Table 2. The Japanese clinical diagnostic criteria 2012 for IgG4-related sclerosing cholangitis.

Diagnostic items
1. Biliary tract imaging reveals diffuse or segmental narrowing of the intrahepatic and/or extrahepatic bile duct associated with the tickening of the bile duct wall
2. Hematological examination shows elevated serum IgG4 concentrations (≥135 mg/dl)
3. Coexistence of autoimmune pancreatiti, IgG4-related dacryoadenitis/sialadenitis, or IgG4-related retroperitoneal fibrosis
4. Histopathological examination shows:
a. Marked lymphocytic and plasmacyte infiltration and fibrosis
b. Infiltration of IgG4-positive plasma cells: >10 IgG4-positive plasma cells/HPF
c. Storiform fibrosis
d. Obliterative phlebitis
Option: effectiveness of steroid therapy
A specialized facility, in which detailed examinations such as endoscopic biliary biopsy and endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) can be administered, may include in its diagnosis the effectiveness of steroid therapy, once pancreatic or biliary cancers have been ruled out
Diagnosis
Definite diagnosis
1. + 3.
1. + 2. + 4.a., b.
4.a., b., c.
4.a., b., d.
Probable diagnosis
1. + 2. + option
Possible diagnosis
1. + 2.
It is necessary to exclude PSC, malignant diseases such as pancreatic or biliary cancers, and secondary sclerosing cholangitis caused by the diseases with obvious pathogenesis. When it is difficult to differentiate from malignant conditions, a patient must not be treated with facile steroid therapy but should be referred to a specialized medical facility.

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previously reported [18], lactoferrin (LF), carbonic anhydrase (CA)-II, CA-IV, and pancreatic secretory trypsin inhibitor (PSTI) are distributed in the pancreas, salivary glands, biliary duct, lungs, and renal tubules. 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-RD [18].

Role of B cells

In addition to steroid and immune-modulators, B cell depletion by rituximab is a useful therapeutic strategy in IgG4-RD. Interestingly, rituximab reduces only the IgG4 subclass but no other subclasses of IgG1, IgG2, or IgG3 [19]. A recent study showed expansion of $IgG4^+$ B cell receptor (BCR) clones in blood and tissue of patients with active IgG4-cholangiopathy, and disappearance by corticosteroid treatment. These findings suggest that specific B cell responses may have a pivotal role in the pathogenesis of IgG4-SC [20].

Th1 and Th2 immune balance

The effector cells in IgG4-related diseases have been poorly understood. The $CD4^+$ T cells differentiate from naïve T cells (Th0) to Th1, Th2, Th17, and regulatory T (Treg) cells [3]. In the livers of IgG4-SC patients, a Th2 type immune reaction [16] is induced in addition to the Th1 responses [18]. Th2 cytokines may be involved in the progression of the disease process, especially the maturation and proliferation of local B cells and plasmacytes.

Regulatory T cells

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 $CD4^+CD25^+$ regulatory T cells (Tregs) classified as naturally occurring $CD4^+CD25^+$ Tregs (nTregs) originating in the thymus and adaptive Tregs (aTregs) induced in the periphery by different antigens [15]. In IgG4-related diseases, circulatory naïve ($CD45RA^+$) Tregs are significantly decreased in the peripheral blood, whereas memory ($CD45RA^-$) Tregs are significantly increased [15]. In addition, prominent infiltration of Tregs with upregulation of IL-10 is observed in the liver of IgG4-SC patients [21]. These findings suggest that increased memory-Tregs in the periphery and local tissues may be an inhibitory immune response against inflammation, although decreased naïve Tregs may be pathogenic. The neonatally thymectomized (nTx)-BALB/c mice with CA-II or LF immunization and WBN/Kob rat models showed depletion of naïve Tregs and multi-organ inflammation similar to human IgG4-RD [5]. These animal models suggested that, in addition to depletion of naïve Tregs, macrophage activation and Th1 immune responses by $CD4^+/CD8^+$ T cells play major roles in the initial development of organ involvement.

Our hypothesis for the pathogenesis of IgG4-SC

Based on the above findings, we propose the pathogenic mechanisms in IgG4-SC/AIP outlined in Fig. 2. The basic concept is the biphasic mechanism of "induction" and "progression." Initially,

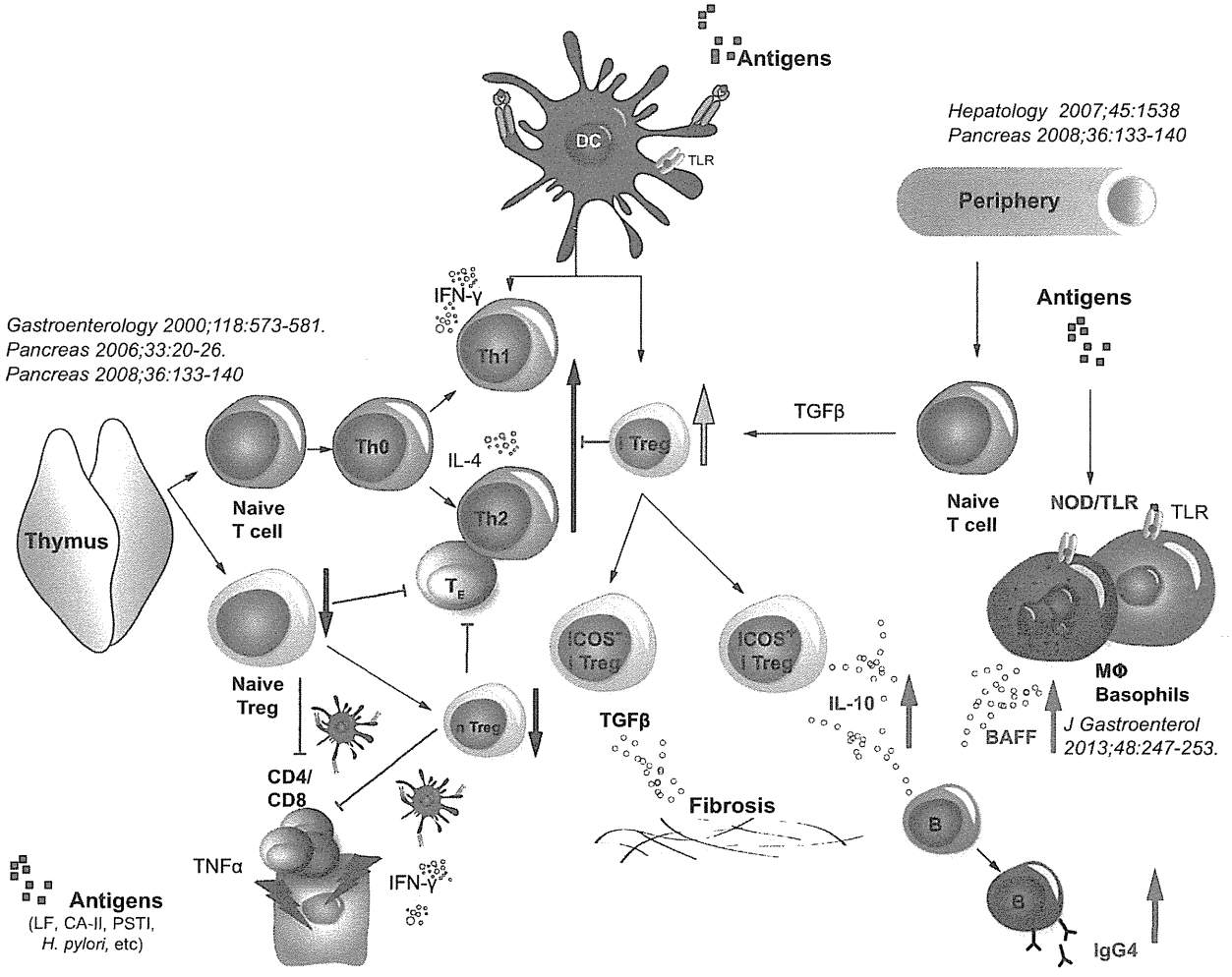

Fig. 2. Hypothesis for the pathogenesis of AIP and IgG4-related disease. In the central tolerance, naïve and natural regulatory T cells (Tregs) derived from the thymus suppress autoreactive CD4 or CD8 cells in the normal state. In the IgG4-related disease, the basic concept is the biphasic mechanism of "induction" and "progression". Initial response to 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-β secreted from inducible memory-Tregs, respectively. On the other hand, activation of NOD receptor or TLRs on monocytes or basophils increases IgG4 via upregulation BAFF and IL-13. iTreg, inducible Treg; TE, effector T cell; nTreg, natural Treg; BAFF, B cell activating factor. Modified from J Gastroenterol. 2011;46:277–288 [5], Copyright © 2012, with permission.

decreased naïve-Tregs may induce a Th1 immune response with the release of pro-inflammatory cytokines (IFN-γ, IL-1beta, IL-2, and TNF-α) to unknown antigens such as self-antigens (LF, CA-II, CA-IV, PSTI, and alpha-amylase) or microorganisms (*Helicobacter pylori*, commensal bacteria, and viruses). Subsequently, Th2 type immune responses may be involved in the disease progression. Production of IgG4 may be upregulated by BAFF from monocytes and basophils, and by IL-10 from inducible memory-Tregs. Tumor growth factor (TGF)-beta secreted from inducible memory-Tregs infiltrating into the involved organ may induce fibrosis.

Conclusion

In conclusion, recent advances support the concept of IgG4-SC, a unique clinical entity as a biliary manifestation of IgG4-RD. Although the pathogenic mechanism remains unclear, we proposed a hypothesis of the pathogenic mechanism of IgG4-SC. Further studies are necessary to clarify the pathogenesis including genetic backgrounds, disease specific antigens, and the role of IgG4.

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Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References

[1] 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;344:732–738.

[2] Ohara H, Okazaki K, Tsubouchi H, Inui K, Kawa S, Kamisawa T, et al. Clinical diagnostic criteria of IgG4-related sclerosing cholangitis 2012. J Hepatobiliary Pancreat Sci 2012;19:536–542.

[3] Okazaki K, Uchida K, Miyoshi H, Ikeura T, Takaoka M, Nishio A. Recent concepts of autoimmune pancreatitis and IgG4-related disease. Clin Rev Allergy Immunol 2011;41:126–138.

[4] Yoshida K, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N, et al. Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. Dig Dis Sci 1995;40:1561–1568.

[5] Okazaki K, Uchida K, Koyabu M, Miyoshi H, Takaoka M. Recent advances in the concept and diagnosis of autoimmune pancreatitis and IgG4-related disease. J Gastroenterol 2011;46:277–288.

[6] Stone JH, Khosroshahi A, Deshpande V, Chan JK, Heathcote JG, Aalberse R, et al. Recommendations for the nomenclature of IgG4-related disease and its individual organ system manifestations. Arthritis Rheum 2012;64: 3061–3067.

[7] Tanaka A, Tazuma S, Okazaki K, Tsubouchi H, Inui K, Takikawa H. A nationwide survey for primary sclerosing cholangitis and IgG4-related sclerosing cholangitis in Japan. J Hepatobiliary Pancreat Sci 2014;21:43–50.

[8] Nakanuma Y, Zen Y. Pathology and immunopathology of immunoglobulin G4-related sclerosing cholangitis: the latest addition to the sclerosing cholangitis family. Hepatol Res 2007;37:S478–S486.

[9] Kamisawa T, Okazaki K, Kawa S, Shimosegawa T, Tanaka M. Research committee for intractable pancreatic disease and Japan pancreas society. Japanese consensus guidelines for management of autoimmune pancreatitis III. Treatment and prognosis of AIP. J Gastroenterol 2010;45:471–477.

[10] Tomiyama T, Uchida K, Matsushita M, Ikeura T, Fukui T, Takaoka M, et al. Comparison of steroid pulse therapy and conventional oral steroid therapy as initial treatment for autoimmune pancreatitis. J Gastroenterol 2011;46:696–704.

[11] 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;134:706–715.

[12] Topazian M, Witzig TE, Smyrk TC, Pulido JS, Levy MJ, Kamath PS, et al. Rituximab therapy for refractory biliary strictures in immunoglobulin G4-associated cholangitis. Clin Gastroenterol Hepatol 2008;6:364–366.

[13] Watanabe T, Yamashita K, Sakurai T, Kudo M, Shiokawa M, Uza N, et al. Toll-like receptor activation in basophils contributes to the development of IgG4 related disease. J Gastroenterol 2013;48:247–253.

[14] Yamashina M, Nishio A, Nakayama S, Okazaki T, Uchida K, Fukui T, et al. Comparative study on experimental autoimmune pancreatitis and its extrapancreatic involvement in mice. Pancreas 2012;41:1255–1262.

[15] 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 2008;36:133–140.

[16] 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;45:1538–1546.

[17] Muraki T, Hamano H, Ochi Y, Komatsu K, Komiyama Y, Arakura N, et al. Autoimmune pancreatitis and complement activation system. Pancreas 2006;32:16–21.

[18] Okazaki K, Uchida K, Ohana M, Nakase H, Uose S, Inai M, et al. Autoimmune-related pancreatitis is associated with autoantibodies and a Th1/Th2-type cellular immune response. Gastroenterology 2000;118:573–581.

[19] Khosroshahi A, Carruthers MN, Deshpande V, Unizony S, Bloch DB, Stone JH. Rituximab for the treatment of IgG4-related disease: lessons from 10 consecutive patients. Medicine (Baltimore) 2012;91:57–66.

[20] Maillette de Buy Wenniger LJ, Doorenspleet ME, Klarenbeek PL, Verheij J, Baas F, Elferink RP, et al. Immunoglobulin G4+ clones identified by next-generation sequencing dominate the B cell receptor repertoire in immunoglobulin G4 associated cholangitis. Hepatology 2013;57:2390–2398.

[21] Koyabu M, Uchida K, Miyoshi H, Sakaguchi Y, Fukui T, Ikeda H, et al. Analysis of regulatory T cells and IgG4-positive plasma cells among patients of IgG4-related sclerosing cholangitis and autoimmune liver diseases. J Gastroenterol 2010;45:732–741.

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Possible involvement of Toll-like receptor 7 in the development of type 1 autoimmune pancreatitis

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Abstract
Background High serum immunoglobulin G4 (IgG4) levels and IgG4-positive plasma cell infiltration are characteristic of type 1 autoimmune pancreatitis (AIP). It is unclear whether innate immunity is a cause of type 1 AIP; the possible involvement of microbial infection has been suggested in its pathogenesis. To clarify the pathogenesis of type 1 AIP, we investigated Toll-like receptors (TLRs) in type 1 AIP patients.
Methods We studied nine cases of type 1 AIP with ten cases of alcoholic chronic pancreatitis (ACP) and three of the samples from non-tumorous lesion of neuroendocrine tumor (NET) as control subjects. We counted the number of TLR1-11-positive cells immunohistochemically stained with anti-TLR1-11 antibodies. To identify TLR-positive cells in pancreata from type 1 AIP patients, we used a double-immunofluorescence method and counted the numbers of identifiable CD68-, CD163-, CD123-, and CD20-positive cells.
Results In type 1 AIP, TLR7 (8.815 ± 1.755), TLR8 (3.852 ± 1.489), and TLR10 (3.852 ± 0.921) were highly expressed. Only the ratio of TLR7 per monocyte was significantly higher in type 1 AIP (0.053 ± 0.012) than in ACP (0.007 ± 0.004; $p < 0.01$) and non-tumorous lesion of NET (0.000 ± 0.000; $p < 0.01$). In type 1 AIP, the CD163 to TLR7 ratio (0.789 ± 0.031) was significantly higher both than that of CD123 to TLR7 ratio (0.034 ± 0.006; $p < 0.001$) and CD20 to TLR7 ratio (0.029 ± 0.010; $p < 0.001$).
Conclusions TLR7 might be key pattern-recognition receptors involved in the development of type 1 AIP.

Keywords type 1 autoimmune pancreatitis · Toll-like receptor 7 · M2 macrophage · IgG4-related disease

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Introduction

In 1961, Sarles et al. [1] first observed a case of idiopathic chronic pancreatitis with hypergammaglobulinemia in which an autoimmune mechanism was supposedly involved. In 1991, Kawaguchi et al. [2] defined the pathologic feature as lymphoplasmacytic sclerosing pancreatitis (LPSP). In 1995, Yoshida et al. [3] proposed the concept of "autoimmune pancreatitis (AIP)". In 2001, Hamano et al. [4] reported that elevated serum immunoglobulin G4 (IgG4) levels were highly specific and sensitive for the diagnosis of AIP. In 2003, Kamisawa et al. [5] suggested that AIP is a systemic disease, based on the findings that the pancreas and other involved organs had abundant infiltration of IgG4-positive plasma cells. Thereafter, many investigators including Japanese investigators have reported many cases of AIP and AIP has been accepted as a new clinical entity [6–9].

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Reports from Europe [10] and the USA [11] described unique histological patterns in the resected pancreata of patients with mass-forming chronic non-alcoholic pancreatitis with epithelial destruction by granulocytes. This is called idiopathic duct centric pancreatitis (IDCP), AIP with granulocyte epithelial lesions (AIP with GEL), or type 2 AIP. In 2011, the International Consensus Diagnostic Criteria for Autoimmune Pancreatitis (ICDC) proposed the classification of AIP into type 1 AIP (LPSP) and type 2 AIP (IDCP) [12]. Most of the Japanese AIP cases are type 1 AIP; very few are type 2 AIP [13].

Although pathogenic mechanisms in both types of AIP remain unclear, possible involvement of an autoimmune mechanism has been suggested in type 1 AIP. Based on genetic factors (such as the susceptibility of class II antigen of the major histocompatibility complex (MHC) [14], cytotoxic T-lymphocyte antigen 4 gene [15] and Fc-receptor-like (FCRL) 3 genes expressed on B cells [16], presence of autoantibody [8, 9], predominant Th2 immune status [17], and the infiltration of CD4-positive T cells and regulatory T cells (Treg) [8, 17, 18], in addition to the presence of IgG4-positive plasma cells that suggest abnormally acquired immune status. In addition to these factors, the possible involvement of the innate immune system has been suggested in the pathogenesis of type 1 AIP [19, 20].

Through pattern-recognition receptors (PRRs), innate immunity is considered to act as a sentinel for the immune system. The innate immunity is promptly activated after recognition of a diverse range of microbial pathogens. Innate immune cells express various PRRs, which recognize signature molecules of pathogens. These PRRs recognize various pathogen-associated molecular patterns (PAMPs) in various cell compartments and trigger the release of inflammatory cytokines and type I interferons for host defense [21–24]. Furthermore, the innate immune system response is important not only to eliminate pathogens but also to develop pathogen-specific adaptive immunity, which is mediated by B and T cells [25]. Toll-like receptors (TLRs), which are a type of PRRs, play key roles in innate immunity and initiate intracellular signaling to macrophages and dendritic cells after stimulation with various antigens [26].

To clarify the pathogenesis of type 1 AIP, we investigated TLRs in pancreata obtained from type 1 AIP patients.

Methods

Subjects

We examined nine patients with type 1 AIP (five women and four men; mean age 65 years; range, 56–75 years), ten patients with alcoholic chronic pancreatitis (ACP) (ten men; mean age 54 years; range, 39–75 years), and three samples from the non-tumor lesion of neuroendocrine tumor (NET) (three men; mean age 63 years; range, 48–70 years). All cases were collected between 1992 and 2010 at Kansai Medical University from surgery cases. All type 1 AIP cases were suspected as pancreatic ductal adenocarcinoma before operation. These cases were diagnosed histopathologically as LPSP after operation. This study was approved by Kansai Medical University's ethics committee.

Histopathology and immunohistochemistry

Formalin-fixed and paraffin-embedded specimens were prepared. Sections 4 μm thick were cut for hematoxylin and eosin (H&E), immunohistochemical, and immunofluorescence staining. Formalin-fixed paraffin-embedded pancreatic sections were deparaffinized and rehydrated using xylene and a graded descending series of alcohol. Endogenous peroxidase activity was blocked for immunohistochemical sections using 3 % H_2O_2/methanol for 10 min. After washing in distilled water, the slides were exposed to microwave pretreatment in a target retrieval solution (Dako Japan, Kyoto, Japan) at 100 °C for 20 min to enhance antigenicity. All slides were incubated for 10 min in protein blocking reagent without serum (ProT-aqs, purchased from Biocyc GmbH & Co., Berlin, Germany). The slides were then incubated overnight at 4 °C with primary antibodies (Table 1). Next, the slides were

Table 1 Antibodies used for immunohistochemical and immunofluorescence staining

Antigen	Host species	Source
TLR1	Rabbit	AbFrontier (Seoul, Korea)
TLR2	Mouse	Hycult Biotech (Uden, The Netherlands)
TLR3	Mouse	Dendritics (Lyon, France)
TLR4	Mouse	Imgenex (San Diego, CA, USA)
TLR5	Rabbit	AbFrontier (Seoul, Korea)
TLR6	Rabbit	AbFrontier (Seoul, Korea)
TLR7	Rabbit	AbFrontier (Seoul, Korea)
TLR8	Rabbit	AbFrontier (Seoul, Korea)
TLR9	Mouse	Abnova (Taipei, Taiwan)
TLR10	Rabbit	AbFrontier (Seoul, Korea)
TLR11	Rabbit	GenWay (San Diego, CA, USA)
CD68	Mouse	Santa Cruz Biotechnology (Santa Cruz, CA, USA)
CD163	Mouse	AbD Serotec (Oxford, UK)
CD123	Mouse	Leica Biosystems (Newcastle Upon Tyne, UK)
CD20	Mouse	Dako Japan (Kyoto, Japan)

incubated with secondary antibodies, using a Chem Envision kit/HRP (Dako Japan), following the manufacturer's instructions. Finally, antibody binding was detected using 3,3′-diaminobenzidine (DAB) (Dojindo, Kumamoto, Japan). Sections were counterstained with hematoxylin [27]. Negative controls were evaluated by replacing the primary antibody with similarly diluted non-immunized serum. Images were obtained with a microscope (Olympus, Tokyo, Japan).

In the samples from patients with type 1 AIP, we counted the number of immunohistochemically identifiable TLR1-11-positive cells in three different fields under the high power fields (hpf) (400×). The fields with the highest density of TLR1-11-positive cells were evaluated. The number of cells in three different fields was compared. The ratios of TLR-positive cells to infiltrated mononuclear cells were compared among samples from patients with type 1 AIP, ACP, and samples from non-tumor lesion surrounding NETs. We used a double-immunofluorescence method. The slides were incubated overnight at 4 °C with rabbit anti-TLR7 as primary antibody (Table 1). The slides were then incubated with Alexa Fluor 488 anti-rabbit immunoglobulins as secondary antibodies (Molecular Probes, Carlsbad, CA, USA). Next, the slides were incubated overnight at 4 °C with primary antibody by a mouse anti-CD68, -CD163, -CD123, or -CD20 as primary antibody (Table 1). The slides were then incubated with Alexa Fluor 546 anti-mouse immunoglobulins as secondary antibodies (Molecular Probes). Following the second round of the primary/secondary antibody step, slides were mounted with VECTASHIELD Mounting Medium with DAPI (Vector Laboratories, Burlingame, CA, USA) to counterstain nuclei and preserve fluorescence. Negative controls were evaluated by replacing the primary antibody with similarly diluted non-immunized serum. Images were obtained with a fluorescence microscope (Olympus).

We counted the number of identifiable TLR7-, CD68-, CD163-, CD123-, and CD20-positive cells with three different hpfs (400×). The fields with the highest density of CD68/TLR7, CD163/TLR7, CD123/TLR7, and CD20/TLR7-positive cells were evaluated. The ratio of TLR7-positive cells to CD68-, CD163-, CD123-, and CD20-positive cells was compared among samples from patients with type 1 AIP and ACP.

Statistical analysis

For all studies, data are expressed as the mean ± standard error of the mean (SEM); differences were analyzed using Fisher's protected least significant difference (PLSD), where $p < 0.05$ was considered significant.

Results

Immunohistochemical findings of TLR1-11-positive cells in type 1 AIP

In samples from patients with type 1 AIP, the number of TLR1-positive cells (TLR1/hpf), TLR2-positive cells (TLR2/hpf), TLR3-positive cells (TLR3/hpf), TLR4-positive cells (TLR4/hpf), TLR5-positive cells (TLR5/hpf), TLR6-positive cells (TLR6/hpf), TLR7-positive cells (TLR7/hpf), TLR8-positive cells (TLR8/hpf), TLR9-positive cells (TLR9/hpf), TLR10-positive cells (TLR10/hpf), TLR11-positive cells (TLR11/hpf) were 1.370 ± 0.349, 2.111 ± 0.518, 1.519 ± 0.405, 1.556 ± 0.539, 2.259 ± 0.509, 1.556 ± 0.261, 8.815 ± 1.755, 3.852 ± 1.489, 1.185 ± 0.261, 3.852 ± 0.921, 0.963 ± 0.263, respectively (Figs. 1, 2). TLR7-, TLR8-, and TLR10-positive cells were dominant in type 1 AIP pancreata as compared to other TLR-positive cells.

Immunohistochemical findings of TLR7-, 8-, and 10-positive cells in type 1 AIP, ACP, and the samples from the non-tumor lesions of NET

The ratio of TLR7-positive cells to infiltrated mononuclear cells (TLR7/Mono) was significantly higher in type 1 AIP (0.053 ± 0.012) than in ACP (0.007 ± 0.004; $p < 0.01$) or samples from the non-tumor lesions of NET (0.000 ± 0.000; $p < 0.01$; Fig. 3a). Among type 1 AIP, ACP and samples from the non-tumor lesion of NET, the ratio of TLR8-positive cells to infiltrated mononuclear cells (TLR8/Mono) (0.030 ± 0.012, 0.009 ± 0.004, and 0.024 ± 0.009, respectively) and TLR10-positive cells to infiltrated mononuclear cells (TLR10/Mono) (0.031 ± 0.008, 0.018 ± 0.005, and 0.005 ± 0.003, respectively) were not different (Fig. 3b, c).

Immunohistochemical findings of CD68-, CD163-, CD123-, CD20-positive cells in type 1 AIP and ACP

The number of CD163-positive cells (CD163/hpf) was significantly higher in samples from patients with type 1 AIP (37.9 ± 5.7) than in samples from patents with ACP (16.2 ± 2.7; $p < 0.01$; Fig. 4a). The number of CD68-positive cells (CD68/hpf) was significantly higher in type 1 AIP (35.2 ± 4.0) than in ACP (18.9 ± 3.1; $p < 0.01$; Fig. 4a). Between type 1 AIP and ACP, the numbers of CD123-positive cells (CD123/hpf) (2.3 ± 0.4 and 2.1 ± 0.5, respectively) and CD20-positive cells (CD20/hpf) (117.9 ± 15.7 and 87.6 ± 19.4, respectively) were not different (Fig. 4a).

In type 1 AIP and ACP, the ratio of CD68-positive cells to infiltrated mononuclear cells (CD68/Mono) (0.19 ± 0.02

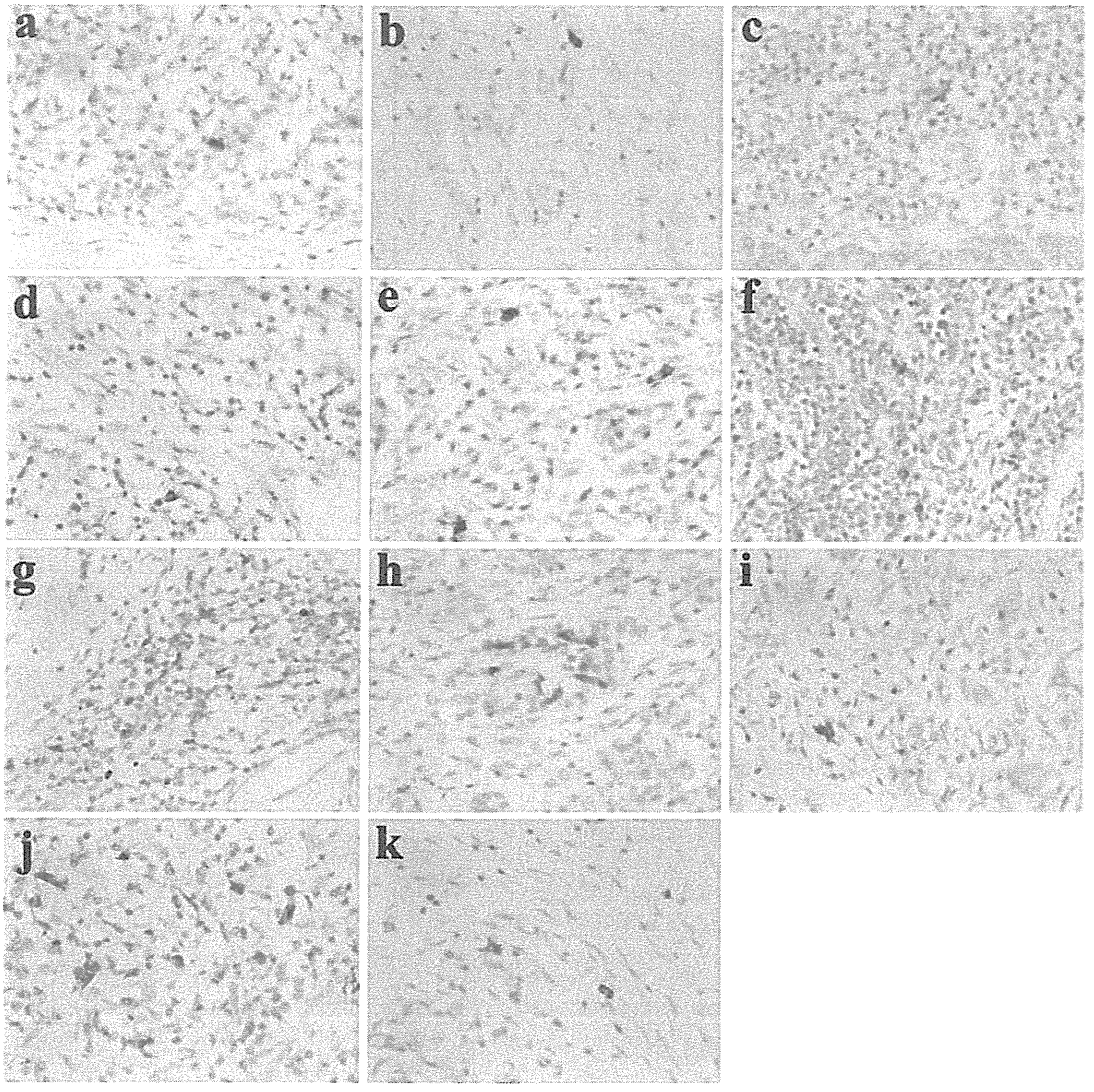

Fig. 1 Immunohistochemical findings in type 1 AIP. TLR1-positive cells [original magnification ×400 (**a**)], TLR2-positive cells (**b**), TLR3-positive cells (**c**), TLR4-positive cells (**d**), TLR5-positive cells (**e**), TLR6-positive cells (**f**), TLR7-positive cells (**g**), TLR8-positive cells (**h**), TLR9-positive cells (**i**), TLR10-positive cells (**j**), and TLR11-positive cells (**k**). TLR7-, TLR8-, and TLR10-positive cells were abundant

and 0.17 ± 0.02, respectively), CD163-positive cells to infiltrated mononuclear cells (CD163/Mono) (0.19 ± 0.02 and 0.15 ± 0.02, respectively), CD123-positive cells to infiltrated mononuclear cells (CD123/Mono) (0.01 ± 0.002 and 0.01 ± 0.003, respectively) and CD20-positive cells to infiltrated mononuclear cells (CD20/Mono) (0.33 ± 0.03 and 0.29 ± 0.04, respectively) were not different (Fig. 4b).

Identification of TLR7-positive cells in type 1 AIP

In type 1 AIP, TLR7-positive cells (green; Fig. 5b) were detected among CD68-positive cells (red; Fig. 5d) as indicated in the merged panel (Fig. 5h), in which DAPI nuclear staining was added (blue; Fig. 5f). TLR7-positive cells (green; Fig. 5a) were detected among CD163-positive cells (red; Fig. 5c) as indicated in the merged panel (Fig. 5g), in which DAPI nuclear staining was added (blue; Fig. 5e).

The ratio of CD68-positive cells to TLR7-positive cells (CD68/TLR7) (0.734 ± 0.021) was significantly higher than both the ratio of CD123-positive cells to TLR7-positive cells (CD123/TLR7) (0.034 ± 0.006; $p < 0.001$) and the ratio of CD20-positive cells to TLR7-positive cells (CD20/TLR7) (0.029 ± 0.010; $p < 0.001$). The ratio of CD163-positive cells to TLR7-positive cells (CD163/TLR7) (0.789 ± 0.031) was significantly higher than both

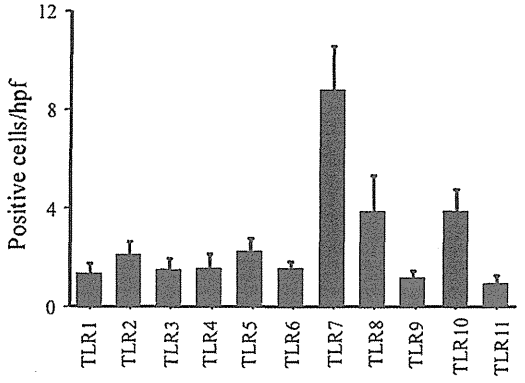

Fig. 2 The number of infiltrated TLR1–11 positive cells in type 1 AIP. TLR7-, TLR8-, and TLR10-positive cells were abundant compared with other TLR-positive cells

the ratio of CD123-positive cells to TLR7-positive cells (CD123/TLR7) ($p < 0.001$) and the ratio of CD20-positive cells to TLR7-positive cells (CD20/TLR7) ($p < 0.001$; Fig. 6).

Discussion

To clarify the involvement of the innate immunity in type 1 AIP, our study focused on the number of TLR1-11-positive cells. In type 1 AIP, TLR7, TLR8, and TLR10 were highly expressed. Only the ratio of TLR7/Mono was significantly higher in typel AIP than in ACP and the samples from non-tumor lesion of NET. The main ligands recognized by different TLRs have been clarified [28]. TLR7 recognizes several synthetic compounds and single-stranded RNA (ssRNA) of viruses. The various ssRNA viruses cause the complication pancreatitis [29–35]. TLR7 recognizes not only exogenous RNA but also endogenous RNA from damaged tissues or apoptotic cells, and can contribute to the pathology of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and psoriasis [36, 37]. In type 1 AIP, TLR7 might recognize self-RNA as one of the candidates of target.

Umemura et al. [38] reported that TLR4 gene polymorphisms were not significantly associated with susceptibility to type 1 AIP in Japanese patients. In this study, TLR4-positive cells were not highly expressed. Watanabe et al. [20] reported that peripheral blood mononuclear cells from IgG4-related diseases produced a large amount of IgG4 upon stimulation with nucleotide-binding oligomerization domain (NOD)-like receptors and TLR ligands. In our study, there was no correlation between TLR7/Mono and IgG4/Mono in type 1 AIP (data not shown). Under normal conditions, viruses are not pathogenic. However, additional factors such as genetic factors including the haplotype of the class II antigen of the major

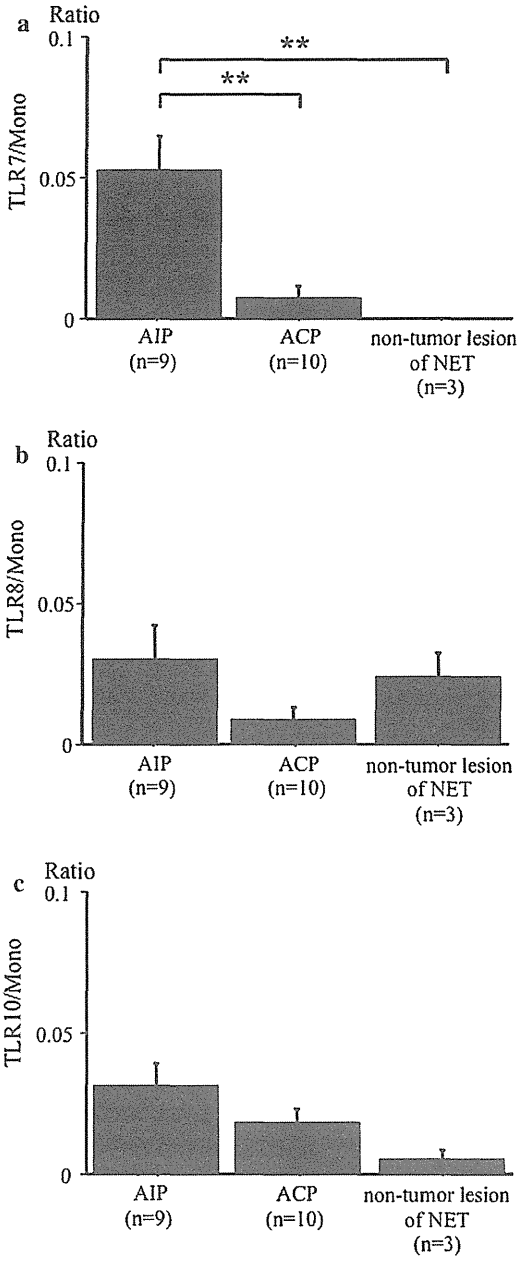

Fig. 3 The ratios of infiltrated TLR7-, TLR8-, and TLR10-positive cells. **a** The ratio of TLR7-positive cells to infiltrated mononuclear cells (TLR7/Mono) in type 1 AIP patients was significantly higher than ACP and the non-tumor lesion of NET. **b** The ratio of TLR8-positive cells to infiltrated mononuclear cells (TLR8/Mono) among type 1 AIP, ACP, and the non-tumor lesion of NET, were not different. **c** The ratio of TLR10-positive cells to infiltrated mononuclear cells (TLR10/Mono) among type 1 AIP, ACP, and the non-tumor lesion of NET, were not different. Data are expressed as the mean ± standard error of the mean (SEM). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

histocompatibility complex (MHC) can lead to a breakdown in immunological tolerance and the development of self-antigen-specific T cell and antibody responses and

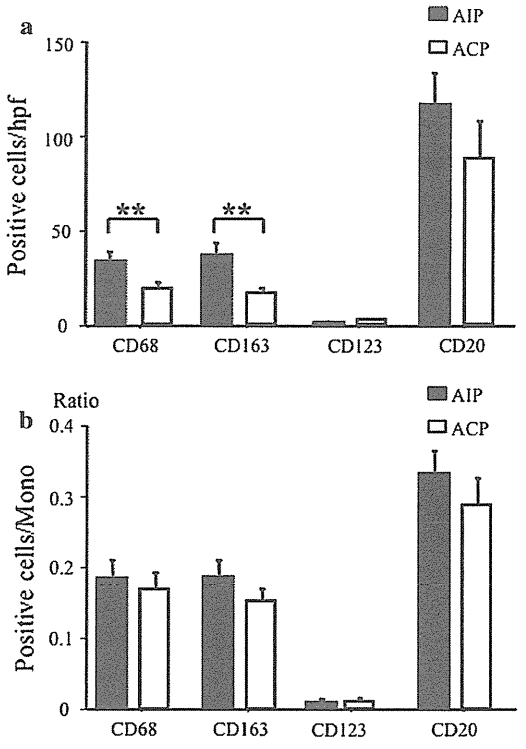

Fig. 4 Infiltrated CD68-, CD163-, CD123-, and CD20-positive cells in type 1 AIP and ACP. **a** The number of infiltrated CD68-, CD163-, CD123-, and CD20-positive cells in type 1 AIP and ACP. In type 1 AIP, the number of infiltrated CD163- and CD68-positive cells were significantly higher than ACP. **b** The ratios of CD68-positive cells to infiltrated mononuclear cells (CD68/Mono), CD163-positive cells to infiltrated mononuclear cells (CD163/Mono), CD123-positive cells to infiltrated mononuclear cells (CD123/Mono) and CD20-positive cells to infiltrated mononuclear cells (CD20/Mono) in type 1 AIP and ACP. In type 1 AIP and ACP, the ratios of CD68, CD163, CD123 and CD20-positive cells to infiltrated mononuclear cells were not different. Data are expressed as the mean ± standard error of the mean (SEM). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

eventually establish the autoimmune disease. Throughout these processes, TLR7 might participate as a key member of the PRRs involved in driving autoimmune inflammation.

Several animal models have been used to avoid the difficulties inherent in the study of the autoimmune mechanism of AIP in human patients [39–44]. As microbe-induced AIP models, C57BL/6 mice infected with the murine leukemia retrovirus LP-BM5, developed histological findings similar to human AIP [45, 46]. The development of the disease is accelerated by administration of polyinosinic polycytidylic acid (poly I:C), a synthetic double-stranded RNA and TLR3 ligand [47–51]. We previously reported that sensitization occurs not only with viral components, such as double-stranded RNA poly I:C, but also with bacterial lipopolysaccharide in interleukin (IL)-10-deficient mice [49]. Haruta et al. [52] reported that when C57BL/6 mice were inoculated intraperitoneally with heat-killed *E. coli*, marked cellular infiltration with fibrosis was observed in the exocrine pancreas. In rat models, pancreatic stellate cells expressed mRNAs for TLR2, TLR3, TLR4, and TLR5 [53]. In murine models of acute pancreatitis, genetic deletion of TLR4 reduces the severity of pancreatic, lung, and acinar cell injury in edematous and necrotizing experimental acute pancreatitis [54, 55]. Pharmacologic antagonism of both TLR7 and TLR9 decrease acinar cell necrosis and lung injury in an experimental model of severe acute pancreatitis [56]. Taken together, animal and human studies, innate immune responses include TLRs might participate in causing pancreatic inflammation.

In the present study, we also examined which cells expressed TLR7. Human TLR7 is expressed predominantly in plasmacytoid dendritic cells (pDC), B cells [57, 58], and macrophages [59]. To identify the TLR7-positive cells in type 1 AIP, we investigated the number of pDCs (CD123), B cells (CD20), and macrophages (CD68, CD163). The ratio of CD68-positive cells to TLR7-positive cells (CD68/TLR7) and the ratio of CD163/TLR7 was significantly higher than both the ratio of CD123/TLR7 and the ratio of CD20/TLR7. From these results, TLR7 was expressed mainly on macrophages in pancreata with type 1 AIP. Several reports have published the importance of TLR7-positive macrophage in immune-related disease [60–63]. From these points of view, TLR7-positive macrophage may play an important role in type 1 AIP.

Macrophages are a heterogeneous cell population that adapt and respond to a large variety of microenvironmental signals. While macrophage activation by T helper 1 (Th1) cytokines, such as interferon-γ, interleukin (IL)-1β, and lipopolysaccharide was named "classical" activation, by Th2 cytokines, such as IL-4 and IL-13 was named "alternative" activation. Mirroring the Th1 and Th2 nomenclature, many researchers refer to polarized macrophages as M1 and M2 cells [64, 65]. These subpopulations of macrophage have different types of receptor expression and cytokine and chemokine production. M1-polarized macrophages have the IL-12^{high}, IL-23^{high}, IL-10^{low} phenotype and produce tumor necrosis factor (TNF)-α and nitric oxide (NO): M1-polarized macrophages are potent effector cells that kill microorganisms and tumor cells. In contrast, M2-polarized macrophages have the IL-12^{low}, IL-23^{low}, IL-10^{high} phenotype and have high expression of receptors, such as mannose receptor (MR, CD163). M2-polarized macrophages scavenge debris and promote angiogenesis, tissue remodeling, and repair [66].

Detlefsen et al. [67] reported that in AIP numerous macrophages were found not only in the periductal and interlobular areas but also between the acinar cells and in areas with focal storiform fibrosis replacing acinar tissue. Notohara et al. [68] reported that CD163-positive spindle-

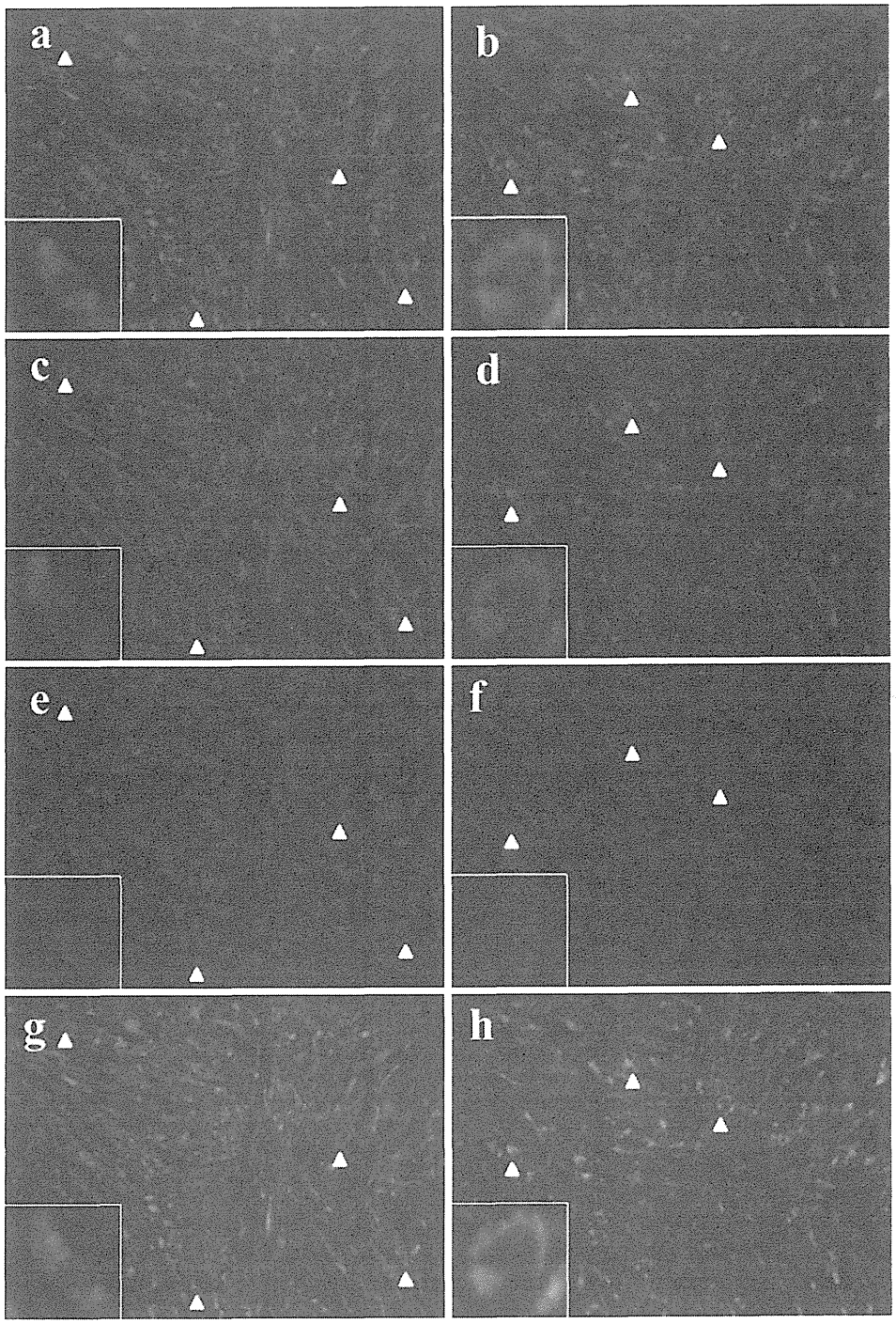

Fig. 5 Double-immunofluorescence staining in type 1 AIP. In type 1 AIP, TLR7-positive cells (*green*; **b**) were detected among CD68-positive cells (*red*; **d**) as indicated in the merged panel (**h**), in which there is added nuclear staining with DAPI (*blue*; **f**). TLR7-positive cells (*green*; **a**) were detected among CD163-positive cells (*red*; **c**) as indicated in merged panel (**g**), in which there is added nuclear staining with DAPI (*blue*; **e**) (original magnification ×400)

shaped macrophages are one of the major inflammatory components of LPSP, and contribute to forming the unique histology of IgG4-related disease, such as storiform fibrosis. Even in this study, a large number of TLR7- and CD163-positive spindle-shaped macrophages infiltrated in pancreata with type 1 AIP. Our results showed a higher

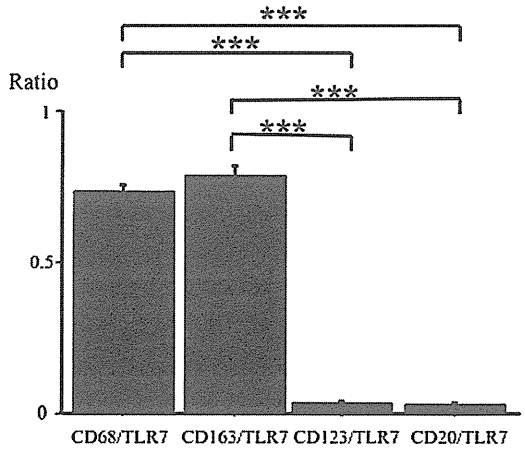

Fig. 6 The ratios of infiltrated CD68/TLR7, CD163/TLR7, CD123/TLR7, and CD20/TLR7. The ratio of CD68-positive cells to infiltrated TLR7-positive cells (CD68/TLR7) in type 1 AIP patients was significantly higher than the ratio of CD123-positive cells to infiltrated TLR7-positive cells (CD123/TLR7) and the ratio of CD20-positive cells to infiltrated TLR7-positive cells (CD20/TLR7). The ratio of CD163-positive cells to infiltrated TLR7-positive cells (CD163/TLR7) in type 1 AIP patients was significantly higher than the ratio of CD123-positive cells to infiltrated TLR7-positive cells (CD123/TLR7) and the ratio of CD20-positive cells to infiltrated TLR7-positive cells (CD20/TLR7). Data are expressed as the mean ± standard error of the mean (SEM). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

number of CD163-positive cells than CD68-positive cells in type 1 AIP. The same phenomenon has been observed in melanoma [69], leiomyosarcoma [70], and Hodgkin's lymphoma [71]. The antibody used against CD163 was able to outline the macrophages more clearly than CD68, thereby allowing for a more precise assessment of the density of macrophages. CD163 is also expressed in some dendritic cells [72]. The difference in staining patterns may be due to the phenomenon of macrophage/dendritic cell heterogeneity [73]. However, the precise pattern of expression of CD163 and CD68 across the different subtypes has not been reported in the literature.

Macrophages are considered to play a pivotal role in the development of renal fibrosis [74, 75]. Th2 cytokines are implicated in fibrosis formation in lung and hepatic models of chronic disease [76–78]. In pigs, analysis of the expression and functionality of TLR7 in peripheral blood leukocyte subpopulations suggests that this receptor is expressed and functional in a CD163-positive monocytic cell subpopulation containing the fibrocyte precursor [79]. Recent reports have shown reduced fibrosis in TLR4-deficient mice in renal [80] and hepatic [81] models of fibrotic disease. In TLR2, TLR4, myeloid differentiation factor 88, and IL-4 KO mice, a decreased number of M2 macrophages presented less fibrosis [82]. Th2 cells and M2 macrophages might participate in fibrosis that is sensed by the innate immunity system. We previously reported the Th1/Th2 balance in type 1 AIP [83, 84]. In the early stage, pro-inflammatory cytokines were released via Th1 immune response. In the chronic stage, Th2 immune responses produced IgG4, IgG, and autoantibodies. Particularly, an increased number of ICOS + Tregs may affect IgG4 production via IL-10 in type 1 AIP [18, 84, 85]. Furthermore, CD163-positive macrophages that express TLR7 may participate in the fibrosis exhibited in type 1 AIP. In conclusion, TLR7 might be a key pattern-recognition receptor involved in the development of type 1 AIP.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Sarles H, Sarles JC, Muratore R, et al. Chronic inflammatory sclerosis of the pancreas—an autonomous pancreatic disease? Am J Dig Dis. 1961;6:688–98.
2. Kawaguchi K, Koike M, Tsuruta K, et al. Lymphoplasmacytic sclerosing pancreatitis with cholangitis: a variant of primary sclerosing cholangitis extensively involving pancreas. Hum Pathol. 1991;22:387–95.
3. Yoshida K, Toki F, Takeuchi T, et al. Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. Dig Dis Sci. 1995;40:1561–8.
4. Hamano H, Kawa S, Horiuchi A, et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med. 2001;344:732–8.
5. Kamisawa T, Funata N, Hayashi Y, et al. A new clinicopathological entity of IgG4-related autoimmune disease. J Gastroenterol. 2003;38:982–4.
6. Ito T, Nakano I, Koyanagi S, et al. Autoimmune pancreatitis as a new clinical entity. Three cases of autoimmune pancreatitis with effective steroid therapy. Dig Dis Sci. 1997;42:1458–68.
7. Horiuchi A, Kawa S, Akamatsu T, et al. Characteristic pancreatic duct appearance in autoimmune chronic pancreatitis: a case report and review of the Japanese literature. Am J Gastroenterol. 1998;93:260–3.
8. Uchida K, Okazaki K, Konishi Y, et al. Clinical analysis of autoimmune-related pancreatitis. Am J Gastroenterol. 2000;95:2788–94.
9. Okazaki K, Uchida K, Chiba T. Recent concept of autoimmune-related pancreatitis. J Gastroenterol. 2001;36:293–302.
10. Zamboni G, Luttges J, Capelli P, et al. Histopathological features of diagnostic and clinical relevance in autoimmune pancreatitis: a study on 53 resection specimens and 9 biopsy specimens. Virchows Arch. 2004;445:552–63.
11. Notohara K, Burgart LJ, Yadav D, et al. Idiopathic chronic pancreatitis with periductal lymphoplasmacytic infiltration:

clinicopathologic features of 35 cases. Am J Surg Pathol. 2003;27:1119–27.
12. Shimosegawa T, Chari ST, Frulloni L, et al. International consensus diagnostic criteria for autoimmune pancreatitis: guidelines of the International Association of Pancreatology. Pancreas. 2011;40:352–8.
13. Okazaki K, Kawa S, Kamisawa T, et al. Japanese consensus guidelines for management of autoimmune pancreatitis: I. Concept and diagnosis of autoimmune pancreatitis. J Gastroenterol. 2010;45:249–65.
14. Ota M, Katsuyama Y, Hamano H, et al. Two critical genes (HLA-DRB1 and ABCF1) in the HLA region are associated with the susceptibility to autoimmune pancreatitis. Immunogenetics. 2007;59:45–52.
15. Umemura T, Ota M, Hamano H, Katsuyama Y, et al. Association of autoimmune pancreatitis with cytotoxic T-lymphocyte antigen 4 gene polymorphisms in Japanese patients. Am J Gastroenterol. 2008;103:588–94.
16. Umemura T, Ota M, Hamano H, et al. Genetic association of Fc receptor-like 3 polymorphisms with autoimmune pancreatitis in Japanese patients. Gut. 2006;55:1367–8.
17. Zen Y, Fujii T, Harada K, Kawano M, et al. Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. Hepatology. 2007;45:1538–46.
18. Kusuda T, Uchida K, Miyoshi H, et al. Involvement of inducible costimulator- and interleukin 10-positive regulatory T cells in the development of IgG4-related autoimmune pancreatitis. Pancreas. 2011;40:1120–30.
19. Watanabe T, Yamashita K, Fujikawa S, et al. Involvement of activation of Toll-like receptors and nucleotide-binding oligomerization domain-like receptors in enhanced IgG4 responses in autoimmune pancreatitis. Arthritis Rheum. 2012;64:914–24.
20. Watanabe T, Yamashita K, Sakurai T, et al. Toll-like receptor activation in basophils contributes to the development of IgG4-related disease. J Gastroenterol. 2013;48:247–53.
21. Janeway CA Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002;20:197–216.
22. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006;124:783–801.
23. Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature. 2007;449:819–26.
24. Beutler BA. TLRs and innate immunity. Blood. 2009;113:1399–407.
25. Pasare C, Medzhitov R. Toll-like receptors and acquired immunity. Semin Immunol. 2004;16:23–6.
26. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010;11:373–84.
27. Koyabu M, Uchida K, Miyoshi H, et al. Analysis of regulatory T cells and IgG4-positive plasma cells among patients of IgG4-related sclerosing cholangitis and autoimmune liver diseases. J Gastroenterol. 2010;45:732–41.
28. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol. 2004;4:499–511.
29. Witte CL, Schanzer B. Pancreatitis due to mumps. JAMA. 1968;203:1068–9.
30. Feldstein JD, Johnson FR, Kallick CA, et al. Acute hemorrhagic pancreatitis and pseudocyst due to mumps. Ann Surg. 1974;180:85–8.
31. Ursing B. Acute pancreatitis in coxsackie B infection. Br Med J. 1973;3:524–5.
32. Bunnell CE, Monif GR. Interstitial pancreatitis in the congenital rubella syndrome. J Pediatr. 1972;80:465–6.
33. Jain P, Nijhawan S, Rai RR, et al. Acute pancreatitis in acute viral hepatitis. World J Gastroenterol. 2007;13:5741–4.
34. Rizzardi GP, Tambussi G, Lazzarin A. Acute pancreatitis during primary HIV-1 infection. N Engl J Med. 1997;336:1836–7.
35. Blum A, Podvitzky O, Shalabi R, et al. Acute pancreatitis may be caused by H1N1 influenza A virus infection. Isr Med Assoc J. 2010;12:640–1.
36. Barrat FJ, Coffman RL. Development of TLR inhibitors for the treatment of autoimmune diseases. Immunol Rev. 2008;223:271–83.
37. Ewald SE, Barton GM. Nucleic acid sensing Toll-like receptors in autoimmunity. Curr Opin Immunol. 2011;23:3–9.
38. Umemura T, Katsuyama Y, Hamano H, et al. Association analysis of Toll-like receptor 4 polymorphisms with autoimmune pancreatitis. Hum Immunol. 2009;70:742–6.
39. Meagher C, Tang Q, Fife BT, et al. Spontaneous development of a pancreatic exocrine disease in CD28-deficient NOD mice. J Immunol. 2008;180:7793–803.
40. Davidson TS, Longnecker DS, Hickey WF. An experimental model of autoimmune pancreatitis in the rat. Am J Pathol. 2005;166:729–36.
41. Vallance BA, Hewlett BR, Snider DP, et al. T cell-mediated exocrine pancreatic damage in major histocompatibility complex class II-deficient mice. Gastroenterology. 1998;115:978–87.
42. Tsubata R, Tsubata T, Hiai H, et al. Autoimmune disease of exocrine organs in immunodeficient alymphoplasia mice: a spontaneous model for Sjögren's syndrome. Eur J Immunol. 1996;26:2742–8.
43. Hosaka N, Nose M, Kyogoku M, et al. Thymus transplantation, a critical factor for correction of autoimmune disease in aging MRL/+ mice. Proc Natl Acad Sci USA. 1996;93:8558–62.
44. Kanno H, Nose M, Itoh J, et al. Spontaneous development of pancreatitis in the MRL/Mp strain of mice in autoimmune mechanism. Clin Exp Immunol. 1992;89:68–73.
45. Watanabe S, Suzuki K, Kawauchi Y, et al. Kinetic analysis of the development of pancreatic lesions in mice infected with a murine retrovirus. Clin Immunol. 2003;109:212–23.
46. Suzuki K, Makino M, Okada Y, et al. Exocrinopathy resembling Sjögren's syndrome induced by a murine retrovirus. Lab Invest. 1993;69:430–5.
47. Qu WM, Miyazaki T, Terada M, et al. A novel autoimmune pancreatitis model in MRL mice treated with polyinosinic:polycytidylic acid. Clin Exp Immunol. 2002;129:27–34.
48. Yamashina M, Nishio A, Nakayama S, et al. Comparative study on experimental autoimmune pancreatitis and its extrapancreatic involvement in mice. Pancreas. 2012;41:1255–62.
49. Nishio A, Asada M, Uchida K, et al. The role of innate immunity in the pathogenesis of experimental autoimmune pancreatitis in mice. Pancreas. 2011;40:95–102.
50. Asada M, Nishio A, Akamatsu T, et al. Analysis of humoral immune response in experimental autoimmune pancreatitis in mice. Pancreas. 2010;39:224–31.
51. Soga Y, Komori H, Miyazaki T, et al. Toll-like receptor 3 signaling induces chronic pancreatitis through the Fas/Fas ligand-mediated cytotoxicity. Tohoku J Exp Med. 2009;217:175–84.
52. Haruta I, Yanagisawa N, Kawamura S, et al. A mouse model of autoimmune pancreatitis with salivary gland involvement triggered by innate immunity via persistent exposure to avirulent bacteria. Lab Invest. 2010;90:1757–69.
53. Masamune A, Kikuta K, Watanabe T, et al. Pancreatic stellate cells express Toll-like receptors. J Gastroenterol. 2008;43:352–62.
54. Sharif R, Dawra R, Wasiluk K, et al. Impact of Toll-like receptor 4 on the severity of acute pancreatitis and pancreatitis-associated lung injury in mice. Gut. 2009;58:813–9.
55. Ding SQ, Li Y, Zhou ZG, et al. Toll-like receptor 4-mediated apoptosis of pancreatic cells in cerulein-induced acute pancreatitis in mice. Hepatobiliary Pancreat Dis Int. 2010;9:645–50.

56. Hoque R, Sohail M, Malik A, et al. TLR9 and the NLRP3 inflammasome link acinar cell death with inflammation in acute pancreatitis. Gastroenterology. 2011;141:358–69.
57. Marques JT, Williams BR. Activation of the mammalian immune system by siRNAs. Nat Biotechnol. 2005;23:1399–405.
58. Hornung V, Rothenfusser S, Britsch S, et al. Quantitative expression of Toll-like receptor 1–10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J Immunol. 2002;168:4531–7.
59. Gantier MP, Tong S, Behlke MA, et al. TLR7 is involved in sequence-specific sensing of single-stranded RNAs in human macrophages. J Immunol. 2008;180:2117–24.
60. Greena NM, Marshak-Rothstein A. Toll-like receptor driven B cell activation in the induction of systemic autoimmunity. Semin Immunol. 2011;23:106–12.
61. Chamberlain ND, Kim SJ, Vila OM, et al. Ligation of TLR7 by rheumatoid arthritis synovial fluid single-strand RNA induces transcription of TNFα in monocytes. Ann Rheum Dis. 2013; 72:418–26.
62. Reed JH, Jain M, Lee K, et al. Complement receptor 3 influences Toll-like receptor 7/8-dependent inflammation: implications for autoimmune diseases characterized by antibody reactivity to ribonucleoproteins. J Biol Chem. 2013;29(288):9077–83.
63. Shikhagaie MM, Andersson CK, Mori M, et al. Mapping of TLR5 and TLR7 in central and distal human airways and identification of reduced TLR expression in severe asthma. Clin Exp Allergy. 2014;44:184–96.
64. Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003;3:23–35.
65. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol. 2009;27:451–83.
66. Van Gorp H, Delputte PL, Nauwynck HJ. Scavenger receptor CD163, a Jack-of-all-trades and potential target for cell-directed therapy. Mol Immunol. 2010;47:1650–60.
67. Detlefsen S, Sipos B, Zhao J, et al. Autoimmune pancreatitis: expression and cellular source of profibrotic cytokines and their receptors. Am J Surg Pathol. 2008;32:986–95.
68. Notohara K, Wani Y, Fujisawa M. Proliferation of CD163+ spindle-shaped macrophages in IgG4-related sclerosing disease: analysis of lymphoplasmacytic sclerosing pancreatitis and sclerosing sialadenitis. Mod Pathol. 2010;23:367A.
69. Jensen TO, Schmidt H, Moller HJ, et al. Macrophage markers in serum and tumor have prognostic impact in American Joint Committee on Cancer stage I/II melanoma. J Clin Oncol. 2009;27:3330–7.
70. Lee CH, Espinosa I, Vrijaldenhoven S, et al. Prognostic significance of macrophage infiltration in leiomyosarcomas. Clin Cancer Res. 2008;14:1423–30.
71. Kamper P, Bendix K, Hamilton-Dutoit S, et al. Tumor-infiltrating macrophages correlate with adverse prognosis and Epstein–Barr virus status in classical Hodgkin's lymphoma. Haematologica. 2011;96:269–76.
72. Maniecki MB, Moller HJ, Moestrup SK, et al. CD163 positive subsets of blood dendritic cells: the scavenging macrophage receptors CD163 and CD91 are coexpressed on human dendritic cells and monocytes. Immunobiology. 2006;211:407–17.
73. Geissmann F, Gordon S, Hume DA, et al. Unravelling mononuclear phagocyte heterogeneity. Nat Rev Immunol. 2010;10: 453–60.
74. Sung SA, Jo SK, Cho WY, et al. Reduction of renal fibrosis as a result of liposome encapsulated clodronate induced macrophage depletion after unilateral ureteral obstruction in rats. Nephron Exp Nephrol. 2007;105:e1–9.
75. Kitamoto K, Machida Y, Uchida J, et al. Effects of liposome clodronate on renal leukocyte populations and renal fibrosis in murine obstructive nephropathy. J Pharmacol Sci. 2009;111: 285–92.
76. Cheever AW, Williams ME, Wynn TA, et al. Anti-IL-4 treatment of *Schistosoma mansoni*-infected mice inhibits development of T cells and non-B, non-T cells expressing Th2 cytokines while decreasing egg-induced hepatic fibrosis. J Immunol. 1994; 153:753–9.
77. Chiaramonte MG, Donaldson DD, Cheever AW, et al. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. J Clin Invest. 1999;104:777–85.
78. Reiman RM, Thompson RW, Feng CG, et al. Interleukin-5 (IL-5) augments the progression of liver fibrosis by regulating IL-13 activity. Infect Immun. 2006;74:1471–9.
79. Balmelli C, Alves MP, Steiner E, et al. Responsiveness of fibrocytes to Toll-like receptor danger signals. Immunobiology. 2007;212:693–9.
80. Pulskens WP, Rampanelli E, Teske GJ, et al. TLR4 promotes fibrosis but attenuates tubular damage in progressive renal injury. J Am Soc Nephrol. 2010;21:1299–308.
81. Pradere JP, Troeger JS, Dapito DH, Mencin AA, Schwabe RF. Toll-like receptor 4 and hepatic fibrogenesis. Semin Liver Dis. 2010;30:232–44.
82. Braga TT, Correa-Costa M, Guise YF, et al. MyD88 signaling pathway is involved in renal fibrosis by favoring a TH2 immune response and activating alternative M2 macrophages. Mol Med. 2012;18:1231–9.
83. Uchida K, Okazaki K, Nishi T, et al. Experimental immune-mediated pancreatitis in neonatally thymectomized mice immunized with carbonic anhydrase II and lactoferrin. Lab Invest. 2002;82:411–24.
84. Uchida K, Kusuda T, Koyabu M, et al. Regulatory T cells in type 1 autoimmune pancreatitis. Int J Rheumatol. 2012;. doi:10.1155/2012/795026.
85. Miyoshi H, Uchida K, Taniguchi T, et al. Circulating naïve and CD4 + CD25high regulatory T cells in patients with autoimmune pancreatitis. Pancreas. 2008;36:133–40.

Autoimmune pancreatitis: pathogenesis, latest developments and clinical guidance

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Abstract: Recently, autoimmune pancreatitis has been classified into two subtypes. Type 1 is related to immunoglobulin G4 and type 2 is related to granulocytic epithelial lesions, but pathogenetic mechanisms in both still remain unclear. Apart from type 2 autoimmune pancreatitis, the pathological features of type 1 autoimmune pancreatitis with increased serum immunoglobulin G4/immunoglobulin E levels, abundant infiltration of immunoglobulin G4+plasmacytes and lymphocytes, fibrosis, and steroid responsiveness are suggestive of abnormal immunity such as allergy or autoimmunity. Although pathophysiological conditions seem to be different in each, both respond well to steroid drugs. After remission, the patients with type 1 autoimmune pancreatitis show high relapse rates (30–50% within 6–12 months), whereas those with type 2 autoimmune pancreatitis seldom relapse. After remission, the steroid maintenance therapy and therapeutic strategy for relapsing patients with type 1 is different among local expertise. In this paper, recent advances in pathogenesis and clinical guidance for therapy are discussed.

Keywords: autoimmune pancreatitis, immunoglobulin G4, immunoglobulin G4-related disease, Mikulicz disease, regulatory T cell, steroid therapy

Introduction

In 1961, Sarles and colleagues first observed a particular case of pancreatitis with hypergammaglobulinemia [Sarles *et al.* 1961]. Yoshida and colleagues first proposed the concept of autoimmune pancreatitis (AIP) [Yoshida *et al.* 1995]. Hamano and colleagues reported increased serum levels of immunoglobulin (Ig) G4 in Japanese patients with AIP [Hamano *et al.* 2001]. The histopathological findings of AIP in Japanese patients are characterized by the periductal localization of predominantly cluster of differentiation (CD) 4 positive T cells, IgGt4-positive plasma cells, storiform fibrosis with acinar cell atrophy frequently resulting in stenosis of the main pancreatic duct, storiform fibrosis and obliterative phlebitis [Okazaki and Chiba, 2002, 2011; Pickartz *et al.* 2007], which is also called lymphoplasmacytic sclerosing pancreatitis (LPSP) [Kawaguchi *et al.* 1991]. Recently, the International Consensus Diagnostic Criteria for AIP classified two distinct subtypes; type 1 and type 2 AIP [Chari *et al.* 2010]. Type 1 AIP is classified as a pancreatic manifestation of IgG4-related disease, probably a systemic disease with an autoimmune process, whereas type 2 AIP is supposed to be a specific pancreatic disease with occasional coexistence alongside ulcerative colitis [Chari *et al.* 2010; Shimosegawa *et al.* 2011]. Although pathogenesis or pathophysiology remains unclear, we will discuss the most recent advances in the concept and therapeutic guidance of AIP.

Recent advances in the concepts of autoimmune pancreatitis subtypes

Recent studies have suggested that AIP manifests as two distinct subtypes, type 1 and type 2 AIP (Table 1) [Chari *et al.* 2010; Shimosegawa *et al.* 2011; Klöppel *et al.* 2010]. In type 1 AIP, whose histologic description is called LPSP, the pancreatic histopathology shows the following characteristic features: (a) abundant infiltration of plasma cells (IgG4+ cells; >10/hpf, 40% > IgG4/IgG cells) and lymphocytes, (b) peculiar storiform or swirling fibrosis, and (c) perivenular

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Table 1. Subtypes of autoimmune pancreatitis.

Subtype of AIP	Type1	Type2
Other nomenclatures	AIP without GEL IgG4-related LPSP	AIP with GEL IgG4-unrelated IDCP
Prevalence	Asia > USA > EU	EU > USA > Asia
Age	high aged	younger
Gender	male>>female	male=female (NS)
Symptoms		
obstructive jaundice	often	often
abdominal pain	rare	common
Pancreas swelling		
diffuse/segmental/focal/mass forming	common	common
Serology	high serum IgG, IgG4, autoAbs (+)	normal IgG, normal IgG4, autoAbs (-)
Other organ involvement (OOI)	sclerosing cholangitis sclerosing sialadenitis reteroperitoneal fibrosis others	unrelated with OOI
Ulcerative colitis	rare	often
Steroid	responsive	responsive
Relapse	high rate	rare

AIP: autoimmune pancreatitis, GEL: granulocytic epithelial lesion, LPSP: lymphoplasmacytic sclerosing pancreatitis, IDCP: idiopathic duct-centric pancreatitis, USA: United States of America, EU: European Union, NS: not significant.

infiltration with lymphocytes and plasma cells often leading to obliterative phlebitis. Clinically, type 1 AIP seems to be the pancreatic manifestation of the recently proposed IgG4-related disease [Kamisawa *et al.* 2006; Yamamoto *et al.* 2006; Masaki *et al.* 2009; Umehara *et al.* 2012], characterized by swelling of the pancreas, elevated serum IgG4 levels and extrapancreatic lesions (e.g. sclerosing cholangitis, sclerosing sialadenitis and retroperitoneal fibrosis) associated with infiltration of abundant IgG4+plasma cells. Although it is not certain that all of them can be related to AIP, extrapancreatic lesions are prevalent in the systemic organs, suggesting that type 1 AIP, but not type 2 AIP, may be a pancreatic manifestation of IgG4-related disease. Elderly male patients with type 1 AIP often have obstructive jaundice, and the pancreatic and extrapancreatic manifestations respond to steroid therapy [Chari *et al.* 2010; Shimosegawa *et al.* 2011].

Type 2 AIP [Chari *et al.* 2010; Shimosegawa *et al.* 2011] was proposed by American and European pathologists from histological examinations of the resected pancreas of patients with chronic nonalcoholic pancreatitis, and they reported another histopathological pattern named as idiopathic duct-centric pancreatitis (IDCP) or AIP with granulocytic epithelial lesion (GEL) [Notohara *et al.* 2003; Zamboni *et al.* 2004; Klöppel *et al.* 2010]. The most characteristic feature of type 2 AIP is GEL, often with destruction and obliteration of the pancreatic duct. Type 2 AIP has swelling of the pancreas but none, or very few, IgG4-positive plasma cells, and clinical features show a distinctly different profile associated with no serum IgG4, IgG elevation, presence of autoantibodies, or other organ involvement except for inflammatory bowel disease (approximately 30%). Patients with type 2 AIP differ from those with type 1 AIP as they have no serological markers of autoimmunity, but deposition of C3c and IgG at the basement membrane of pancreatic ducts and acini suggests immune complex-mediated destruction of ducts and acini in type 2 AIP as well as in type 1 AIP [Detlefsen *et al.* 2010]. Although it is still in debate as to whether type 2 AIP should be classified as one clinical entity of AIP or not, the nomenclature of the two subtypes and international diagnostic criteria were proposed at the consensus meeting of the International Association of Pancreatology held at Fukuoka in 2010 [Shimosegawa *et al.* 2011].

Pathophysiological conditions in autoimmune pancreatitis

IgG4 and humoral immunity

The pathogenesis and pathophysiology of AIP have been studied mainly from immunological approaches and focused for the most part on IgG4-related type 1 AIP, because few incidences of abnormal immunity have been reported in type 2 AIP. In healthy subjects, the ratios for each IgG subclass are 65% of IgG1, 25% of IgG2, 6% of IgG3, and 4% of IgG4 [Roitt, 1997]. 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 [Hamano *et al.* 2001; Yamamoto *et al.* 2006; Masaki *et al.* 2009; Taguchi *et al.* 2009]. Although the association of IgE-mediated allergy and IgG4 antibodies is well known [Robinson *et al.* 2004], IgG4 characteristics are still poorly understood. Basically, IgG4 has nonacting characteristics for immune responses involved in a continuous process referred to as 'Fab-arm exchange' by swapping a heavy chain and attached a light chain with a heavy-light chain pair from another molecule as monovalent antibodies [van der Neut Kolfschoten *et al.* 2007]. Another aspect of IgG4 mimics IgG rheumatoid factor activity by interacting with IgG on a solid support [Kawa *et al.* 2008]. In contrast to conventional rheumatoid factor, which binds via its variable domains, the activity of IgG4 is located in its constant domains, but is inefficient in activating potentially dangerous effector systems due to its low affinity for C1q and the classical Fcγ receptors. As the patients in active stages of AIP occasionally show decreased complement (C3, C4) with elevated circulating immune complex [Hamano *et al.* 2001; Cornell *et al.* 2007], 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 [Muraki *et al.* 2006]. Moreover, IgG4 bound to other isotypes such as IgG1, 2, and 3 with an Fc–Fc interaction immune complex in patients with AIP [Kawa *et al.* 2008], suggests that 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. Patients with type 1 AIP generally show several nonspecific antibodies, such as an antinuclear antibody, in addition to increased IgG and IgG4 [Okazaki *et al.* 2001, 2011]. From the view of IgG4 function, the big mystery is whether type 1 AIP is an autoimmune or an allergic disease. 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 antilactoferrin (anti-LF) [Uchida *et al.* 2000; Okazaki *et al.* 2000], anticarbonic anhydrase-II (anti-CA-II) [Uchida *et al.* 2000; Okazaki *et al.* 2000; Nishi *et al.* 2007; Aparisi *et al.* 2005], anti-CA-IV [Nishimori *et al.* 2005], antipancreatic secretory trypsin inhibitor (anti-PSTI) [Asada *et al.* 2006], antiamylase-α [Endo *et al.* 2009], anti-HSP-10 [Takizawa *et al.* 2009], and antiplasminogen-binding protein (anti-PBP) peptide autoantibodies [Frulloni *et al.* 2009] 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 [Asada *et al.* 2006], CA-II [Uchida *et al.* 2000; Okazaki *et al.* 2000], CA-IV [Nishimori *et al.* 2005], LF [Uchida *et al.* 2000; Okazaki *et al.* 2000] and PSTI [Frulloni *et al.* 2009] which are distributed in the ductal cells of several exocrine organs, including the pancreas, salivary glands, biliary duct, lungs and renal tubules [Uchida *et al.* 2000; Okazaki *et al.* 2000]. Although not all peptides have been studied, immunization with CA-II or LF induced systemic lesions similar to human AIP, such as pancreatitis, sialadenitis, cholangitis and interstitial nephritis, in the mice models [Nishimori *et al.* 1995]. The high prevalence of the above antibodies suggests that they may be candidates for the target antigens in AIP [Ueno *et al.* 1995]. 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 that infectious agents cause bystander activation of immune cells with autoaggressive potential [Kountouras *et al.* 2005; Guarneri *et al.* 2005]. Guarneri and colleagues showed significant homology between human CA-II and α-CA of *Helicobacter pylori*, a fundamental enzyme for bacterial survival and proliferation in the stomach [Kountouras *et al.* 2005; Guarneri *et al.* 2005]. Moreover, the homologous segments contain the binding motif of DRB1*0405, which confers a risk for AIP development. The PBP peptide identified in European patients with AIP shows homology with an amino acid sequence of the PBP of *H. pylori* and with the

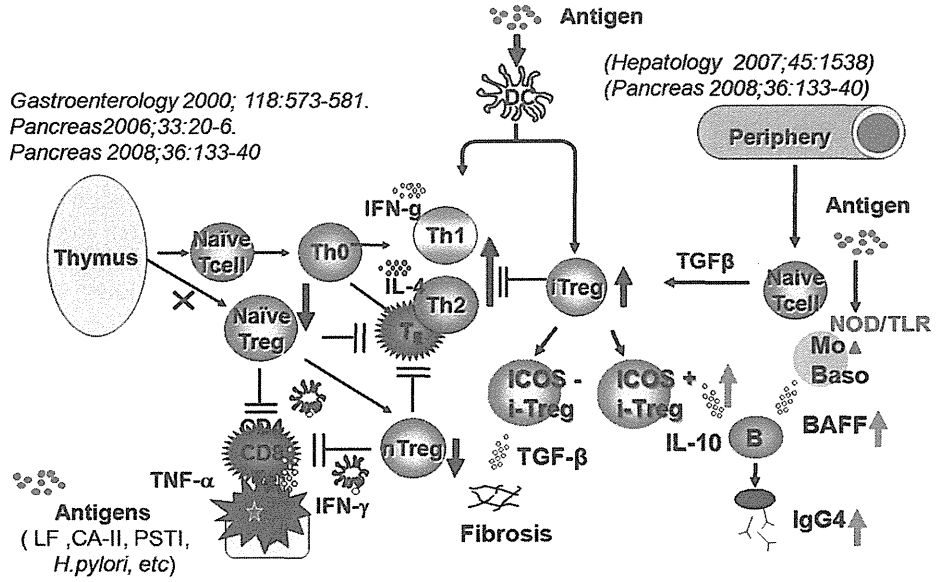
ubiquitin-protein ligase E3 component, n-recognin 2, an enzyme highly expressed in acinar cells of the pancreas, while European patients with AIP did not necessarily show LPSP as the typical histopathology of type 1 AIP in IgG4-related diseases [Frulloni *et al.* 2009]. These findings suggest that gastric *H. pylori* infection might trigger AIP in genetically predisposed subjects [Kountouras *et al.* 2005; Guarneri *et al.* 2005].

Th1 and Th2 immune balance

The presence of autoantibodies, the predominant infiltration of CD4+ and CD8+T-cells, and the expression of HLA-DR antigens in the pancreas [Uchida *et al.* 2000; Okazaki *et al.* 2011; Pickartz *et al.* 2007], 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 regulatory T cells (Treg) [Kountouras *et al.* 2005]. Interleukin (IL)-12 induces Th1 cells, which produce IL-2, tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), mediate cellular immunity, macrophage activation, cytotoxicity and help with B cell production of opsonizing and complement fixing antibodies [Okazaki *et al.* 2001]. IL-4 induces Th2 cells which produce IL-4, 5, 6 and 10, promoting humoral and allergic responses [Okazaki *et al.* 2001]. Transforming growth factor-beta (TGF-β), IL-6 IL-21 and IL-23 induce Th17 cells, which secrete IL-17, and may induce inflammation in mice [McGeachy and Cua, 2007; Oukka, 2007]. Although Th1-immune responses derived from the peripheral blood cells have been reported to be predominant over Th2 in some patients [Okazaki *et al.* 2000; Yamamoto *et al.* 2005], Th2 type immune responses are dominated in the local tissues in IgG4-related sclerosing cholangitis patients [Zen *et al.* 2007]. Mice models with depletion of Tregs by neonatally thymectomy (nTx) showed Th1 cells act mainly as effectors in the initial early stage [Okazaki *et al.* 2011]. Taken together, Th1 cytokines may be essential in the induction of AIP, while Th2 cytokines are involved in the progression of the disease process, especially the maturation and proliferation of local B cells and plasmacytes [Okazaki *et al.* 2011].

Regulatory T cells and innate immunity

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 [Oukka, 2007]. 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 both TGF-β and IL-10, and cell-to-cell contact via ligation of cytotoxic T lymphocyteantigen-4. Naturally occurring $CD4^+CD25^+$ Tregs (nTregs) from the thymus decreased in the peripheral blood of patients with AIP, whereas inducible memory Tregs (iTregs) (CD45RA-)-Tregs in major populations are significantly increased [Zen *et al.*2007]. In addition, prominent infiltration of Tregs with upregulation of IL-10 is observed in the liver of IgG4-related sclerosing cholangitis patients [Uchida *et al.* 2002]. These findings suggest that iTregs in the periphery and local tissues may be inhibitory immune responses against inflammation in the patients with AIP, although decreased nTregs may be pathogenetic. In AIP, increased peripheral iTregs are positively correlated with serum levels of IgG4 [Miyoshi *et al.* 2008]. These findings suggest that IgG4 or IgG4-immune complexes do not act as a pathogenetic factor but as an anti-inflammatory factor in IgG4-related diseases [Kawa *et al.* 2008]. On the other hand, recent studies suggested that abnormal innate immunity may be involved in production of IgG4 by upregulating B-cell activation factor family (BAFF) [Watanabe *et al.* 2012, 2013]. Further studies are necessary to clarify the role of IgG4 in type 1 AIP as an IgG4-related disease. Our recent hypothesis for the pathogenesis of type 1 AIP is shown in Figure 1. The basic concept is the biphasic mechanism of 'induction' and 'progression.' An initial response to self-antigens (LF, CA-II, CA-IV, PSTI, amylase-α, 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-1β, IL-2, TNF-α). In progression, Th2 type immune responses to 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 iTregs, with inducible T-cell co-stimulator molecules, respectively. The classical pathway of the complement system may be activated by the IgG1 immune complex. Recently, another mechanism of upregulation of IgG4 may be mediated by increased BAFF secreted from monocyte or basophils, which suggests that abnormal innate immunity may be involved in the development of IgG4-related disease [Watanabe *et al.* 2012, 2013].



(Okazaki K, et al. J Gastroenterol. 2011;46(3):277-88)

Figure 1. 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 IgG4-related disease, the basic concept is the biphasic mechanism of 'induction' and 'progression'. Initial response to self-antigens (lactoferrin, CA-II, CA-IV, PSTI, amylase-α, PBP peptide of *Helicobacter pylori*, etc.) might be induced by decreased naïve Tregs. Th2 immune responses were followed by Th1 type immune responses with the release of proinflammatory cytokines (IFN-γ, IL-1b, IL-2, TNF-α). In progression, 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-β secreted from inducible memory Tregs, respectively.
AIP, autoimmune pancreatitis; CA, carbonic anhydrase; CD, cluster of differentiation; IL, interleukin; PSTI, pancreatic secretory trypsin inhibitor ; PBP, plasminogen-binding protein; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; IgG, immunoglobulin G; TGF-β, transforming growth factor-beta.

Recent therapeutic strategy for AIP

About 10% of AIP patients improve spontaneously without steroid therapy; thus, steroid therapy in AIP is recommended in patients who have symptoms such as obstructive jaundice, abdominal and back pain, and the presence of symptomatic extrapancreatic lesions. A distinct difference from type 2 AIP patients is that type 1 AIP patients rarely have the severe abdominal pain that occurs in acute pancreatitis, but persistent abdominal or back pain in type 1 AIP appears to be an indication for steroid therapy. Associated symptomatic extrapancreatic lesions, such as retroperitoneal fibrosis, interstitial pneumonia, tubulointerstitial nephritis, and hepatic or pulmonary pseudotumor are indications for steroid therapy. A facile steroid trial to diagnose AIP is permitted only after negative work-up for malignancy by histological examinations under endoscopic ultrasound-fine needle aspiration/core biopsy [Okazaki *et al.* 2009; Kamisawa *et al.* 2010].

Before steroid therapy, biliary drainage for obstructive jaundice should be performed and blood glucose levels should be controlled in patients with diabetes mellitus. For the initial oral prednisolone dose for induction of remission, 0.5–0.6 mg/kg/day is recommended. The initial dose is administered for 2–4 weeks and then gradually tapered. After 2–4 weeks at the initial dose, the dose is tapered by 5 mg every 1–2 weeks over 3–4 months, based on changes in the clinical manifestations, biochemical blood tests, and repeated imaging studies [Okazaki *et al.* 2009; Kamisawa *et al.* 2010].

Most AIP patients (more than 90%) respond well to initial steroid therapy and achieve a remission state. After remission, the patients with type 1 AIP show a high rate of relapsing (30–50% within 6–12 months), whereas those with type 2 AIP seldom relapse. After remission in type 1 AIP, steroid maintenance therapy is still controversial, although the relapsing rate of 18–32% in

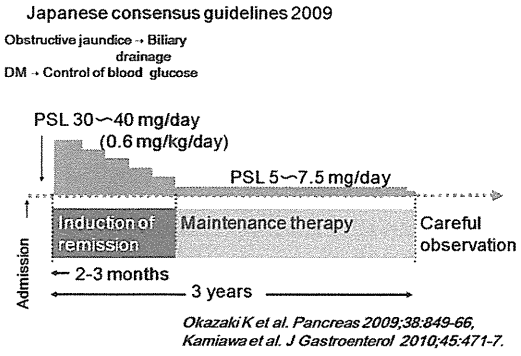


Figure 2. Proposal of steroid treatment of autoimmune pancreatitis (AIP) in Japan.
The Japanese consensus guidelines proposed the treatment of type1 AIP patients in Japan. Prior to steroid treatment, biliary drainage or control of blood sugar is recommended in patients with obstructive jaundice or diabetes. After remission by the initial steroid therapy for 2–3 months, a maintenance therapy with a dose of 5–7.5 mg /day for 3 years is recommended, if there are no major side effects.

Table 2. Medical treatment of AIP.

Steroid (first line for remission)
Oral (Kamisawa *et al.* 2010)
Mini-pulse (Tomiyama *et al.* 2011)
Immunomodulator
AZA/6-MP (Ghazale *et al.* 2008)
Mycofenolate mofetile (Church *et al.* 2007)
Rituximab (Topazian *et al.* 2008)

maintenance therapy is relatively low (53%) in cases without maintenance therapy [Okazaki *et al.* 2009; Kamisawa *et al.* 2010]. To prevent relapse, maintenance therapy (2.5–5 mg/day) within 3 years is recommended in the Japanese consensus guidelines (Figure 2) [Okazaki *et al.* 2009; Kamisawa *et al.* 2010], but not in Western countries (Europe and the United States). Similarly, the therapeutic strategy for relapsing patients with type 1 AIP is different among local expertise. In Japan, re-administration or increased dose of steroid is recommended for the second line in relapsing AIP patients, whereas immune-modulators such as thiopurine (azathioprine/6-mercaptoethanol) [Ghazale *et al.* 2008; Church *et al.* 2007] or a molecular targeting agent (rituximab) [Topazian *et al.* 2008] is commonly used in Western countries (Table 2). At this moment, molecular biological mechanism of the treatments, such as steroid and immunomodulator still remains unclear.

Conclusion

The pathophysiological conditions in the two subtypes of AIP seem to be different, although both of them respond well to steroid treatment. After remission, the steroid maintenance therapy and therapeutic strategy for relapsing patients with type 1 AIP are different among local expertise. As Tregs seem to take important roles in progression as well as induction of the disease, further studies are necessary to clarify the pathogenesis of AIP, including genetic backgrounds, disease specific antigens, and the role of IgG4.

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Conflict of interest statement

The author declares that there is no conflict of interest.

References

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: 703–709.

Asada, M., Nishio, A., Uchida, K., Kido, M., Ueno, S., Uza, N. *et al.* (2006) Identification of a novel autoantibody against pancreatic secretory trypsin inhibitor in patients with autoimmune pancreatitis. *Pancreas* 33: 20–26.

Chari, S., Kloeppel, G., Zhang, L., Notohara, K., Lerch, M., Shimosegawa, T. *et al.* (2010) Histopathologic and clinical subtypes of autoimmune pancreatitis: the Honolulu consensus document. *Pancreas* 39: 549–554.

Church, N., Pereira, S., Deheragoda, M., Sandanayake, N., Amin, Z., Lees, W. *et al.* (2007) Autoimmune pancreatitis: clinical and radiological features and objective response to steroid therapy in a UK series. *Am J Gastroenterol* 102: 2417–2425.

Cornell, L., Chicano, S., Deshpande, V., Collins, A., Selig, M., Lauwers, G. *et al.* (2007) Pseudotumors due to IgG4 immune-complex tubulointerstitial nephritis associated with autoimmune

pancreatocentric disease. *Am J Surg Pathol* 31: 1586–1597.

Detlefsen, S., Bräsen, J., Zamboni, G., Capelli, P. and Klöppel, G. (2010) Deposition of complement C3c, immunoglobulin (Ig)G4 and IgG at the basement membrane of pancreatic ducts and acini in autoimmune pancreatitis. *Histopathology* 57: 825–835

Endo, T., Takizawa, S., Tanaka, S., Takahashi, M., Fujii, H., Kamisawa, T. *et al.* (2009) Amylase alpha-2A autoantibodies: novel marker of autoimmune pancreatitis and fulminant type 1 diabetes. *Diabetes* 58: 732–737.

Frulloni, L., Lunardi, C., Simone, R., Dolcino, M., Scattolini, C., Falconi, M. *et al.* (2009) Identification of a novel antibody associated with autoimmune pancreatitis. *N Engl J Med* 361: 2135–2142.

Ghazale, A., Chari, S., Zhang, L., Smyrk, T., Takahashi, N., Levy, M. *et al.* (2008) Immunoglobulin G4-associated cholangitis: clinical profile and response to therapy. *Gastroenterology* 134: 706–715

Guarneri, F., Guarneri, C. and Benvenga, S. (2005) Helicobacter pylori and autoimmune pancreatitis: role of carbonic anhydrase via molecular mimicry? *J Cell Mol Med* 9: 741–744.

Hamano, H., Kawa, S., Horiuchi, A., Unno, H., Furuya, N., Akamatsu, T. *et al.* (2001) High serum IgG4 concentrations in patients with sclerosing pancreatitis. *New Engl J Med* 344: 732–738.

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: 982–984.

Kamisawa, T. and Okamoto, A. (2006) Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease. *J Gastroenterol* 41: 613–625.

Kamisawa, T., Okazaki, K., Kawa, S., Shimosegawa, T. and Tanaka, M. and Research Committee for Intractable Pancreatic Disease and Japan Pancreas Society. (2010) Japanese consensus guidelines for management of autoimmune pancreatitis: III. Treatment and prognosis of AIP. *J Gastroenterol* 45: 471–477.

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: e1637.

Kawaguchi, K., Koike, M., Tsuruta, K., Okamoto, A., Tabata, I. and Fujita, N. (1991). Lymphoplasmacytic sclerosing pancreatitis with cholangitis: a variant of primary sclerosing cholangitis extensively involving pancreas. *Hum Pathol* 22; 387–395.

Klöppel, G., Detlefsen, S., Chari, S., Longnecker, D. and Zamboni, G. (2010) Autoimmune pancreatitis: the clinicopathological characteristics of the subtype with granulocytic epithelial lesions. *J Gastroenterol* 45: 787–793.

Kountouras, J., Zavos, C. and Chatzopoulos, D. (2005) A concept on the role of Helicobacter pylori infection in autoimmune pancreatitis. *J Cell Mol Med* 9: 196–207.

Masaki, Y., Dong, L., Kurose, N., Kitagawa, K., Morikawa, Y., Yamamoto, M. *et al.* (2009) Proposal for a new clinical entity, IgG4-positive multi-organ lymphoproliferative syndrome: Analysis of 64 cases of IgG4-related disorders. *Ann Rheum Dis* 68: 1310–1315.

McGeachy, M. and Cua, D. (2007) The link between IL-23 and Th17 cell-mediated immune pathologies. *Semin Immunol* 19: 372–376.

Miyoshi, H., Uchida, K., Taniguchi, T., Yazumi, S., Matsushita, M., Takaoka, M. *et al.* (2008) Circulating naive and CD4+CD25 high regulatory T cells in patients with autoimmune pancreatitis. *Pancreas* 36: 133–140.

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.

Nishi, H., Tojo, A., Onozato, M., Jimbo, R., Nangaku, M., Uozaki, H. *et al.* (2007) Anti-carbonic anhydrase II antibody in autoimmune pancreatitis and tubulointerstitial nephritis. *Nephrol Dial Transplant* 22: 1273–1275.

Nishimori, I., Bratanova, T., Toshkov, I., Caffrey, T., Mogaki, M., Shibata, Y. *et al.* (1995) Induction of experimental autoimmune sialoadenitis by immunization of PL/J mice with carbonic anhydrase II. *J Immunol* 154: 4865–4873.

Nishimori, I., Miyaji, E., Morimoto, K., Nagao, K., Kamada, M. and Onishi, S. (2005) Serum antibodies to carbonic anhydrase IV in patients with autoimmune pancreatitis. *Gut* 54: 274–281.

Oukka, M. (2007) Interplay between pathogenic Th17 and regulatory T cells. *Ann Rheum Dis* 66(Suppl. 3): iii87–90.

Notohara, K., Burgart, L., Yadav, D., Chari, S. and Smyrk, T. (2003) Idiopathic chronic pancreatitis with periductal lymphoplasmacytic infiltration: clinicopathologic features of 35 cases. *Am J Surg Pathol* 27: 1119–1127.

Okazaki, K., Uchida, K., Ohana, M., Nakase, H., Uose, S., Inai, M. *et al.* (2000) Autoimmune-related pancreatitis is associated with autoantibodies and a Th1/Th2-type cellular immune response. *Gastroenterology* 118: 573–581.