apparent pancreatic changes or other organ

involvement [26, 27]. The cholangiographic findings in patients with IgG4-SC and PSC have been compared and summarized in a number of studies [12, 28, 29]; the following are only found in PSC patients: a band-like stricture, a beaded appearance, a pruned-tree appearance, and a diverticulum-like formation. Long stenosis, segmental stricture, and a long stricture with prestenotic dilatation are significantly more common in IgG4-SC patients. A shaggy appearance and stricture of the hilar region are occasionally present in both groups. Histological studies have revealed that IgG4-SC patients have fibrosis with lymphoplasmacytic and eosinophil infiltration with mild fibrosis and a significantly increased number of IgG4-positive plasma cells than PSC patients [30, 31]. In one recent study, the immunohistochemical analysis of the liver biopsy specimens revealed that IgG4-positive plasma cell infiltration was significantly more severe in IgG4-SC than in PSC patients [28, 32]. Our immunohistochemical findings on IgG4 are consistent with the results of these earlier studies. However, we did not observed abundant IgG4-positive plasma cell infiltration in all our cases of IgG4-SC. In our study, we classified IgG4-SC patients into two groups based on the intra-/extra-hepatic (intra-IgG4-SC) or extra-hepatic (extra-IgG4-SC) nature of the biliary strictures. The infiltration of IgG4-positive plasma cells was more severe in patients with intra-IgG4-SC than in those with extra-IgG4-SC (data not shown). In terms of IgG4 serum levels, there were no significant differences between intra- (556 ± 224; $n = 8$) and extra-IgG4-SC (341 ± 61; $n = 8$). Moreover, IgG4-positive plasma cell infiltration in both classes of IgG4-SC was high compared to that of IgG1-positive plasma cell infiltration, whereas in other liver diseases (PSC, AIH, PBC), IgG1-positive plasma cell infiltration was higher than that of IgG4. Taken together, our study shows that the ratio of IgG4/Mono to IgG1/Mono (IgG4/G1) in intra-IgG4-SC patients was significantly higher than that in patients with other liver diseases (PSC, AIH, and PBC). In recent studies, IgG4-associated AIH was differentiated from other recognized types of AIH [33, 34]. Chung et al. [34] reported that patients with IgG4-associated AIH showed increased IgG serum levels and a marked response to prednisolone therapy. In our study, one of the nine AIH patients showed infiltration of abundant IgG4-positive plasma cells in the liver (>10 IgG4-positive plasma cells/hpf) and increased serum levels of IgG (2886 mg/dl).

In our study, we also examined the local infiltration of $Foxp3^+$ Tregs in the liver of intra-IgG4-SC, AIH, PSC, and PBC patients because $Foxp3^+$ Tregs have been reported to be involved in the development of various autoimmune diseases. Several recent studies have demonstrated the presence of $CD4^+CD25^{high}$ Tregs in patients with autoimmune liver diseases, such as AIH and PBC [35–39], but these results are still open to discussion in terms of PBC [35–37]. Lan et al. [35] recently reported a decrease in the level of Tregs in PBC patients and suggested that Tregs may play a role in the loss of immune tolerance in PBC; however, other investigators [36, 37] have reported a relative increase of Tregs in PBC patients. Sasaki et al. [36] reported that the level of Foxp3, interleukin (IL)-10, and transforming growth factor beta (TGF-β) mRNA expression was higher in the livers of PBC patiens than in normal healthy livers and that the amount of infiltrating $Foxp3^+$ Tregs in portal tracts paralleled the degree of portal inflammation. In contrast, the level of $CD4^+CD25^+$ Tregs suppressing the effector Th1 and Th2 responses were decreased in peripheral blood samples from AIH patients [39]. Our data show a significantly increased infiltration of $Foxp3^+$ Tregs in the liver of intra-IgG4-SC, AIH, and PBC patients compared with PSC patients and a significantly increased number of $Foxp3^+$ Tregs in PBC patients than in those with intra-IgG4-SC and PSC. In addition, we found that the number of infiltrated Foxp3-positive cells was positively correlated with the number of IgG4-positive cells in intra-IgG4-SC patients, but not in those with other liver diseases (PSC, AIH, and PBC). Data obtained in previous studies showed that the level of $Foxp3^+$ Tregs decreased in the liver of PBC patients as the histological stage of the disease advanced [35–37]. Of our 26 cases of PSC, 23 liver specimens were obtained during liver transplantation surgery, indicating that the histological stages in our cases were advanced. Further studies are necessary in order to be able to draw a reliable conclusion on the relationship between $Foxp3^+$ Tregs and PBC because there is a possibility that staging of PSC may affect the severity of infiltration of Tregs similar to that observed in PBC.

We previously reported that circulating Th1 type $CD4^+$ T cells, but not Th2 type $CD4^+$ T cells [40], and $CD4^+CD25^{high}$ Tregs were increased in the peripheral blood of AIP patients [17]. Recent studies of immune tolerance and allergy show that high-dose antigen exposures can cause both immune deviations of the Th2 response in favor of a Th0/Th1 and the generation of IL-10- and TGF-β-producing Tregs. During high-dose antigen exposures, the activation and/or maintenance of the usual Th2 T cell response is inhibited. Additionally, IL-10 induces preferential switching of the B cell response in favor of producing IgG4 antibodies, and possibly IgA antibodies, under the influence of TGF-β [41]. $CD4^+CD25^+$ Tregs also produce IL-10 to educate antigen presenting cells [42]. Therefore, increased Treg levels may correlate with the production of IL-10 in the involved organs, which in turn may influence the switching of B cells to IgG4-producing plasmacytes and the production of serum IgG4. We have previously reported that serum levels of IL-10 and TGF-β in AIP patients were not different from those in healthy and

other disease controls (alcoholic and idiopathic chronic pancreatitis patients) [17]. In other experiments, there was no difference in the serum levels of TGF-β among patients with PSC [43], PBC [44], and healthy controls but serum TGF-β levels in AIH patients were higher than those in healthy controls [45]. Zen et al. [11] reported that Tregs producing IL-10 and TGF-β infiltrated the liver of patients with IgG4-SC and that Foxp3-positive cells were lower in PSC patients than in IgG4-SC patients. However, different from IgG4-SC, our data suggest that such a mechanism is unlikely in AIH or PBC. It still remains unclear why there is difference in the relationship between IgG4-positive cells and Tregs in patients with IgG4-SC, PSC, AIH, and PBC. There are at least two possibilities explaining these differences: (1) it may be due to an originally different subpopulation of Tregs [46–49]; (2) it may be due to different activity of Tregs, such as acting Tregs and resting Tregs [50]. In contrast to murine $Foxp3^+$ Tregs, human $Foxp3^+$ cells may not be functionally homogenous [46, 47]. In general, high amounts of IL-10-producing Tregs, which also produce TGF-β, are well known as type 1 regulatory (Tr1) cells. There is some evidence (based on CD25 expression on $CD4^+$Tr1 cells) in adult humans that constitutive $CD4^+CD25^+$ Tregs and inducible IL-10- and TGF-β-secreting Tr1 cells represent overlapping populations [41]. Furthermore, some $Foxp3^+$ cells are phenotypically naive (e.g., $CD45RA^+$), being present in cord blood as well as in the peripheral blood of adults, and suppressive in vitro [48], whereas other $Foxp3^+$ cells phenotypically resemble memory T cells (e.g., $CD45RA^-$) and possibly originate from peripheral memory $Foxp3^-CD4^+$ T cells [49], in which case they may use different suppressive mechanisms by secreting different immunosuppressive cytokines, such as IL-10 and TGF-β [51]. Taken together, our data may support a hypothesis that decreased naive Tregs may be involved in the pathogenesis of IgG4-SC, resulting in the activation of Th1 type immune responses, while a high-dose antigen (carbonic anhydarase II or lactoferrin, etc.) may induce $CD4^+CD25^{high}$ Tregs from the peripheral blood [52]. This mechanism correlates with the production of IL-10 switching B cells to IgG4-producing plasmacytes in the chronic active phase, resulting in the suppression of both Th2 and Th2 type immune cells [53].

In conclusion, the IgG4/G1 ratio may be another useful marker for the differential diagnosis between intra-IgG4-SC and PSC. We have also demonstrated that the infiltration of $Foxp3^+$ Tregs in the liver was significantly increased in the livers of patients with intra-IgG4-SC, AIH, and PBC relative to those of patients with PSC and that there is a possibility that the function of infiltrated $Foxp3^+$ Tregs is different in intra-IgG4-SC and other liver diseases (PSC, AIH, and PBC). Further studies are needed to clarify the real function of $Foxp3^+$ Tregs infiltrating into the liver of patients with intra-IgG4-SC and other autoimmune liver diseases as well as whether IL-10 or TGF-β is upregulated in the local microenvironment or not.

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References

1. Sarles H, Sarles JC, Muratore R, Guien C. Chronic inflammatory sclerosis of the pancreas—an autonomous pancreatic disease? Am J Dig Dis. 1961;6:688–98.
2. Yoshida K, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N. Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. Dig Dis Sci. 1995;40:1561–8.
3. Horiuchi A, Kawa S, Akamatsu T, Aoki Y, Mukawa K, Furuya N, et al. Characteristic pancreatic duct appearance in autoimmune chronic pancreatitis: a case report and review of Japanese literature. Am J Gastroenterol. 1998;93:260–3.
4. Uchida K, Okazaki K, Konishi Y Ohana M, Takakuwa H, Hajiro K, et al. Clinical analysis of autoimmune-related pancreatitis. Am J Gastroenterol. 2000;95:2788–94.
5. Okazaki K, Kawa S, Kamisawa T, Naruse S, Tanaka S, Nishimori I, et al. Clinical diagnostic criteria of autoimmune pancreatitis: revised proposal. J Gastroenterol. 2006;41:626–31.
6. Okazaki K, Uchida K, Matsushita M, Takaoka M. Autoimmune pancreatitis. Intern Med. 2005;44:1215–23.
7. Kamisawa T, Egawa N, Nakajima H, Tsuruta K, Okamoto A. Extrapancreatic lesions in autoimmune pancreatitis. J Clin Gastroenterol. 2005;39:904–7.
8. Nakazawa T, Ohara H, Sano H, Ando T, Aoki S, Kobayashi S, et al. Clinical differences between primary sclerosing cholangitis and sclerosing cholangitis with autoimmune pancreatitis. Pancreas. 2005;30:20–5.
9. Hamano H, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T, et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med. 2001;344:732–8.
10. Kamisawa T, Okamoto A. Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease. J Gastroenterol. 2006;41:613–25.
11. Zen Y, Fujii T, Harada K, Kawano M, Yamada K, Takahira M, et al. Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. Hepatology. 2007;45:1538–46.
12. Nakazawa T, Ohara H, Sano H, Aoki S, Kobayashi S, Okamoto T, et al. Cholangiography can discriminate sclerosing cholangitis with autoimmune pancreatitis from primary sclerosing cholangitis. Gastrointest Endosc. 2004;60:937–44.
13. Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. $CD4^+$ CD25high regulatory cells in human peripheral blood. J Immunol. 2001;167:1245–53.
14. Dieckmann D, Plottner H, Berchtold S, Berger T, Schuler G. Ex vivo isolation and characterization of CD4(+)CD25(+) T cells with regulatory properties from human blood. J Exp Med. 2001;193:1303–10.

15. Wing K, Ekmark A, Karlsson H, Rudin A, Suri-Payer E. Characterization of human $CD25^+$ $CD4^+$ T cells in thymus, cord and adult blood. Immunology. 2002;106:190–9.
16. Makita S, Kanai T, Oshima S, Uraushihara K, Totsuka T, Sawada T, et al. $CD4^+$ CD25bright T cells in human intestinal lamina propria as regulatory cells. J Immunol. 2004;173:3119–30.
17. Miyoshi H, Uchida K, Taniguchi T, Yazumi S, Matsushita M, Takaoka M, et al. Circulating Naive and $CD4^+$ CD25high regulatory T cells in patients with autoimmune pancreatitis. Pancreas. 2007;36:133–40.
18. Otsuki M, Chung JB, Okazaki K, Kim MH, Kamisawa T, Kawa S, et al. Asian diagnostic criteria for autoimmune pancreatitis: consensus of the Japan-Korea Symposium on Autoimmune Pancreatitis. J Gastroenterol. 2008;43:403–8.
19. Ghazale A, Chari ST, Zhang L, Smyrk TC, Takahashi N, Levy MJ, et al. Immunoglobulin G4-associated cholangitis: clinical profile and response to therapy. Gastroenterology. 2008;134:706–15.
20. Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol. 1999;31:929–38.
21. Vergani D, Alvarez F, Bianchi FB, Cançado EL, Mackay IR, Manns MP, et al. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. J Hepatol. 2004;41:677–83.
22. Kaplan MM, Gershwin ME. Primary biliary cirrhosis. N Engl J Med. 2005;353:1261–73.
23. LaRusso NF, Weisnor RH, Ludwig J, MacCarty RL. Current concept: primary sclerosing cholangitis. N Engl J Med. 1984;310:899–903.
24. Yamamoto M, Takahashi H, Ohara M, Suzuki C, Naishiro Y, Yamamoto H, et al. A new conceptualization for Mikulicz's disease as an IgG4-related plasmacytic disease. Mod Rheumatol. 2006;16:335–40.
25. Masaki Y, Dong L, Kurose N, Kitagawa K, Morikawa Y, Yamamoto M, et al. Proposal for a new clinical entity, IgG4-positive multi-organ lymphoproliferative syndrome: Analysis of 64 cases of IgG4-related disorders. Ann Rheum Dis. 2009; 68:1310–5.
26. Hayashi K, Nakazawa T, Ohara H, Ando T, Takada H, Tanaka H, et al. Autoimmune sclerosing cholangiopancreatitis with little pancreatic involvements by imaging findings. Hepatogastroenterology. 2007;54:2150–5.
27. Hamano H, Kawa S, Uehara T, Ochi Y, Takayama M, Komatsu K, et al. Immunoglobulin G4–related lymphoplasmacytic sclerosing cholangitis that mimics infiltrating hilar cholangiocarcinoma: part of a spectrum of autoimmune pancreatitis? Gastrointest Endosc. 2005;62:152–7.
28. Nishino T, Oyama H, Hashimoto E, Toki F, Oi I, Kobayashi M, et al. Clinicopathological differentiation between sclerosing cholangitis with autoimmune pancreatitis and primary sclrosing cholangitis. J Gastroenterol. 2007;42:550–9.
29. Ohara H, Nakazawa T, Ando T, Joh T. Systemic extrapancreatic lesions associated with autoimmune pancreatitis. J Gastroenterol. 2007;42:15–21.
30. Uehara T, Hamano H, Kawa S, Sano K, Honda T, Ota H. Distinct clinicopathological entity autoimmune pancreatitis-associated sclerosing cholangitis. Pathol Int. 2005;55:405–11.
31. Zen Y, Harada K, Sasaki M, Sato Y, Tsuneyama K, Haratake J, et al. IgG4–related sclerosing cholangitis with and without hepatic inflammatory pseudotumor, and sclerosing pancreatitis-associated sclerosing cholangitis: Do they belong to a spectrum of sclerosing pancreatitis? Am J Surg Pathol. 2004;28:1193–203.
32. Umemura T, Zen Y, Hamano H, Kawa S, Nakanuma Y, Kiyosawa K. Immunoglobin G4-hepatopathy association of immunoglobin G4-bearing plasma cells in liver with autoimmune pancreatitis. Hepatology. 2007;46:463–71.
33. Umemura T, Zen Y, Hamano H, Ichijo T, Kawa S, Nakanuma Y, et al. IgG4 associated autoimmune hepatitis: a differential diagnosis for classical autoimmune hepatitis. Gut. 2007;56:1471–2.
34. Chung H, Watanabe T, Kudo M, Maenishi O, Wakatsuki Y, Chiba T. Identification and characterization of IgG4-associated autoimmune hepatitis. Liver Int. 2010;30:222–31.
35. Lan RY, Cheng C, Lian ZX, Tsuneyama K, Yang GX, Moritoki Y, et al. Liver-targeted and peripheral blood alterations of regulatory T cells in primary biliary cirrhosis. Hepatology. 2006;43:729–37.
36. Sasaki M, Ikeda H, Sawada S, Sato Y, Nakanuma Y. Naturally-occurring regulatory T cells are increased in inflamed portal tracts with cholangiopathy in primary biliary cirrhosis. J Clin Pathol. 2007;60:1102–7.
37. Sakaki M, Hiroishi K, Baba T, Ito T, Hirayama Y, Saito K, et al. Intrahepatic status of regulatory T cells in autoimmune liver diseases and chronic viral hepatitis. Hepatol Res. 2008;38:354–61.
38. Longhi MS, Hussain MJ, Mitry RR, Arora SK, Vergani GM, Vergani D, et al. Functional study of $CD4^+$ $CD25^+$ regulatory T cells in health and autoimmune hepatitis. J Immunol. 2006;176:4484–91.
39. Longhi MS, Ma Y, Bogdanos DP, Cheeseman P, Mieli-Vergani G, Vergani D. Impairment of CD4(+)CD25(+) regulatory T-cells in autoimmune liver disease. J Hepatol. 2004;41:31–7.
40. Okazaki K, Uchida K, Ohana M, Nakase H, Uose S, Inai M, et al. Autoimmune-related pancreatitis is associated with autoantibodies and Th1/Th2 cellular immune response. Gastroenterology. 2000;118:573–81.
41. Robinson DS, Larche M, Durhan SR. Tregs and allergic disease. J Clin Invest. 2004;114:1389–97.
42. Alpan O, Bachelder E, Isil E, Arnheiter H, Matzinger P. 'Educated' dendritic cells act as messengers from memory to naive T helper cells. Nat Immunol. 2004;5:615–22.
43. Watt JK, Hawkins K, Zhang M, Lipschitz J, Sandha G, Gong Y, et al. Transforming growth factor-beta (TGF-beta) protein levels are not elevated in the blood or bile of patients with primary sclerosing cholangitis: a pilot study. Dig Dis Sci. 2004;49:5–8.
44. Rong G, Zhou Y, Xiong Y, Zhou L, Geng H, Jiang T, et al. Imbalance between T helper type 17 and T regulatory cells in patients with primary biliary cirrhosis: the serum cytokine profile and peripheral cell population. Clin Exp Immunol. 2009;156:217–25.
45. Sakaguchi K, Kitano M, Nishimura M, Senoh T, Ohta T, Terao M, et al. Serum level of transforming growth factor-beta1 (TGF-beta1) and the expression of TGF-beta receptor type II in peripheral blood mononuclear cells in patients with autoimmune hepatitis. Hepatogastroenterology. 2004;51:1780–3.
46. Allan SE, Crome SQ, Crellin NK, Passerini L, Steiner TS, Bacchetta R, et al. Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production. Int Immunol. 2007;19:345–54.
47. Tran DQ, Ramsey H, Shevach EM. Induction of FOXP3 expression in naive human $CD4^+$ FOXP3 T cells by T-cell receptor stimulation is transforming growth factor-beta dependent but does not confer a regulatory phenotype. Blood. 2007; 110:2983–90.
48. Valmori D, Merlo A, Souleimanian NE, Hesdorffer CS, Ayyoub M. A peripheral circulating compartment of natural naive CD4 Tregs. J Clin Invest. 2005;115:1953–62.
49. Vukmanovic-Stejic M, Zhang Y, Cook JE, Fletcher JM, McQuaid A, Masters JE, et al. Human $CD4^+$ CD25hiFoxp3$^+$ regulatory T cells are derived by rapid turnover of memory populations in vivo. J Clin Invest. 2006;116:2423–33.

50. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, et al. Functional Delineation and Differentiation Dynamics of Human CD4(+) T Cells Expressing the FoxP3 Transcription Factor. Immunity. 2009;30:1–13.
51. Ito T, Hanabuchi S, Wang YH, Park WR, Arima K, Bover L, et al. Two functional subsets of FOXP3$^+$ regulatory T cells in human thymus and periphery. Immunity. 2008;28:870–80.
52. Uchida K, Okazaki K, Nishi T, Uose S, Nakase H, Ohana M, et al. Experimental immune-mediated pancreatitis in neonatally thymectomized mice immunized with carbonic anhydarase II and lactoferrin. Lab Invest. 2002;82:411–24.
53. Okazaki K, Uchida K, Fukui T. Recent advances in autoimmune pancreatitis: concept, diagnosis, and pathogenesis. J Gastroenterol. 2008;43:409–18.

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NEW SURGICAL HORIZONS

Autoimmune pancreatitis—a new evolving pancreatic disease?

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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

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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	

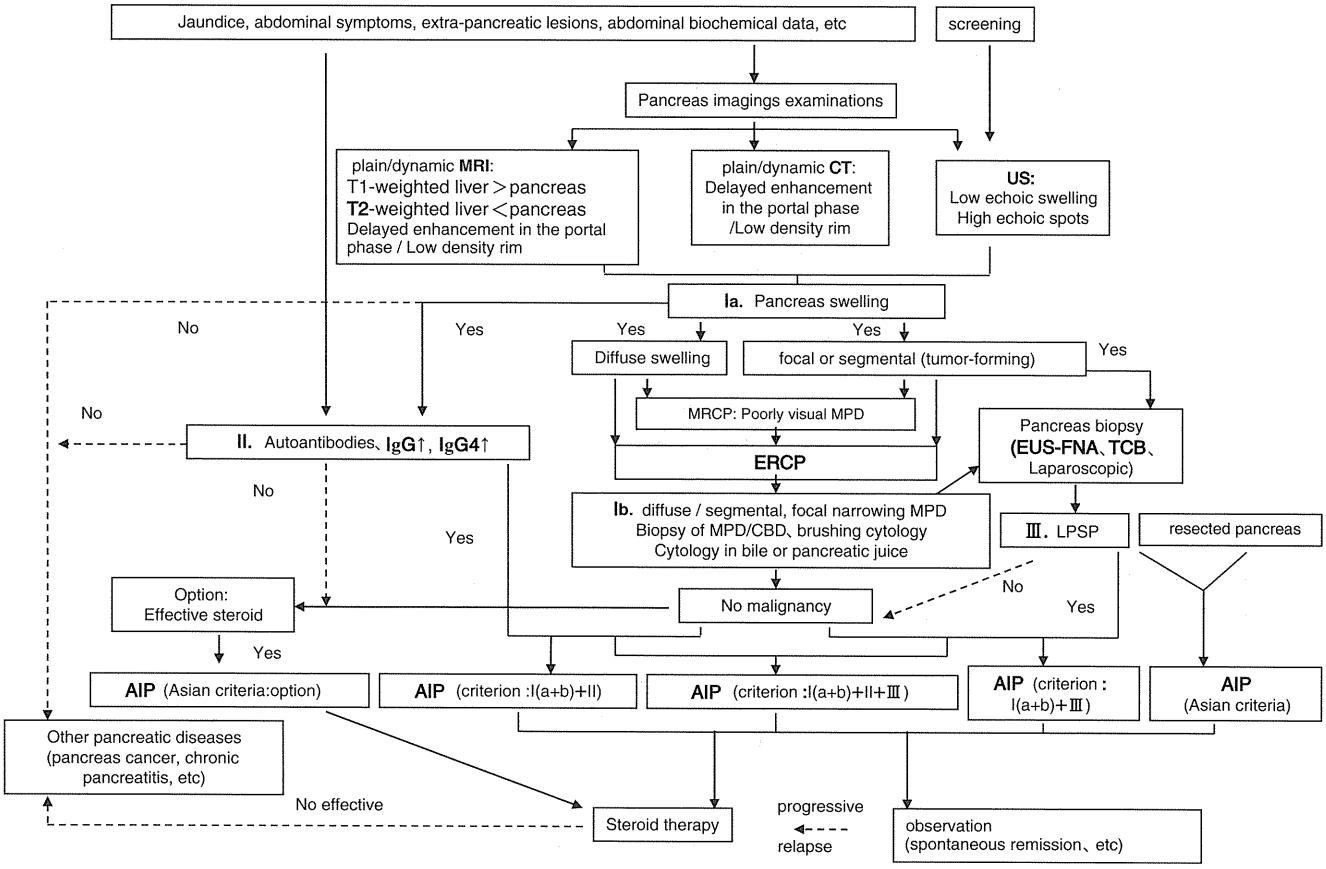

Fig. 1 Algorithm of diagnosis and management of AIP based on Asian diagnostic criteria proposed by the Japanese guidelines. A comprehensive diagnosis is performed based on pancreatic imaging, and serological and histopathological findings. In Japan, as defined by the Clinical Diagnostic Criteria 2006, 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 Thl 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.

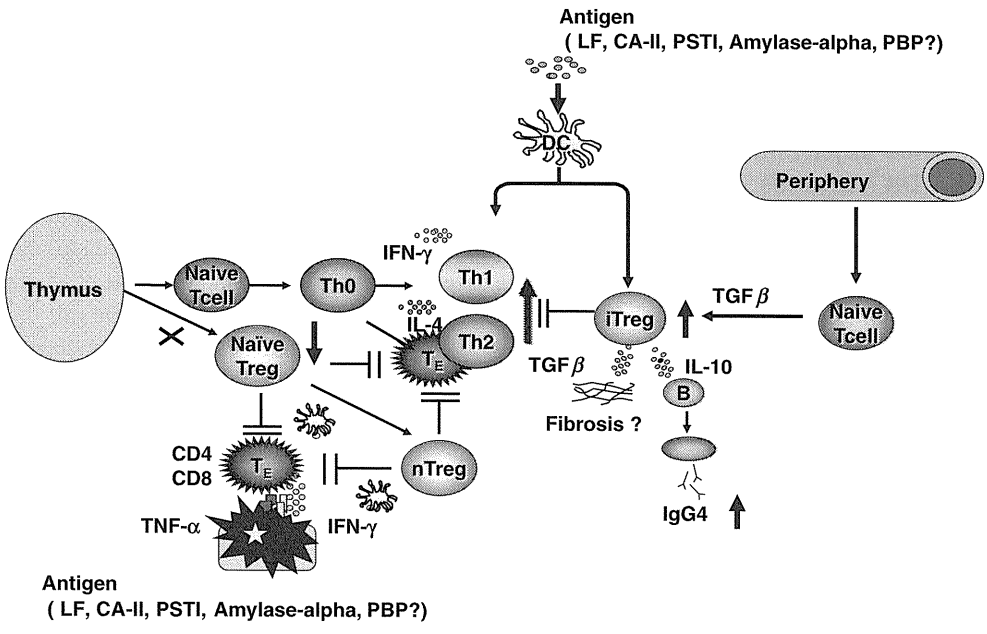

Fig. 2 Hypothesis for the pathogenesis of AIP. In the early stage, initial response to self-antigens (LF, CA-II, CA-IV, PSTI, or α-fodrin) or molecular mimicry (*H. pylori*) is induced by decreased naive Tregs, and Th1 cells release proinflammatory cytokines (IFN-γ, IL-1b, IL-2, and TNF-α). In the chronic 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.

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Conflicts of interest None.

References

1. Sarles H, Sarles JC, Muratore R et al (1961) Chronic inflammatory sclerosis of the pancreas—an autoimmune pancreatic disease? Am J Dig Dis 6:688–698
2. Toki F, Kozu T, Oi I (1992) An unusual type of chronic pancreatitis showing diffuse irregular narrowing of the entire main pancreatic duct on ERC—a report of four cases. Endoscopy 24:640
3. Yoshida K, Toki F, Takeuchi T et al (1995) Chronic pancreatitis caused by autoimmune abnormality. Proposal of concept of autoimmune pancreatitis. Dig Dis Sci 40:1561–1568
4. Okazaki K, Uchida K, Chiba T (2001) Recent concept of autoimmune-related pancreatitis. J Gastroenterol 36:293–302
5. Okazaki K (2006) In: Pour Parviz M (ed) Autoimmune pancreatitis—recent concept. Target organ toxicology series, toxicology of the pancreas. Taylor & Francis, Boca Raton, pp 459–473
6. Kawaguchi K, Koike M, Tsuruta K et al (1991) Lymphoplasmacytic sclerosing pancreatitis with cholangitis: a variant primary sclerosing cholangitis extensively involving pancreas. Human Pahtol 22:387–395
7. Pickartz T, Mayerle J, Lerch MM (2007) Autoimmune pancreatitis. Nat Clin Pract Gastroenterol Hepatol 4:314–23
8. Members of the Criteria Committee for Autoimmune Pancreatitis of the Japan Pancreas Society (2002) Diagnostic criteria for autoimmune pancreatitis by the Japan Pancreas Society. J Jpn Pancreas Soc 17:587
9. Okazaki K, Kawa S, Kamisawa T, Naruse S, Tanaka S, Nishimori I et al (2006) Clinical diagnostic criteria of autoimmune pancreatitis: revised proposal. J Gastroenterol 41:626–31
10. Kim KP, Kim MH, Kim JC, Lee SS, Seo DW, Lee SK (2006) Diagnostic criteria for autoimmune chronic pancreatitis revisited. World J Gastroenterol 12:2487–96
11. Kim MH, Lee TY (2007) Diagnostic criteria for autoimmune pancreatitis: a proposal of revised Kim criteria. J Gastroenterol Hepatol 22:A104
12. Otsuki M, Chung JB, Okazaki K, Kim MH, Kamisawa T, Kawa S et al (2008) Asian diagnostic criteria for autoimmune pancreatitis: consensus of the Japan–Korea Symposium on Autoimmune Pancreatitis. J Gastroenterol 43:403–8
13. Chari ST, Smyrk TC, Levy MJ et al (2006) Diagnosis of autoimmune pancreatitis: the Mayo Clinic experience. Clin Gastroenterol Hepatol 4:1010–6
14. Chari ST, Takahashi N, Levy MJ et al (2009) A diagnostic strategy to distinguish autoimmune pancreatitis from pancreatic cancer. Clin Gastroenterol Hepatol 7:1097–1103
15. Pearson RK, Longnecker DS, Chari ST et al (2003) Controversies in clinical pancreatology: autoimmune pancreatitis: does it exist? Pancreas 27:1–13
16. Frulloni L, Scattolini C, Falconi M et al (2009) Autoimmune pancreatitis: differences between the focal and diffuse forms in 87 patients. Am J Gastroenterol 104:2288–94
17. Kawa S, Ota M, Yoshizawa K, Horiuchi A, Hamano H, Ochi Y et al (2002) HLA DRB10405-DQB10401 haplotype is associated with autoimmune pancreatitis in the Japanese population. Gastroenterology 122:1264–69
18. Park do H, Kim MH, Oh HB, Kwon OJ, Choi YJ, Lee SS et al (2008) Substitution of aspartic acid at position 57 of the DQbeta1 affects relapse of autoimmune pancreatitis. Gastroenterology 134:440–6
19. Hamano H, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T et al (2001) High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med 344:732–738
20. Uchida K, Okazaki K, Konishi Y et al (2000) Clinical analysis of autoimmune-related pancreatitis. Am J Gastroenterol 95:2788–2794
21. Okazaki K, Uchida K, Ohana M et al (2000) Autoimmune-related pancreatitis is associated with autoantibodies and Th1/Th2-type cellular immune response. Gastroenterology 118:573–581
22. Asada M, Nishio A, Uchida K et al (2006) Identification of a novel autoantibody against pancreatic secretory trypsin inhibitor in patients with autoimmune pancreatitis. Pancreas 33:20–6
23. Horiuchi A, Kawa S, Hamano H, Hayashi Y, Kiyosawa K (2002) Does a lack of reactivity to alpha-fodrin indicate the existence of primary autoimmune pancreatitis? Am J Gastroenterol 97:1275–7
24. Muraki T, Hamano H, Ochi Y, Komatsu K, Komiyama Y, Arakura N et al (2006) Autoimmune pancreatitis and complement activation system. Pancreas 32:16–21
25. Tanaka S, Kobayashi T, Nakanishi K et al (2000) Corticosteroid-responsive diabetes mellitus associated with autoimmune pancreatitis. Lancet 356:910–911
26. Erkelens GW, Vleggaar FP, Lesterhuis W et al (1999) Sclerosing pancreato-cholangitis responsive to steroid therapy. Lancet 354:43–44
27. Chutaputti A, Burrell MI, Boyer JL (1995) Pseudotumor of the pancreas associated with retroperitoneal fibrosis: a dramatic response to corticosteroid therapy. Am J Gastroenterol 90:1155–1158
28. Petter LM, Martin JK Jr, Menke DM (1998) Localized lymphoplasmacellular pancreatitis forming a pancreatic inflammatory pseudotumor. Mayo Clin Proc 73:447–450
29. Uchida K, Satoi S, Miyoshi H et al (2007) Inflammatory pseudotumors of the pancreas and liver with infiltration of IgG4-positive plasma cells. Intern Med 46:1409–12
30. Kamisawa T, Funata N, Hayashi Y et al (2003) A new clinicopathological entity of IgG4-related autoimmune disease. J Gastroenterol 38:982–984
31. Fukui T, Okazaki K, Yoshizawa H et al (2005) A case of autoimmune pancreatitis associated with sclerosing cholangitis, retroperitoneal fibrosis and Sjogren's syndrome. Pancreatology 5:86–91
32. Kamisawa T, Funata N, Hayashi Y, Eishi Y, Koike M, Tsuruta K et al (2003) A new clinicopathological entity of IgG4-related autoimmune disease. J Gastroenterol 38:982–4
33. Yamamoto M, Takahashi H, Ohara M, Suzuki C, Naishiro Y, Yamamoto H, Shinomura Y, Imai K (2006) A new conceptualization for Mikulicz's disease as an IgG4-related plasmacytic disease. Mod Rheumatol 16:335–340
34. Masaki Y, Dong L, Kurose N, Kitagawa K, Morikawa Y, Yamamoto M, Takahashi H, Shinomura Y, Imai K, Saeki T, Azumi A, Nakada S, Sugiyama E, Matsui S, Origuchi T, Nishiyama S, Nishimori I, Nojima T, Yamada K, Kawano M,

Zen Y, Kaneko M, Miyazaki K, Tsubota K, Eguchi K, Tomoda K, Sawaki T, Kawanami T, Tanaka M, Fukushima T, Sugai S, Umehara H (2009) Proposal for a new clinical entity, IgG4-positive multiorgan lymphoproliferative syndrome: analysis of 64 cases of IgG4-related disorders. Ann Rheum Dis 68:1310–1315
35. Notohara K, Burgart LJ, Yadav D, Chari S, Smyrk TC (2003) Idiopathic chronic pancreatitis with periductal lymphoplasmacytic infiltration: clinicopathologic features of 35 cases. Am J Surg Pathol 27:1119–27
36. Zamboni G, Luttges J, Capelli P, Frulloni L, Cavallini G, Pederzoli P et al (2004) Histopathological features of diagnostic and clinical relevance in autoimmune pancreatitis: a study on 53 resection specimens and 9 biopsy specimens. Virchows Arch 445:552–63
37. Okazaki K, Kawa S, Kamisawa T, Ito T, Inui K, Irie H et al (2009) Japanese clinical guidelines for autoimmune pancreatitis. Pancreas 38:849–66
38. Inoue H, Miyatani H, Sawada Y et al (2006) A case of pancreas cancer with autoimmune pancreatitis. Pancreas 33:208–9
39. Fukui T, Mitsuyama T, Takaoka M et al (2008) Pancreatic cancer associated with autoimmune pancreatitis in remission. Intern Med 47:151–5
40. Kochi Y, Yamada R, Suzuki A et al (2005) A functional variant in FCRL3, encoding Fc receptor-like 3, is associated with rheumatoid arthritis and several autoimmunities. Nat Genet 37:478–485
41. Umemura T, Ota M, Hamano H et al (2006) Genetic association of Fc receptor-like 3 polymorphisms with autoimmune pancreatitis in Japanese patients. Gut 55:1367–1368
42. Umemura T, Katsuyama Y, Hamano H et al (2009) Association analysis of toll-like receptor 4 polymorphisms with autoimmune pancreatitis. Hum Immunol 7:742–6
43. Chang MC, Chang YT, Tien YW, Liang PC, Jan IS, Wei SC et al (2007) T-cell regulatory gene CTLA-4 polymorphism/haplotype association with autoimmune pancreatitis. Clin Chem 53:1700–5
44. Umemura T, Ota M, Hamano H, Katsuyama Y, Muraki T, Arakura N, Kawa S, Kiyosawa K (2008) Association of autoimmune pancreatitis with cytotoxic T-lymphocyte antigen 4 gene polymorphisms in Japanese patients. Am J Gastroenterol 103:588–94
45. Ueda H, Howson JM, Esposito L et al (2003) Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 423:506–11
46. Roitt IM (1997) Antibodies. In: Roitt IM (ed) Roitt's essential immunology, 9th edn. Blackwell Science, London, pp 43–62
47. Taguchi M, Kihara Y, Nagashio Y et al (2009) Decreased production of immunoglobulin M and A in autoimmune pancreatitis. J Gastroenterol 44:1133–9
48. Robinson DS, Larché M, Durham SR (2004) Tregs and allergic disease. J Clin Invest 114:1389–97
49. Kolfschoten NM, Schuurman J, Losen M et al (2007) Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. Science 317:1554–7
50. Kawa S, Kitahara K, Hamano H et al (2008) A novel immunoglobulin-immunoglobulin interaction in autoimmunity. PLoS ONE 3:e1637
51. Cornell LD, Chicano SL, Deshpande V et al (2007) Pseudotumors due to IgG4 immune-complex tubulointerstitial nephritis associated with autoimmune pancreatocentric disease. Am J Surg Pathol 31:1586–97
52. Uchiyama-Tanaka Y, Mori Y, Kimura T et al (2004) Acute tubulointerstitial nephritis associated with autoimmune-related pancreatitis. Am J Kidney Dis 43:e18–25
53. Kawa S, Kitahara K, Hamano H, Ozaki Y, Arakura N, Yoshizawa K et al (2008) A novel immunoglobulin-immunoglobulin interaction in autoimmunity. PLoS ONE 3(2):e1637
54. Nishi H, Tojo A, Onozato ML, Jimbo R, Nangaku M, Uozaki H, Hirano K et al (2007) Anti-carbonic anhydrase II antibody in autoimmune pancreatitis and tubulointerstitial nephritis. Nephrol Dial Transplant 22:1273–5
55. Aparisi L, Farre A, Gomez-Cambronero L, Martinez J, De Las Heras G, Corts J et al (2005) Antibodies to carbonic anhydrase and IgG4 levels in idiopathic chronic pancreatitis: relevance for diagnosis of autoimmune pancreatitis. Gut 54:703–9
56. Nishimori I, Miyaji E, Morimoto K, Nagao K, Kamada M, Onishi S (2005) Serum antibodies to carbonic anhydrase IV in patients with autoimmune pancreatitis. Gut 54:274–81
57. Endo T, Takizawa S, Tanaka S, Takahashi M, Fujii H, Kamisawa T, Kobayashi T (2009) Amylase alpha-2A autoantibodies: novel marker of autoimmune pancreatitis and fulminant type 1 diabetes. Diabetes 58:732–7
58. Takizawa S, Endo T, Wanjia X, Tanaka S, Takahashi M, Kobayashi T (2009) HSP 10 is a new autoantigen in both autoimmune pancreatitis and fulminant type 1 diabetes. Biochem Biophys Res Commun 386:192–6
59. Frulloni L, Lunardi C, Simone R et al (2009) Identification of a novel antibody associated with autoimmune pancreatitis. N Engl J Med 361:2135–42
60. Nishimori I, Bratanova T, Toshkov I, Caffrey T, Mogaki M, Shibata Y, Holingworth MA (1995) Induction of experimental autoimmune sialoadenitis by immunization of PL/J mice with carbonic anhydrase II. J Immunol 154:4865–73
61. Ueno Y, Ishi M, Takahashi S, Igarashi T, Toyoda T, LaRusso FN (1998) Different susceptibility of mice to immune-mediated cholangitis induced by immunization with carbonic anhydrase II. Lab Invest 78:629–37
62. Kountouras J, Zavos C, Gavalas E et al (2007) Challenge in the pathogenesis of autoimmune pancreatitis: potential role of *Helicobacter pylori* infection via molecular mimicry. Gastroenterology 133:368–369
63. Kountouras J, Zavos C, Chatzopoulos D (2005) A concept on the role of *Helicobacter pylori* infection in autoimmune pancreatitis. J Cell Mol Med 9:196–207
64. Guarneri F, Guarneri C, Benvenga S (3) Helicobacter pylori and autoimmune pancreatitis: role of carbonic anhydrase via molecular mimicry? J Cell Mol Med 9(3):741–4
65. Okazaki K (2005) Autoimmune pancreatitis: etiology, pathogenesis, clinical findings and treatment. The Japanese experience. JOP 6(1 Suppl):89–96
66. McGeachy MJ, Cua DJ (2007) The link between IL-23 and Th17 cell-mediated immune pathologies. Semin Immunol 19:372–6
67. Oukka M (2007) Interplay between pathogenic Th17 and regulatory T cells. Ann Rheum Dis 66(Suppl 3):iii87–90
68. Yamamoto M, Harada S, Ohara M et al (2005) Clinical and pathological differences between Mikulicz's disease and Sjogren's syndrome. Rheumatology 44:227–34
69. Zen Y, Fujii T, Harada K et al (2007) Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. Hepatology 45:1538–46
70. Uchida K, Okazaki K, Nishi T et al (2002) Experimental immune-mediated pancreatitis in neonatally thymectomized mice immunized with carbonic anhydrase II and lactoferrin. Lab Invest 82:411–24
71. Ajjan RA, McIntosh RS, Waterman EA et al (1998) Analysis of the T-cell receptor Valpha repertoire and cytokine gene expression in Sjogren's syndrome. Br J Rheumatol 37:179–185
72. Dienes HP, Lohse AW, Gerken G et al (1997) Bile duct epithelia as target cells in primary biliary cirrhosis and primary sclerosing cholangitis. Virchows Arch 431:119–124
73. Valencia X, Lipsky PE (2007) $CD4^+$ $CD25^+$ $FoxP3^+$ regulatory T cells in autoimmune diseases. Nat Clin Pract Rheumatol 3:619–26
74. Gottenberg JE, Lavie F, Abbed K et al (2005) CD4 CD25high regulatory T cells are not impaired in patients with primary Sjogren's syndrome. J Autoimmun 24:235–42

75. Miyoshi H, Uchida K, Taniguchi T et al (2008) Circulating Naive and $CD4^+$ CD25 high regulatory T cells in patients with autoimmune pancreatitis. Pancreas 36:133–140
76. Stanley JR, Amagai M (2006) Pemphigus, bullous impetigo, and the staphylococcal scalded-skin syndrome. N Engl J Med 355:1800–10
77. Fujii H, Nakatani K, Arita N et al (2003) Internalization of antibodies by endothelial cells via fibronectin implicating a novel mechanism in lupus nephritis. Kidney Int 64:1662–70
78. Aalberse RC, Stapel SO, Schuurman J, Rispens T (2009) Immunoglobulin G4: an odd antibody. Clin Exp Allergy 39:469–77
79. Ruiter B, Knol EF, Van Neerven RJ et al (2007) Maintenance of tolerance to cow's milk in atopic individuals is characterized by high levels of specific immunoglobulin G4. Clin Exp Allergy 37:1103–10
80. Hussain R, Poindexter RW, Ottesen EA (1992) Control of allergic reactivity in human filariasis: predominant localization of blocking antibody to the IgG4 subclass. J Immunol 148:2731–7
81. Yazdanbakhsh M, van den Biggelaar A, Maizels RM (2001) Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. Trends Immunol 22:372–7
82. Sakaguchi S, Fukuma K, Kuribayashi K, Masuda T (1985) Organ-specific autoimmune diseases in mice by elimination of T cell subset. 1. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease. J Exp Med 161:72–87
83. Demols A, Moine OL, Desalle F, Quertinmont E, Laethem JLV, Deviere J (2000) $CD4^+$ T cells play an important role in acute experimental pancreatitis in mice. Gastroenterology 118:582–590
84. Vallance BA, Hewlett BR, Snider DP, Collins SM (1998) T cell-mediated exocrine pancreatic damage in major histocompatibility complex class II-deficient mice. Gastroenterology 115:978–987
85. Sakaguchi Y, Inaba M, Tsuda M, Quan GK, Omae M, Ando Y et al (2008) The Wistar Bonn Kobori rat, a unique animal model for autoimmune pancreatitis with extrapancreatic exocrinopathy. Clin Exp Immunol 152:1–12
86. Marth T, Strober W, Kelsall BL (1996) High dose oral tolerance in ovalbumin TCR-transgenic mice: systemic neutralization of IL-12 augments TGF-beta secretion and T cell apoptosis. J Immunol 157:2348–57
87. Hahm KB, Im YH, Lee C, Park WT, Bang YJ, Grenn JE, Kim SJ (2000) Loss of TGF-β signaling contributes to autoimmune pancreatitis. J Clin Invest 105:1057–1065