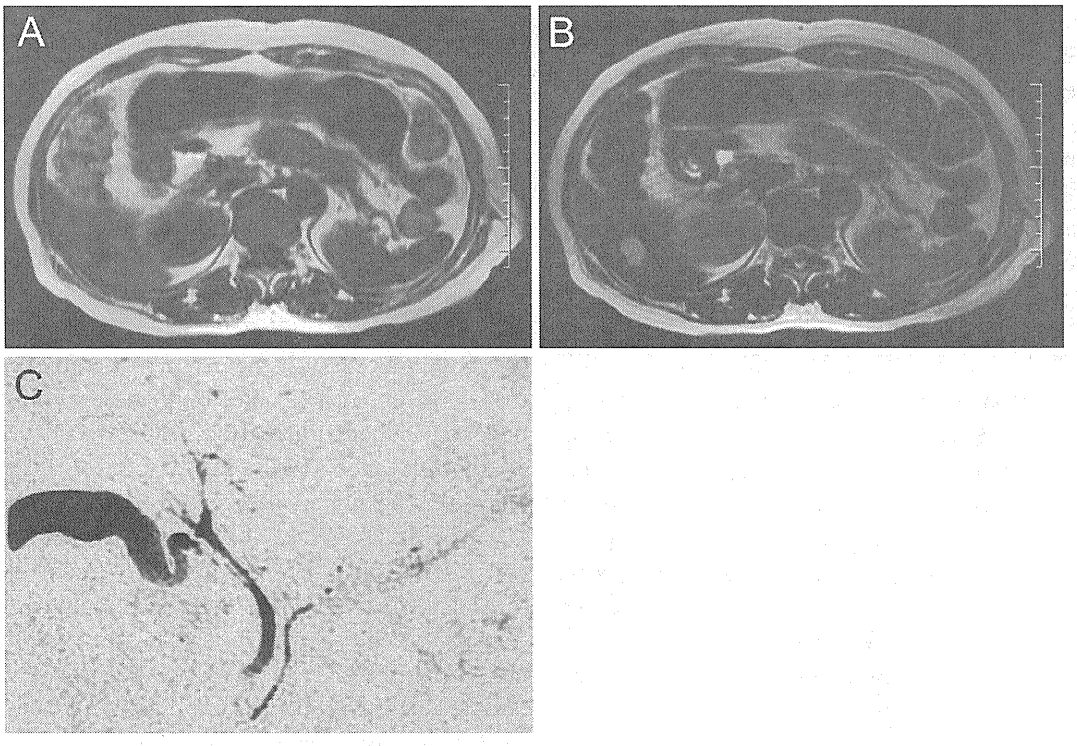

Figure 3. **Magnetic resonance imaging (MRI) of the pancreas. MRI demonstrated swelling in the body and tail of the pancreas with no obvious intensity of change (A; T1 intensive image, B; T2 intensive image). Magnetic resonance cholangiopancreatography (MRCP) revealed obstruction of the main pancreatic duct in the body (C).**

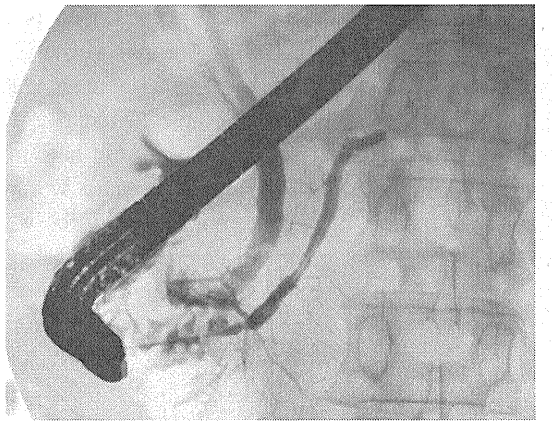

Figure 4. **Endoscopic retrograde cholangiopancreatography (ERCP). ERCP image demonstrated abrupt ending of the main pancreatic duct in the body compatible with pancreatic cancer.**

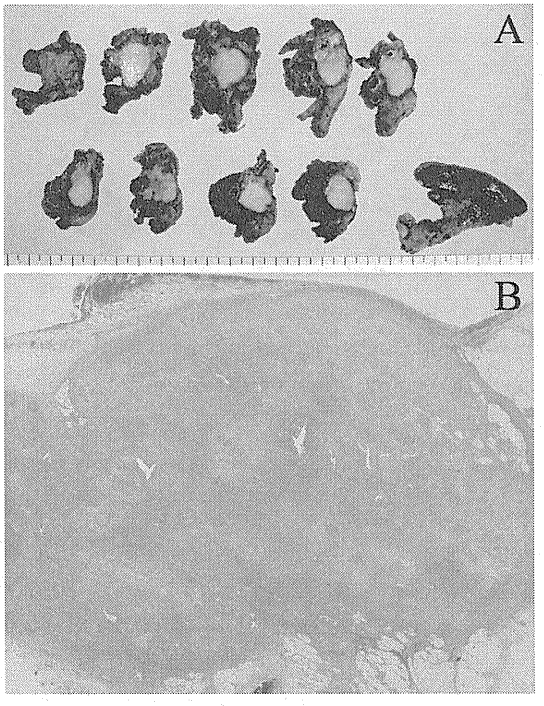

Figure 5. **Macroscopic findings of the pancreas. The cut surface of the resected specimen showed swelling of parenchyma with the whitish indurated tissue (Fig. 5A). The picture of loupe of the greatest surface of cut specimen (Fig. 5B).**

of parenchyma with the whitish indurated tissue (Fig. 5)). Histologically, prominent lobular inflammation consisted of edema and infiltrating neutrophils, lymphocytes, and plasma cells. Although fibroblastic proliferation and fibrosis were seen, inflammatory infiltrate cells were scarce between the lobules. Neutrophils were sometimes prominent in and around the intralobular duct. Numerous microabscesses were found in the intralobular duct. Neutrophils involved the duct epithelium and lumen, and the epithelial cells were destroyed. Obliterative phlebitis was not observed (Fig. 6). From these findings, the histopathological diagnosis made was IDCP. On immunohistochemical staining, IgG1-positive plasma cells were abundant (Fig. 7B), but IgG4-positive plasma cells were not (Fig. 7A). Abundant infiltration of

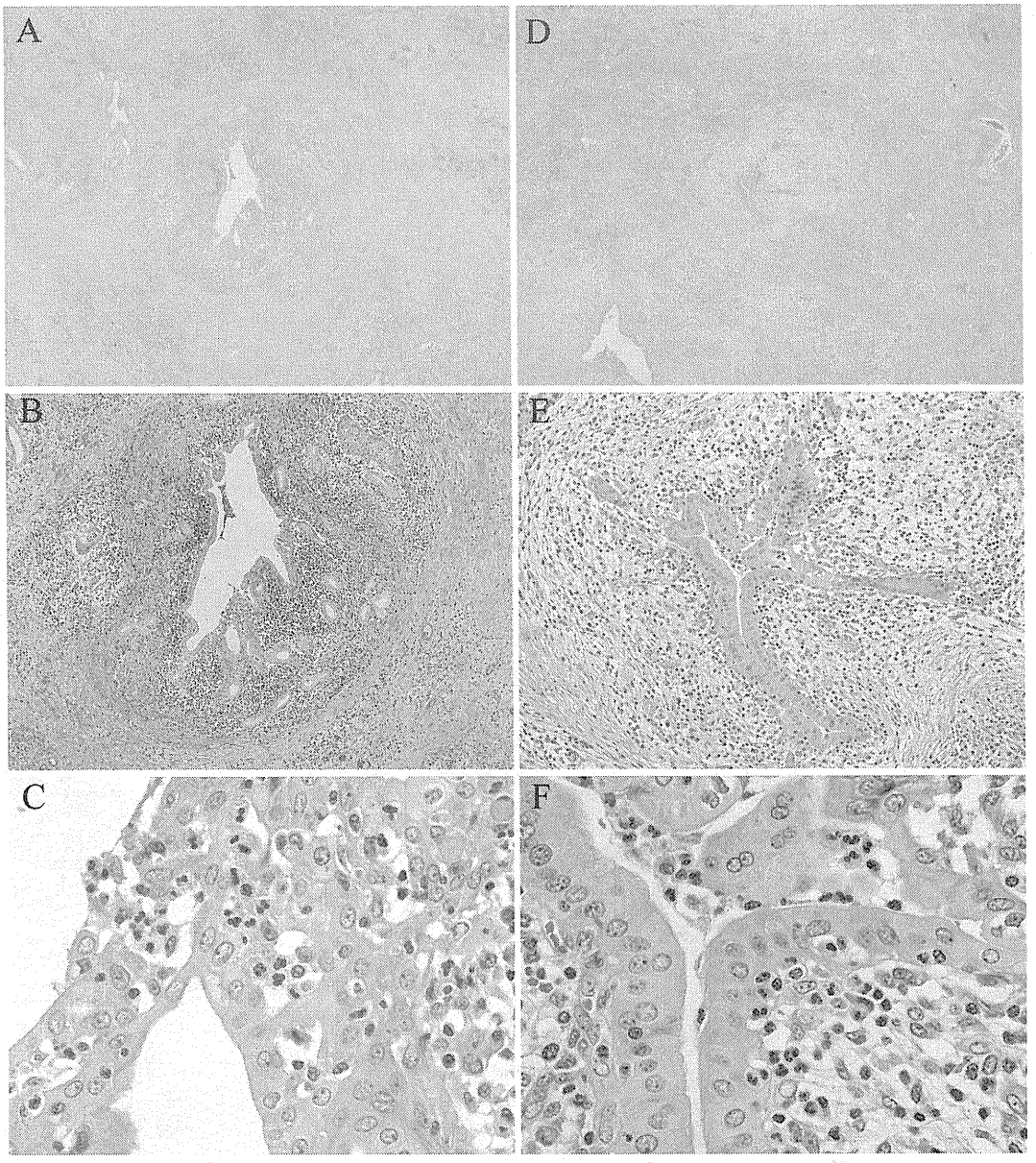

Figure 6. Histological findings of the pancreas (Hematoxylin and Eosin staining). Although fibroblastic proliferation and fibrosis are seen, inflammatory infiltrates are scarce between the lobules (A, ×20; D, ×20). Periductal inflammation with destruction of pancreatic epithelia by abundant neutrophils suggested a granulocyte epithelial lesion (GEL) (B, ×40; C, ×400). Inflammatory cells were few in fibrosis. Microabscess was seen in the intralobular duct (E, ×100; F, ×400).

Foxp3-positive T lymphocytes was observed around the intralobular ducts (Fig. 7C). Predominant infiltration of CD3-positive, CD4-positive and CD8-positive T lymphocytes was seen around the interlobular ducts (Fig. 7D-F). In addition, the infiltrated cells contained CD79a-positive plasma cells (Fig. 7G).

We examined 9 patients with LPSP (6 women and 3 men; mean age 54 years; range, 56-73 years), 9 patients with alcoholic pancreatitis (9 men; mean age, 53 years; range, 39-75 years), and only one patient with IDCP (woman, age; 65). The numbers of IgG4-positive plasma cells (IgG4/HPF) were significantly higher in LPSP (20.0 ± 6.0 cells/HPF) than in alcoholic chronic pancreatitis (2.1 ± 0.9 cells/HPF; $p<0.05$). The numbers of IgG1-positive plasma cells (IgG1/HPF) were significantly lower in LPSP (7.6 ± 2.4 cells/HPF) than in alcoholic chronic pancreatitis (12.1 ± 1.8 cells/HPF; $p<0.05$). The ratio of IgG4/HPF to IgG1/HPF (IgG4/G 1 ratio) was significantly higher in AIP (2.72 ± 0.76) than in alcoholic chronic pancreatitis (0.18 ± 0.09; $p<0.05$). The numbers of Foxp3-positive cells (Foxp3/HPF) in patients with LPSP (15.3 ± 3.0 cells/HPF) were significantly increased compared with alcoholic chronic pancreatitis (1.7 ± 0.5 cells/HPF; $p<0.05$). However, the IDCP case showed Foxp3-positive cells; 9.7 cells/HPF, IgG4-positive plasma cells; 8.0 cells/HPF, IgG4/G1 ratio; 0.39, IgG1-positive plasma cells; 20.7 cells/HPF, respectively (Table 1).

Discussion

Since Sarles et al reported a case of idiopathic pancreatitis

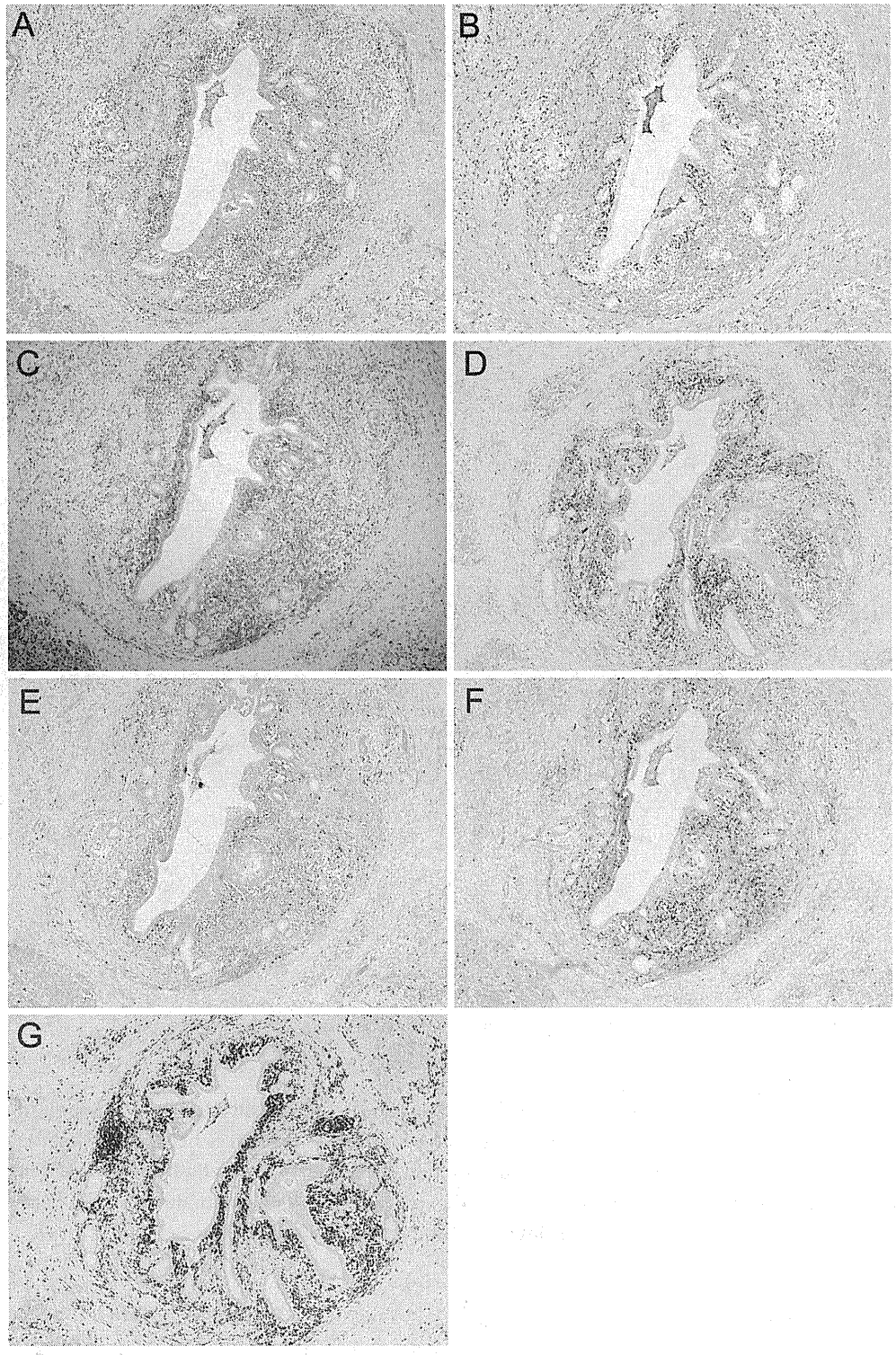

Figure 7. Immunohistochemistry of the pancreas (A, IgG4; B, IgG1; C, Foxp3; D, CD3; E, CD4; F, CD8; G, CD79a). IgG4-positive plasma cells were scattered, whereas IgG1-positive plasma cells were abundant (A, B). Foxp3-positive T lymphocytes were observed around the intralobular duct (C). A predominance of CD3-positive, CD4-positive and CD8-positive T lymphocytes were abundant in the interlobular ducts (D, E, F). In addition, also infiltrated CD79a-positive plasma cells were seen (G).

with hypergammaglobulinemia (1), many investigators have suggested that an autoimmune mechanism is involved in some instances of idiopathic pancreatitis. We previously reported that patients with AIP frequently have autoantibodies (3, 4). Hamano et al reported that patients with AIP show a high serum IgG4 concentration, and that the values are closely associated with the disease activity (11). Japanese AIP cases are almost exclusively LPSP (4, 7).

In contrast, AIP with neutrophilic infiltration in the epithelium of the pancreatic duct (idiopathic duct-centric pan-

Table 1. Immunohistochemical Findings of 19 Cases of Tumor-forming Type of Pancreatitis

Infiltrated cells	Non-alcoholic (n=10)		Alcoholic (n=9)
	IDCP (n=1)	LPSP (n=9)	Chronic pancreatitis (n=9)
IgG1	20.7	7.6±2.4*	12.1±1.8*
IgG4	8.0	20.0±6.0*	2.1±0.9*
IgG4/IgG1	0.39	2.72±0.76*	0.18±0.09*
Foxp3	9.7	15.3±3.0*	1.7±0.5*

IgG1-, IgG4-, and Foxp3-positive cells contained within the portal tracts selected in each specimen were counted under five different high power fields (HPF). IgG4/IgG1; the ratio of IgG4/HPF to IgG1/HPF was calculated in each case. Values are the mean ± SD. * $p<0.05$

creatitis: IDCP, or granulocyte epithelial lesion: GEL) has been reported by American and European pathologists (6, 8, 9). In a recent study, Zhang et al reported that while LPSP consistently shows moderate to severe infiltration with IgG4-positive cells, IDCP rarely shows excess IgG4-positive cells (12). In the present case IgG4-positive cells were not abundant. Prior to the concept of IDCP or GEL, in 1997 Ectors et al (13) reported the concept of non-alcoholic duct destructive chronic pancreatitis (NDCP) characterized by histological findings distinguishable from LPSP: a neutrophil predominant lobular inflammation and a duct destructive infiltrate without obliterative phlebitis (8, 13). The features seen in patients with NDCP are similar to those of IDCP although it still remains unclear whether these two entities represent different manifestations of the same disease or not. The clinical features of AIP in Western countries have been reported to be elderly males, frequent association with inflammatory bowel disease, and a weaker association with other sclerosing diseases, which seems to be different from Japanese AIP (LPSP). Frulloni et al recently reported that the focal type of AIP (63%) is more common than the diffuse type (37%) of the 87 Italian patients with AIP patients (54 males and 33 females, mean age 43.4 ± 15.3 years). Of total patients, 30% had ulcerative colitis, and 66% of focal AIP and 27% of diffuse AIP showed increased serum levels of IgG4. Although the histopathological findings were not evaluated in their series, IDCP may be predominant in the diffuse type of AIP (14). In Japan, the above Western type of AIP cases has not been confirmed yet owing to the limited number of studies.

Therefore, AIP might be a heterogeneous disease with different clinical aspects, and these patients with young onset might be another subtype distinguishable from the usual AIP as defined in Japan (15). Although a single study of young Japanese patients with AIP reported more frequent abdominal pain and increased serum amylase elevations compared with aged patients (16), it was unclear whether these young patients had IDCP or not.

The present patient had no abdominal pain, but did have an elevated level of serum amylase. Serum IgG4 was not measured. She had no other organ involvement. Radiological findings did not demonstrate the typical findings of AIP as shown in the Japanese diagnostic criteria (17, 18). In the US, CT, and MRI images of the present case, the pancreas was slightly swollen, but it lacked a capsular-like low density rim on the enhanced CT images. Pancreatogram on ERCP showed the abrupt ending of the MPD without irregular narrowing of MPD. Immunochemically, in this IDCP case, the density of IgG1-positive cells was higher than the density of IgG4- and Foxp3-positive cells. The ratio of IgG4/HPF to IgG1/HPF (IgG4/G1) was higher in LPSP than in IDCP (Table 1). Miyoshi et al reported that the numbers of circulating regulatory T cells (Tregs) is increased in AIP (19). Koyabu et al reported that the ratio of IgG4/IgG1-positive plasma cells in specimens obtained from patients with IgG4-related sclerosing cholangitis (IgG4-SC) was significantly higher than in specimens from patients with primary sclerosing cholangitis (PSC), autoimmune pancreatitis (AIP), and primary biliary cirrhosis (PBC). The Foxp3/Mono ratio in patients with PBC was significantly higher than that in patients with IgG4-SC and PSC (20). Certainly, in our LPSP cases, Foxp3-positive cells (Tregs) were abundant, but not in our IDCP case (Table 1). LPSP is consistent with the definition of autoimmune disease (AID) (21), but IDCP is not. Therefore, we feel that LPSP and IDCP should be considered as completely different diseases immunologically. Further studies are necessary to establish the concept of IDCP in Japan and to clarify the mechanism in the development of IDCP.

Acknowledgement

This study was partially supported by (1) Grant-in-Aid for Scientific Research (C) of Ministry of Culture and Science of Japan (20590810), (2) Intractable Diseases, the Health and Labor Sciences Research Grants (KO) from the Ministry of Labor and Welfare of Japan.

References

1. Sarles H, Sarles JC, Muratore R, Guien C. Chronic inflammatory sclerosis of the pancreas--an autonomous pancreatic disease? Am J Dig Dis **6**: 688-698, 1961.
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

40: 1561-1568, 1995.
3. Okazaki K, Uchida K, Ohana M, et al. Autoimmune-related pancreatitis is associated with autoantibodies and a Th1/Th2-type cellular immune response. Gastroenterology **118**: 573-581, 2000.
4. Uchida K, Okazaki K, Konishi Y, et al. Clinical analysis of autoimmune-related pancreatitis. Am J Gastroenterol **95**: 2788-2794, 2000.
5. Kawaguchi K, Koike M, Tsuruta K, Okamoto A, Tabata I, Fujita N. Lymphoplasmacytic sclerosing pancreatitis with cholangitis: a variant of primary sclerosing cholangitis extensively involving pancreas. Hum Pathol **22**: 387-395, 1991.
6. Notohara K, Burgart LJ, Yadav D, Chari S, Smyrk TC. Idiopathic chronic pancreatitis with periductal lymphoplasmacytic infiltration: clinicopathologic features of 35 cases. Am J Surg Pathol **27**: 1119-1127, 2003.
7. Okazaki K, Uchida K, Matsushita M, Takaoka M. Autoimmune pancreatitis. Intern Med **44**: 1215-1223, 2005.
8. Yadav D, Notohara K, Smyrk TC, et al. Idiopathic tumefactive chronic pancreatitis: clinical profile, histology, and natural history after resection. Clin Gastroenterol Hepatol **1**: 129-135, 2003.
9. 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 **445**: 552-563, 2004.
10. Nagahisa Y, Taebun P, Ogasawara K. A case of idiopathic duct-centric type chronic pancreatitis. Kurashiki Cyuuou Byouin Nennpou **68**: 87-91, 2005 (in Japanese, Abstract in English).
11. Hamano H, Kawa S, Horiuchi A, et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med **344**: 732-738, 2001.
12. Zhang L, Notohara K, Levy MJ, Chari ST, Smyrk TC. IgG4-positive plasma cell infiltration in the diagnosis of autoimmune pancreatitis. Mod Pathol **20**: 23-28, 2007.
13. Ectors N, Maillet B, Aerts R, et al. Non-alcoholic duct destructive chronic pancreatitis. Gut **41**: 263-268, 1997.
14. Frulloni L, Scattolini C, Falconi M, et al. Autoimmune pancreatitis: differences between the focal and diffuse forms in 87 patients. Am J Gastroenterol **104**: 2288-2294, 2009.
15. Kamisawa T. Clinical subtypes of autoimmune pancreatitis. Intern Med **44**: 785-786, 2005.
16. Kamisawa T, Wakabayashi T, Sawabu N. Autoimmune pancreatitis in young patients. J Clin Gastroenterol **40**: 847-850, 2006.
17. Okazaki K, Kawa S, Kamisawa T, et al. Clinical diagnostic criteria of autoimmune pancreatitis: revised proposal. J Gastroenterol **41**: 626-631, 2006.
18. Okazaki K, Uchida K, Matsushita M, Takaoka M. How to diagnose autoimmune pancreatitis by the revised Japanese clinical criteria. J Gastroenterol **42**(Suppl 18): 32-38, 2007.
19. Miyoshi H, Uchida K, Taniguchi T, et al. Circulating naive and CD4+CD25high regulatory T cells in patients with autoimmune pancreatitis. Pancreas **36**: 133-140, 2008.
20. 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 **45**: 732-741, 2010.
21. Mackay IR. Autoimmune disease. Med J Aust **1**: 696-699, 1969.

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

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

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

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

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

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

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

(*Pancreas* 2011;40: 95–102)

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

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

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

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

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

MATERIALS AND METHODS

Mice

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

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

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

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

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

Induction of Pancreatitis

Six-week-old female MRL/Mp mice were injected intraperitoneally with poly I:C or lipopolysaccharide (LPS; Sigma Chemical Co, St Louis, Mo) at doses of 5 mg/kg body weight twice weekly for 12 weeks (12 mice in each group) or were given a single injection of poly I:C or LPS at the same doses (6 mice in each group). Six-week-old female IL-10KO mice were injected intraperitoneally with poly I:C at a dose of 5 mg/kg body weight or LPS at a dose of 0.5 mg/kg body weight twice weekly for 8 weeks (12 mice in each group) or were given single injections of poly I:C or LPS at the same doses (6 mice in each group). Control mice were injected with phosphate-buffered saline (PBS). All experiments were conducted with the approval of the Ethics Committee for the Use of Experimental Animals of Kyoto University.

Histological Examination

MRL mice were sacrificed after 12 weeks of treatment, and IL-10KO mice were sacrificed after 8 weeks of treatment. Blood was collected, and serum was stored at −20°C until use. Pancreatic tissue was excised for histopathological examination. Tissues were fixed in 10% phosphate-buffered formaldehyde (pH 7.2) and embedded in paraffin. The sections were stained with hematoxylin and eosin, and histopathological examination was performed using light microscopy. The severity of pancreatitis was scored on a scale of 0 to 4, which was based on the histopathological scoring system described by Kanno et al[14] (briefly, 0 = no mononuclear cell infiltration; 1 = mononuclear cell aggregation and/or infiltration within the interstitium without any parenchymal destruction; 2 = focal parenchymal destruction with mononuclear cell infiltration; 3 = diffuse parenchymal destruction but with retention of some intact parenchymal residue; and 4 = almost all pancreatic tissue, except pancreatic islets, destroyed or replaced with fibrosis or adipose tissue).

Measurement of Serum Cytokine Levels

Serum levels of interferon (IFN)-α, IFN-γ, tumor necrosis factor (TNF)-α, IL-4, IL-10, and IL-12p70 were measured in mice in which AIP was induced by serial injections of the TLR ligands (6 mice in each group) and mice that received single doses of the TLR ligands (6 mice in each group) using enzyme-linked immunosorbent assay kits (R&D Systems Inc, Minneapolis, Minn). To study the effect of TLR stimulation on cytokine production, mice were sacrificed 3 hours after a single injection of the various TLR ligands, and serum was collected.

Gene Expression of TLRs

The gene expression of TLRs in pancreatic tissues was examined using mice that were given a single injection of the TLR ligands (5 mice in each group). To analyze messenger RNA (mRNA) expression of TLR3, TLR4, and TLR9 using the reverse transcription–polymerase chain reaction (PCR), total RNA was extracted from the pancreas using an RNA extraction solution (RNeasy, Qiagen, Tokyo, Japan) and then reverse transcribed into complementary DNA using the SuperScript Preamplification System (Gibco-BRL, Gaithersburg, Md). The reaction mixture was heated for 50 minutes at 42°C and 15 minutes at 70°C, and was then chilled on ice. Polymerase chain reaction was performed using a mixture of complementary DNAs, 20 mmol/L of Tris-HCl (pH 8.4), 50 mmol/L of KCl, 2.5 mmol/L of $MgCl_2$, 200 mmol/L of each deoxynucleotide triphosphate (PerkinElmer, Branchburg, NJ), 50 pmol/L of each specific primer, and 1.0 U of *Taq* DNA polymerase (AmpliTaq Gold; PerkinElmer). The primer sequences used in this study were TLR3, (forward) 5′-GGT GGT CCC GTT AAT TTC CT-3′, (reverse) 5′-CAG GAG CAT ACT GGT GCT GA-3′; TLR4, (forward) 5′-AGA GTC AGG TGA TGG ATG TCG-3′, (reverse) 5′-CAA GGG ATA AGA ACG CTG AGA-3′; and TLR9, (forward) 5′-GCA AGC TCA ACC TGT CCT TC-3′, (reverse) 5′-CAG GCT AAG ACA CTG GAG GC-3′. Amplification was performed using a thermal cycler (GeneAmp PCR System 9600R; PerkinElmer) set at 30 to 40 cycles for 20 seconds at 95°C, 1 minute at 55°C, and 1 minute at 72°C. A 10-μL aliquot of each PCR product was electrophoresed on a 2.0% agarose gel containing ethidium bromide. The densities of bands on the gels were measured using an image autoanalysis system (Fotodyne, FOTOanalyst and Archive ECLIPSE; Advanced American Biotechnology, Fullerton, Calif) and were expressed as absorbance levels. The semiquantitative value for each product was corrected according to the β-actin density of the sample.

Statistical Analysis

Student *t* test was used to determine differences between 2 groups. One-way analysis of variance followed by Fisher protected least significant difference test was used to determine differences between multiple groups. A 2-tailed $P < 0.05$ was considered significant.

RESULTS

Pancreatitis in MRL/Mp Mice

Polyinosinic polycytidylic acid administration accelerated the development of pancreatitis in MRL/Mp mice, but PBS did not (Fig. 1A). After 12 weeks of poly I:C injections, marked inflammatory cell infiltration accompanied by severe destruction of the acini, irregular fibrosis, and fatty changes were observed (Fig. 1B). In addition, some of the acinar cells showed cellular vacuolization. However, the endocrine glands showed little change, and the tissues were well preserved. In contrast, administration of LPS induced only mild pancreatitis (Fig. 1C). Histological scores for pancreatitis were 3.5 ± 0.2 in mice treated with poly I:C and 1.3 ± 0.2 in mice treated with LPS (Fig. 1D).

Serum Cytokine Levels in MRL/Mp Mice

To study the role of proinflammatory cytokines in the induction of pancreatitis, we compared serum cytokine levels between mice treated with PBS, poly I:C, or LPS. There were no significant differences in IFN-α, TNF-α, or IL-4 levels between the PBS, poly I:C, and LPS groups (Figs. 2A, C, D). Serum levels of IFN-γ were elevated in mice treated with LPS, but the increase was not significant (Fig. 2B). Serum levels of IL-10 and IL-12p70 were significantly elevated in poly I:C-treated mice compared with PBS-treated or LPS-treated mice (Figs. 2E, F).

Next, we measured serum cytokines in mice after single injections of the TLR ligands to investigate the early response to TLR stimulation. An increase in serum IFN-α levels was observed only in the poly I:C-treated mice (Fig. 3A). Single injections of poly I:C or LPS significantly increased serum levels of IFN-γ (Fig. 3B). The increase in IFN-γ was higher in LPS-injected mice than in poly I:C-injected mice. Similar increases in serum TNF-α level were observed after single injections of poly I:C or LPS (Fig. 3C). There were no significant differences in IL-4 level between the PBS-treated, poly I:C-treated, and LPS-treated mice (Fig. 3D). Interleukin 10 levels were elevated in poly I:C-treated and LPS-treated mice, and increase in IL-10 levels was much greater in LPS-treated mice than in poly I:C-treated mice (Fig. 3E). Lipopolysaccharide administration

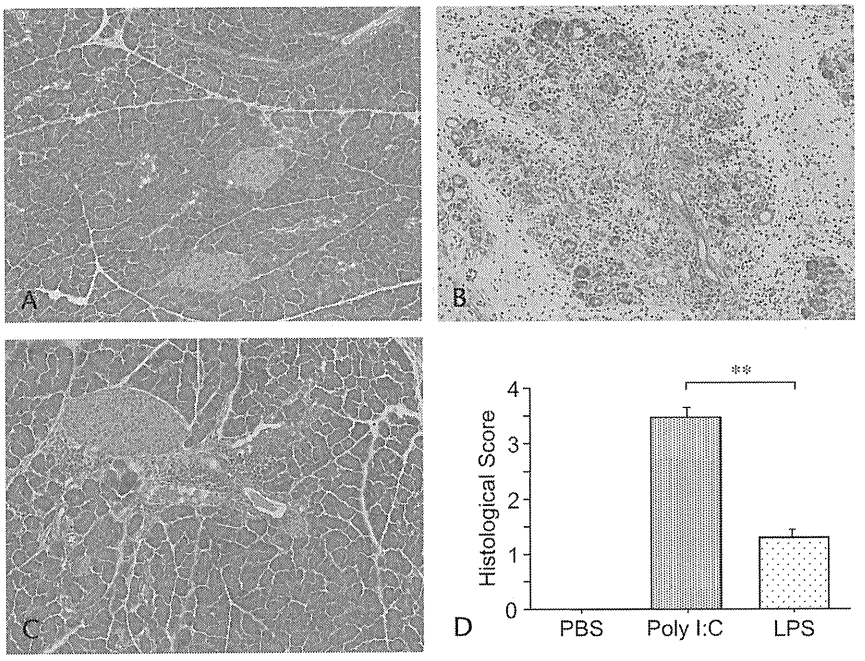

FIGURE 1. Histopathological examination of the pancreas and histological scoring of pancreatitis in MRL/Mp mice. Representative pancreatic sections stained with hematoxylin and eosin: 12-week treatment with PBS (A), 12-week treatment with poly I:C (B), and 12-week treatment with LPS (C). After the mice were injected with poly I:C for 12 weeks, marked inflammatory cell infiltration with severe destruction of the acini, irregular fibrosis, and fatty changes in the pancreas were observed. In contrast, mild inflammatory cell infiltration with slight interstitial edema was observed in mice treated with LPS (original magnification ×100). The severity of pancreatitis was scored on a 0 to 4 scale based on a histological scoring system. The histological score for pancreatitis was higher in poly I:C-treated mice than in LPS-treated mice, 3.5 ± 0.2 vs 1.3 ± 0.2, respectively; $^{**}P < 0.01$ (D).

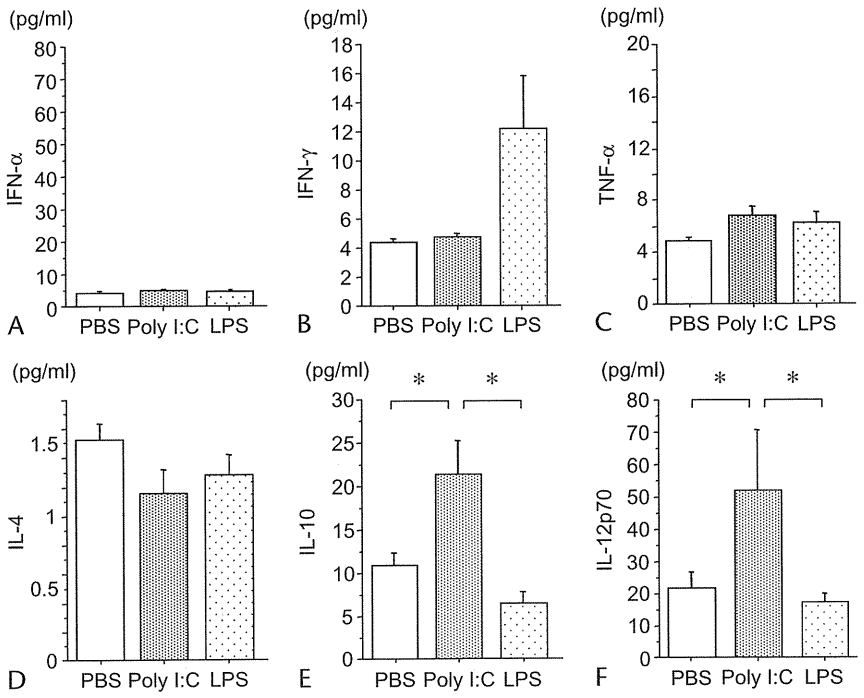

FIGURE 2. Serum cytokine levels in MRL/Mp mice treated with PBS, poly I:C, or LPS for 12 weeks: IFN-α (A), IFN-γ (B), TNF-α (C), IL-4 (D), IL-10 (E), and IL-12p70 (F). There were no significant differences in IFN-α, TNF-α, or IL-4 levels between PBS-treated, poly I:C-treated, and LPS-treated mice. Interferon-γ levels were elevated in LPS-treated mice, but the increase was not significant. Serum levels of IL-10 and IL-12p70 were significantly elevated in poly I:C-treated mice compared with PBS-treated or LPS-treated mice ($^{*}P < 0.05$).

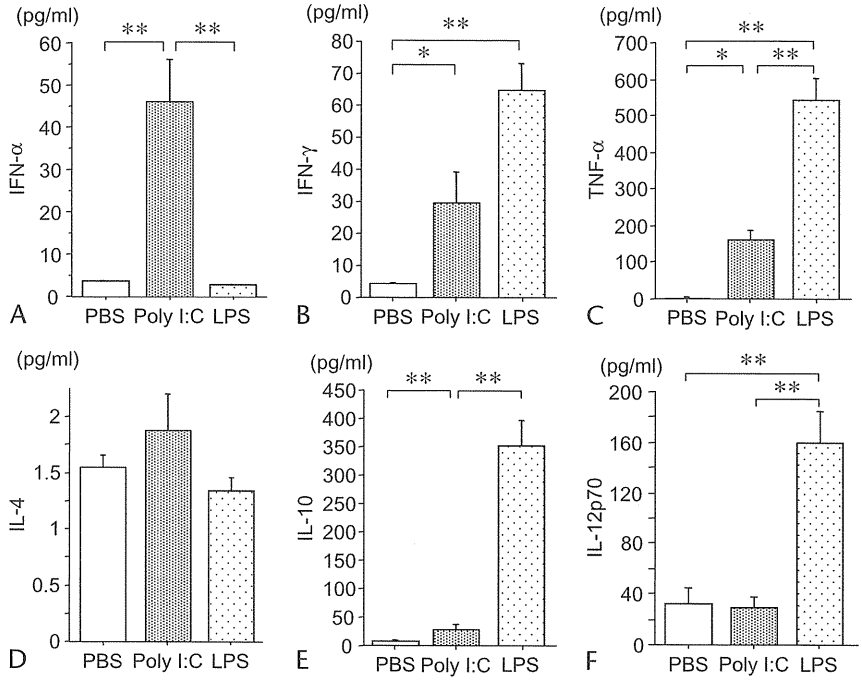

FIGURE 3. Serum cytokine levels in MRL/Mp mice treated with a single injection of PBS, poly I:C, or LPS: IFN-α (A), IFN-γ (B), TNF-α (C), IL-4 (D), IL-10 (E), and IL-12p70 (F). Interferon-α levels were increased in mice treated with poly I:C. Interferon-γ and TNF-α levels were elevated in poly I:C-treated and LPS-treated mice. There were no significant differences in IL-4 level between the PBS-treated, poly I:C-treated, and LPS-treated mice. Interleukin 10 levels were elevated in poly I:C-treated and LPS-treated mice. Lipopolysaccharide administration markedly increased IL-12p70 levels compared with PBS or poly I:C injection (*$P < 0.05$, **$P < 0.01$).

markedly increased serum IL-12p70 level compared with administration of PBS or poly I:C (Fig. 3F).

Toll-Like Receptor Gene Expression in the Pancreas

Gene expression of TLRs in pancreatic tissue 3 hours after administration of TLR ligands was evaluated using semiquantitative reverse transcription–PCR. Toll-like receptor 3 mRNA expression was significantly increased in the pancreas of mice treated with poly I:C compared with mice treated with PBS or LPS (Fig. 4A). Lipopolysaccharide injection increased TLR4 mRNA expression, but the increase was not significant compared with that in mice treated with PBS (Fig. 4B). Toll-like receptor 9 mRNA expression was significantly increased in mice treated with poly I:C compared with mice treated with PBS or poly I:C (Fig. 4C).

Pancreatitis in IL-10KO Mice

Administration of PBS, poly I:C, or LPS did not induce pancreatitis in C57BL/6 WT mice (Figs. 5A–C). In addition, injection of PBS did not cause any inflammation of the pancreas in IL-10KO mice (Fig. 5D). However, poly I:C and LPS administration both induced pancreatitis associated with marked inflammatory cell infiltration and destruction of the acini in IL-10KO mice (Figs. 5E, F). In addition to infiltration of lymphocytes and plasmacytes, stronger neutrophil infiltration, which infiltrated into even the ductule lumen, and fibrosis were observed in the pancreas of LPS-treated mice. The severity of

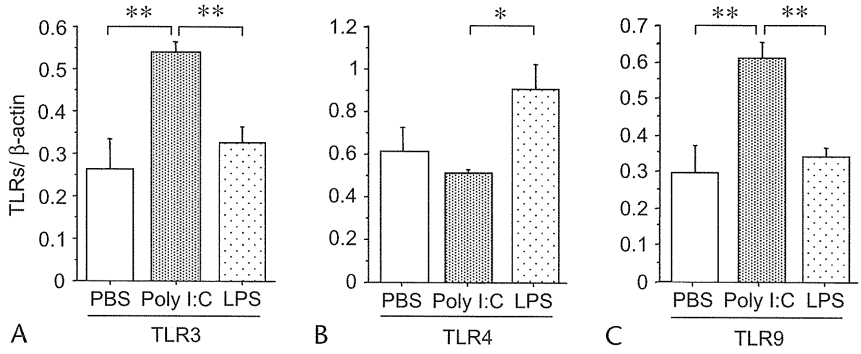

FIGURE 4. Toll-like receptor mRNA expression in pancreatic tissues of MRL/Mp mice treated with single injections of PBS, poly I:C, or LPS: TLR3 (A), TLR4 (B), and TLR9 (C). Toll-like receptor 3 and TLR9 mRNA expression were significantly increased in mice treated with poly I:C. Lipopolysaccharide injection augmented TLR4 mRNA expression (*$P < 0.05$, **$P < 0.01$).

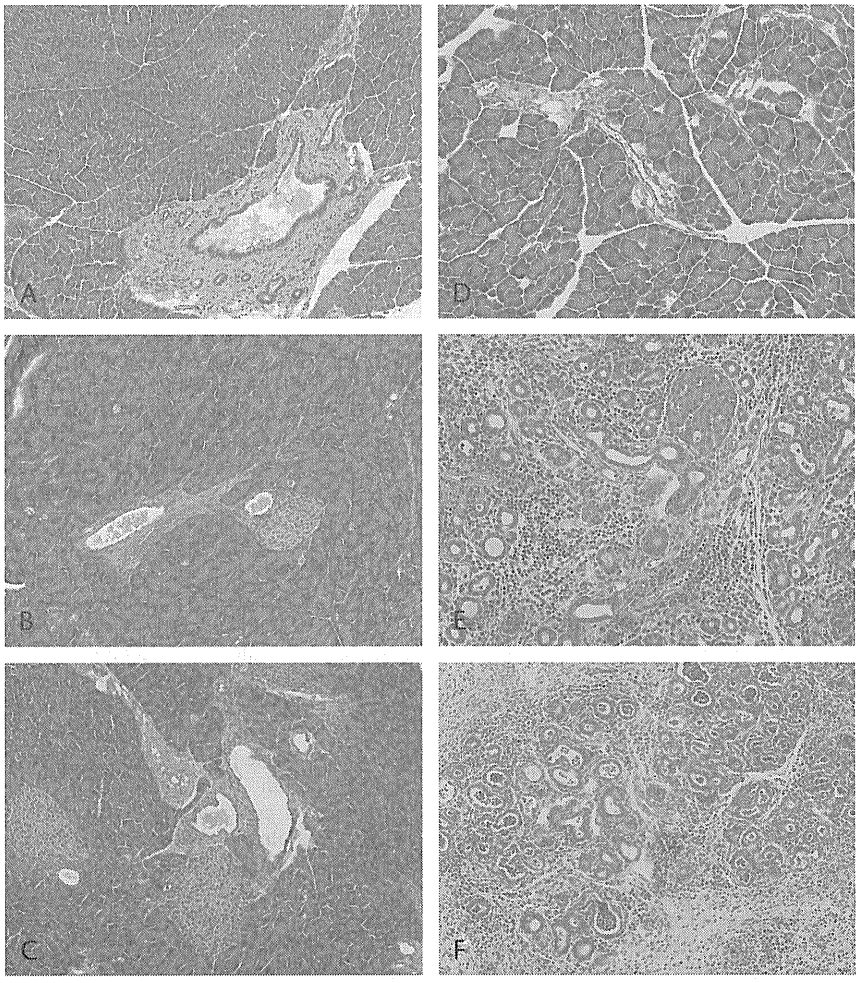

FIGURE 5. Histopathological examination of the pancreas of C57BL/6 WT and IL-10KO mice. Representative pancreatic sections stained with hematoxylin and eosin: 8-week treatment of WT mice with PBS (A), poly I:C (B), or LPS (C); 8-week treatment of IL-10KO mice with PBS (D), poly I:C (E), or LPS (F). Eight-week treatment with PBS, poly I:C, or LPS did not induce pancreatitis in WT mice. Treatment with PBS did not induce pancreatitis in IL-10KO mice. In contrast, administration of poly I:C or LPS induced severe pancreatitis associated with marked inflammatory cell infiltration and destruction of the acini (original magnification ×100).

pancreatitis was greater in LPS-treated mice than in poly I:C-treated mice (4.0 ± 0 vs 3.3 ± 0.2, respectively; Fig. 6).

Serum Cytokine Levels in IL-10KO Mice

Serum levels of IFN-γ were increased in mice treated with poly I:C or LPS compared with control mice treated with PBS, but their increase was not statistically significant (Fig. 7A). Serum levels of TNF-α were significantly higher in mice treated with poly I:C or LPS than in control mice (Fig. 7B). However, serum IL-12p70 concentrations were elevated only in mice treated with LPS (Fig. 7C). To evaluate the effect of TLR3 stimulation on cytokine production, serum cytokine levels were measured 3 hours after a single injection of poly I:C. A single injection of poly I:C caused a marked elevation in TNF-α level in WT and IL-10KO mice (Fig. 8A). In contrast, the poly I:C injection elevated serum IL-12p70 concentration only in IL-10KO mice (Fig. 8B).

DISCUSSION

Significant progress has been made in elucidating the clinical, radiological, serological, and histological features of AIP. However, the pathogenesis of AIP is still unclear because of the difficulty of studying patients with an early stage of the disease. Therefore, we used a murine experimental model of AIP that resembles human AIP to study this issue.

We demonstrated that administration of poly I:C, a TLR3 ligand, accelerated the development of pancreatitis in association with a significant elevation in serum IL-12p70 level. Interleukin 12 is a Th1 cell-inducing cytokine and is produced by antigen-presenting cells such as monocytes/macrophages and dendritic cells in response to TLR stimulation.[24,25] In contrast, there was no significant difference in serum IL-4 level despite the development of pancreatitis. These results are consistent with those of our previous report, which revealed a dominant Th1-type immune response in patients with AIP,[26] and substantiate the similarity between the pathogenesis of murine pancreatitis and human AIP. In addition, the serum concentration of IL-10, an anti-inflammatory cytokine, was increased by poly I:C in the present study, which is consistent with a previous report.[18] Interleukin 10 has been reported to have multiple immunosuppressive effects,[27] and its protective role in acute and chronic pancreatitis has been demonstrated using several experimental

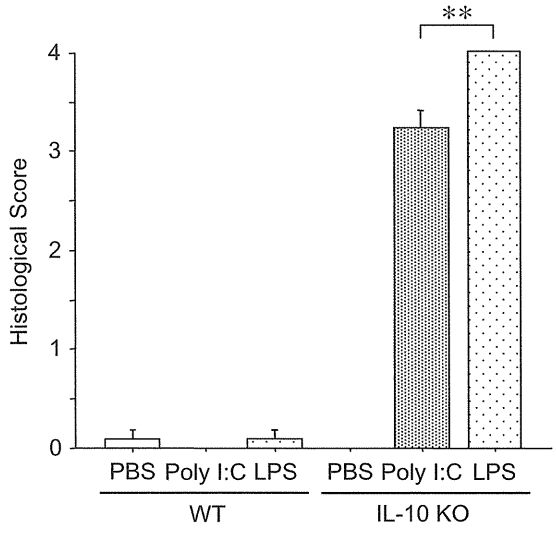

FIGURE 6. Histological pancreatitis scores for WT and IL-10KO mice. The severity of pancreatitis was scored using a scale of 0 to 4, which was based on a histological scoring system. Histological scores for pancreatitis were highest in LPS-treated mice (4.0 ± 0) and were higher than in IL-KO mice (3.3 ± 0.2, $^{**}P < 0.01$).

models.[28,29] As the increases in serum IL-12p70 and IL-10 levels may merely have reflected ongoing pancreatitis and the self-protective responses to inflammation, we next investigated the effects of single injections of various TLR ligands on cytokine production.

Single injections of poly I:C or LPS increased serum INF-γ, TNF-α, and IL-12p70 levels, indicating that poly I:C and LPS treatment caused a Th1 cell-inducing condition; levels of these cytokines were higher in LPS-injected mice than in poly I:C-injected mice. Stimulation of TLR4 activates 2 signaling pathways, myeloid differentiation factor 88–dependent and myeloid differentiation factor 88–independent pathways. Myeloid differentiation factor 88–dependent signaling leads to early activation of NF-κB and activator protein (AP)-1, which initiates the transcription of proinflammatory cytokine genes. Myeloid differentiation factor 88–independent pathway involves a different adaptor molecule, Toll/IL-1 receptor domain-containing adaptor inducing IFN β (TRIF). TRIF-dependent signaling pathway results in late activation of NF-κB and activator protein-1 and upregulation of IFN-regulatory factor 3. Both TLR3 and TLR4 can stimulate TRIF-dependent pathway.[30] Therefore, the variety of serum cytokine production after TLR stimulation may arise from the difference in signaling pathways between TLR3 and TLR4. Furthermore, administration of poly I:C or LPS increased mRNA expression of these TLRs in pancreatic tissues, as was previously observed when pancreatic duct cells were stimulated with poly I:C.[22] This suggests that immune responses induced by TLR stimulation are augmented through a positive feedback mechanism. However, pancreatitis was not induced by LPS administration despite the predominant Th1 condition. Interestingly, administration of LPS markedly increased serum IL-10 concentration in addition to increasing the level of proinflammatory cytokines, as reported previously.[31] It was shown that TLR3 and TLR4 signals induce macrophages and myeloid dendritic cells to produce IL-10 in addition to proinflammatory cytokines.[32] Taken together, it is most likely that the marked increase in IL-10 overwhelmed the effect of Th1 cell-inducing cytokines and prevented the development of pancreatitis in mice treated with LPS. Thus, an imbalance between proinflammatory and anti-inflammatory cytokines may induce murine pancreatitis.

To confirm the preventive role of IL-10 in the development of pancreatitis, we investigated the effect of TLR stimulation in IL-10KO mice. Although administration of poly I:C or LPS did not cause any histological change in the pancreas of C57BL/6 WT mice, pancreatitis developed in the LPS-treated and poly I:C-treated IL-10KO mice. The severity of pancreatitis was greater in mice treated with LPS than in mice treated with poly I:C. Abundant infiltration of neutrophils may be caused by increased production of chemokines in LPS-treated mice because chemokine production was greater in tissue macrophages stimulated with TLR4 than those of TLR3.[33] Such histological difference is consistent with the changes in serum cytokine levels. This clearly shows that IL-10 is necessary for the prevention of pancreatitis.

An association between genetic factors and autoimmune diseases has been reported.[34] Kawa et al[35] reported that the HLA DRB1*0405-DQB1*0401 haplotype is associated with AIP in the Japanese population. An association between adenosine triphosphate-binding cassette, subfamily F gene (ABCF1) and AIP was also reported by this group.[36] Recently, Park et al[37] noted that substitution of aspartic acid with nonaspartic acid at the DQβ1 57 locus is a genetic predictor of relapse in Korean patients with AIP. These reports suggest that genetic factors contribute to the development of AIP. Although MRL/Mp mice are prone to autoimmune disease, their predisposition to it is not as strong as that of MRL/lpr^{+} mice, which have a defective Fas gene and spontaneously develop systemic lupus erythematosus-like diseases.[38,39] An autoimmune-prone genetic background may be closely linked

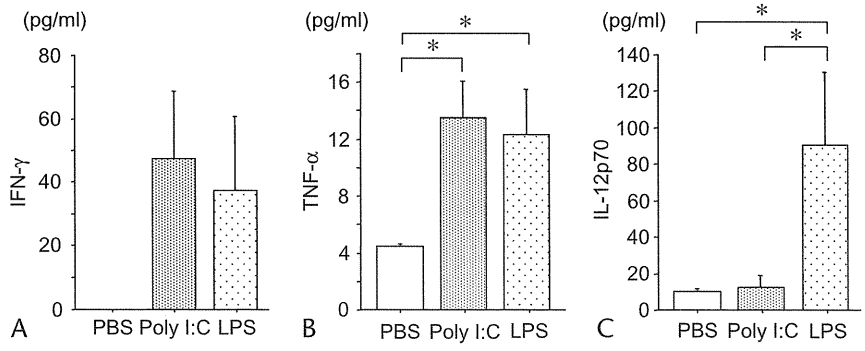

FIGURE 7. Serum cytokine levels in IL-10KO mice treated with PBS, poly I:C, or LPS for 8 weeks: IFN-γ (A), TNF-α (B), and IL-12p70 (C). Although the increase in IFN-γ levels was not significant, TNF-α levels increased in mice treated with poly I:C or LPS ($^{*}P < 0.05$). Interleukin 12p70 levels were elevated only in mice treated with LPS ($^{*}P < 0.05$).

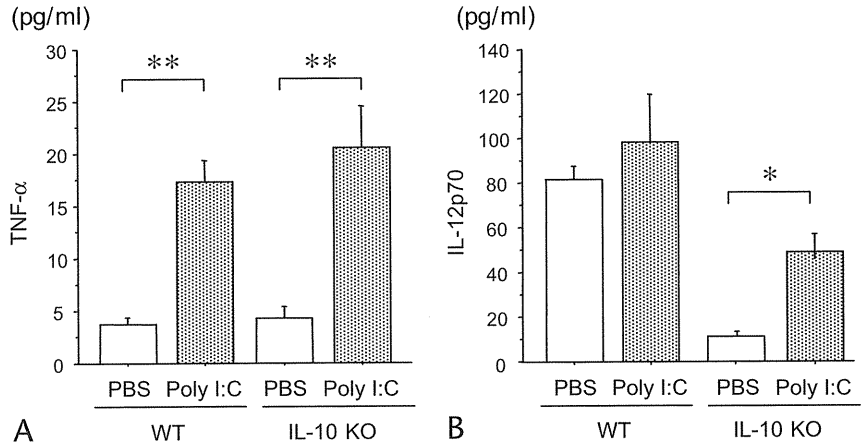

FIGURE 8. Serum cytokine levels in IL-10KO mice treated with single injections of PBS, poly I:C, or LPS: TNF-α (A) and IL-12p70 (B). Tumor necrosis factor-α levels were elevated in WT and IL-KO mice after a single injection of poly I:C. However, an elevated IL-12p70 level was observed only in IL-10KO mice that received a single injection of poly I:C (*$P < 0.05$, **$P < 0.01$).

to the spontaneous development of pancreatitis in MRL/Mp mice. Furthermore, the development of pancreatitis in the IL-10-deficient condition suggests that an IL-10 gene polymorphism may be associated with susceptibility to this disease.

Polyinosinic polycytidylic acid is a double-stranded RNA and is detected by TLR3. In addition to constituting the genome of 1 class of viruses, double-stranded RNAs are also generated during the lifecycle of most other viruses. Viral components may trigger autoimmune diseases.[40] It is believed that during viral infections, pathogen recognition and subsequent induction of adaptive immune responses interfere with the control of self-tolerance in susceptible individuals. Therefore, pattern-recognition receptors that bind pathogen-associated molecular patterns may stimulate both host defense and, under certain circumstances, autoimmune activity. Indeed, it has been shown that the RNA virus Coxsackievirus B4, a prevalent human pathogen associated with pancreatitis, autoimmune diabetes, and myocarditis, induces TLR3 signaling.[41] In addition to TLR3 and TLR4 stimulation, CpG-DNA stimulation of TLR9 induces pancreatitis in IL-10KO mice (unpublished data). Therefore, although exposure to various pathogens, including bacteria and viruses, may cause transient inflammation in the pancreas via TLR signaling, repeated stimulation of TLRs could cause sustained immune responses that result in the development of AIP in genetically susceptible individuals.

In conclusion, we demonstrated that repeated stimulation of innate immunity induced a cytokine imbalance, resulting in autoimmunity. The absence of IL-10 also rendered mice susceptible to pancreatitis in the presence of TLR stimulation. Further studies on environmental and genetic factors such as IL-10 gene polymorphisms are required to elucidate the pathogenesis of AIP.

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;6:688–698.
2. 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;40:1561–1568.
3. Okazaki K, Chiba T. Autoimmune related pancreatitis. *Gut.* 2005;40:1–4.
4. Pickartz T, Mayerle J, Lerch MM. Autoimmune pancreatitis. *Nat Clin Pract Gastroenterol Hepatol.* 2007;4:314–323.
5. Uchida K, Okazaki K, Konishi Y, et al. Clinical analysis of autoimmune-related pancreatitis. *Am J Gastroenterol.* 2000;95: 2788–2794.
6. Okazaki K, Uchida K, Chiba T. Recent concept of autoimmune-related pancreatitis. *J Gastroenterol.* 2001;36:293–302.
7. Nishimori I, Miyaji E, Morimoto K, et al. Serum antibodies to carbonic anhydrase IV in patients with autoimmune pancreatitis. *Gut.* 2005;54: 274–281.
8. Asada M, Nishio A, Uchida K, et al. Identification of a novel autoantibody against pancreatic secretory trypsin inhibitor in patients with autoimmune pancreatitis. *Pancreas.* 2006;33:20–26.
9. Okazaki K, Kawa S, Kamisawa T, et al. Japanese clinical guidelines for autoimmune pancreatitis. *Pancreas.* 2009;38:849–866.
10. Otsuki M, Chung JB, Okazaki K, et al. Asian diagnostic criteria for autoimmune pancreatitis: consensus of the Japan-Korea Symposium on Autoimmune Pancreatitis. *J Gastroenterol.* 2008;43:403–408.
11. Chari ST, Smyrk TC, Levy MJ, et al. Diagnosis of autoimmune pancreatitis: the Mayo Clinic experience. *Clin Gastroenterol Hepatol.* 2006;4:1010–1016.
12. Zen Y, Fujii T, Harada K, et al. Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. *Hepatology.* 2007;45:1538–1546.
13. Kawa S, Kitahara K, Hamano H, et al. A novel immunoglobulin-immunoglobulin interaction in autoimmunity. *PLoS One.* 2008;3:e1637.
14. 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.
15. 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;93:8558–8562.
16. Tsubata R, Tsubata T, Hiai H, et al. Autoimmune disease of exocrine organs in immunodeficient alymphoplasia mice: a spontaneous model of Sjögren's syndrome. *Eur H Immunol.* 1996;26:2742–2748.
17. Valance BA, Hewlett BR, Snider DP, et al. T cell-mediated exocrine pancreatic damage in major histocompatibility complex class II-deficient mice. *Gastroenterology.* 1998;115:978–987.
18. 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.
19. Davidson TS, Longnecker DS, Hickey WF. An experimental model of autoimmune pancreatitis in the rat. *Am J Pathol.* 2005;166:729–736.
20. Meagher C, Tang Q, Fife BT, et al. Spontaneous development of a pancreatic exocrine disease in CD28-deficient NOD mice. *J Immunol.* 2008;180:7793–7803.
21. Asada M, Nishio A, Akamatsu T, et al. Analysis of humoral immune

response in experimental autoimmune pancreatitis in mice. *Pancreas*. 2010;39:224–231.
22. 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;217:175–184.
23. Takeda K, Akira S. Toll-like receptors in innate immunity. *Int Immunol*. 2005;17:1–14.
24. Kaiko GE, Horvat JC, Beagley KW, et al. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? *Immunology*. 2008;123:326–338.
25. Li M, Zhou Y, Feng G, et al. The critical role of toll-like receptor signaling pathways in the induction and progression of autoimmune diseases. *Curr Mol Med*. 2009;9:365–374.
26. Okazaki K, Uchida K, Ohana M, et al. Autoimmune-related pancreatitis is associated with autoantibodies and a Th1/Th2-type cellular immune response. *Gastroenterology*. 2000;118:573–581.
27. Mosmann TR. Properties and functions of interleukin-10. *Adv Immunol*. 1994;56:1–26.
28. Van Laethem JL, Marchant A, Delvaux A, et al. Interleukin 10 prevents necrosis in murine experimental acute pancreatitis. *Gastroenterology*. 1995;108:1917–1922.
29. Demols A, Van Laethem JL, Quertinmont E, et al. Endogenous interleukin-10 modulates fibrosis and regeneration in experimental chronic pancreatitis. *Am J Physiol Gastrointest Liver Physiol*. 2002;282:G1105–G1112.
30. Kaisho T, Akira S. Toll-like receptor function and signaling. *J Allergy Clin Immunol*. 2006;117:979–987.
31. Moore KW, O'Garra A, de Waal MR, et al. Interleukin-10. *Ann Rev Immunol*. 1993;11:165–190.
32. Boonstra A, Rajsbaum R, Holman M, et al. Macrophages and myeloid dendritic cells, but not plasmacytoid dendritic cells, produce IL-10 in response to MyD88- and TRIF-dependent TLR signals, and TLR-independent signals. *J Immunol*. 2006;177:7551–7558.
33. Filippo KD, Henderson RB, Laschinger M, et al. Neutrophil chemokines KC and macrophage-inflammatory protein-2 are newly synthesized by tissue macrophages using distinct TLR signaling pathways. *J Immunol*. 2008;180:4308–4315.
34. Luppi P, Rossiello MR, Faas S, et al. Genetic background and environment contribute synergistically to the onset of autoimmune diseases. *J Mol Med*. 1995;73:381–393.
35. Kawa S, Ota M, Yoshizawa K, et al. HLA DRB1*405-DQB1*401 haplotype is associated with autoimmune pancreatitis in the Japanese population. *Gastroenterology*. 2002;122:1264–1269.
36. 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.
37. Park do H, Kim MH, Oh HB, et al. Substitution of aspartic acid at position 57 of the DQbeta1 affects relapse of autoimmune pancreatitis. *Gastroenterology*. 2008;134:440–446.
38. Andrews BS, Eisenberg RA, Theofilopoulos AN, et al. Spontaneous murine lupus-like syndromes. Clinical and immunological manifestations in several strains. *J Exp Med*. 1978;148:1198–1215.
39. Theofilopoulos AN, Dixon FJ. Murine models of systemic lupus erythematosus. *Adv Immunol*. 1985;37:269–390.
40. Older SA, Battafarano DF, Enzenauer RJ, et al. Can immunization precipitate connective tissue disease? Report of five cases of systemic lupus erythematosus and review of literature. *Semin Arthritis Rheum*. 1999;29:131–139.
41. Richer MJ, Lavalléé DJ, Shanina I, et al. Toll-like receptor 3 signaling on macrophages is required for survival following coxsakievirus B4 infection. *Plos ONE*. 2009;4:e4127.

Visual Field Deficit: A Rare Initial Symptom of Autoimmune Pancreatitis

Kensuke Takuma[1], Terumi Kamisawa[1], Taku Tabata[1], Yosihiko Inaba[1], Naoto Egawa[1], Noriko Ozaki[2], Shin-ichiro Horiguchi[3] and Sanae Takuma[4]

Abstract

An autoimmune pancreatitis (AIP) patient with metachronous and multiple extrapancreatic lesions is reported. Initial symptoms were proptosis, oculomotor deficits, and a visual field deficit of the left eye, and swelling of bilateral lacrimal glands. Swelling of the right salivary gland and elevated serum levels of hepatobiliary enzymes were detected. AIP associated with IgG4-related orbital pseudotumor, IgG4-related sclerosing dacryoadenitis and sialadenitis, and IgG4-related sclerosing cholangitis was diagnosed. All symptoms and lesions improved with steroid therapy. Although an orbital pseudotumor is a rare extrapancreatic lesion of AIP, we should know that AIP patients may describe unusual symptoms such as abnormal visual field.

Key words: autoimmune pancreatitis, IgG4, orbital pseudotumor, sclerosing sialadenitis, sclerosing dacryoadenitis

(Intern Med 50: 887-891, 2011)
(DOI: 10.2169/internalmedicine.50.5012)

Introduction

Autoimmune pancreatitis (AIP) is a peculiar type of pancreatitis of presumed autoimmune etiology. It is characterized clinically by a preponderance of elderly males, jaundice as a frequent initial symptom, and responsiveness to steroid therapy; serologically by elevation of serum IgG or IgG4 levels; radiologically by enlargement of the pancreas and irregular narrowing of the main pancreatic duct; and histopathologically by dense fibrosis with lymphoplasmacytic infiltration in the pancreas (1, 2). Other prominent features of this disease involve a variety of extrapancreatic complications (1-3).

We found dense fibrosis with abundant infiltration of T lymphocytes and IgG4-positive plasma cells and obliterative phlebitis in extrapancreatic lesions associated with AIP, such as sclerosing cholangitis, sclerosing cholecystitis, sclerosing sialadenitis, and retroperitoneal fibrosis. Furthermore, we also found dense infiltration of IgG4-positive plasma cells and T lymphocytes in various organs of AIP patients, such as the periportal area of the liver, gastric mucosa, colonic mucosa, dermis, lymph nodes, and bone marrow (1, 2, 4, 5). Therefore, we proposed the existence of a novel clinicopathological entity, "IgG4-related sclerosing disease" (1, 2, 4), which is a systemic disease characterized by extensive IgG4-positive plasma cell and T lymphocyte infiltration of various organs. In some cases, only 1 or 2 organs are clinically involved, while in others, 3 or 4 organs are affected (1, 2).

From this point of view, both AIP and the extrapancreatic lesions of AIP may occur randomly. We report an AIP patient who developed a visual field deficit of the left eye and swelling of bilateral salivary glands, which were metachronously associated with sclerosing sialadenitis and sclerosing cholangitis.

Case Report

A 76-year-old man noticed swelling in the left upper eyelid in October 2007 and visited another hospital. The patient was suspected to be having an allergic reaction at the initial

[1]Department of Internal Medicine, Tokyo Metropolitan Komagome Hospital, Japan, [2]Department of Ophthalmology, Tokyo Metropolitan Komagome Hospital, Japan, [3]Department of Pathology, Tokyo Metropolitan Komagome Hospital, Japan and [4]Department of Ophthalmology, Omori Medical Center, Toho University School of Medicine, Japan
Received for publication December 15, 2010; Accepted for publication January 12, 2011
Correspondence to Dr. Terumi Kamisawa, kamisawa@cick.jp

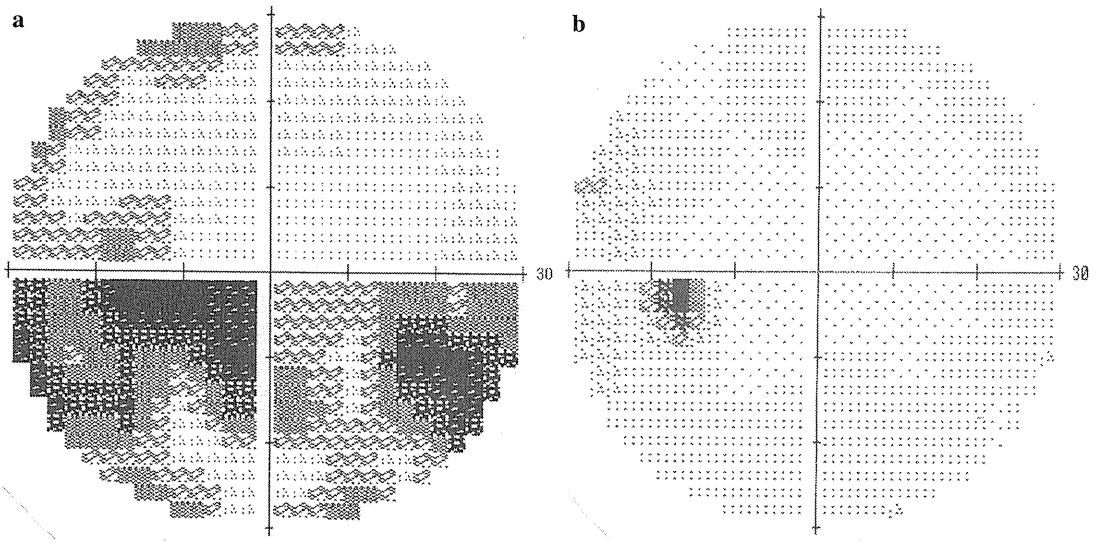

Figure 1. (a) Visual field examination shows lower visual field deficit of the left eye before steroid therapy. (b) The visual field deficit improved significantly after steroid therapy.

visit, but the eyelid swelling gradually increased. In April 2009, the patient felt proptosis, oculomotor deficits, and a visual field deficit of the left eye, as well as swelling of bilateral lacrimal glands. The left orbital lesion was biopsied on suspicion of malignant tumor, but there was no malignancy, and he was followed without treatment. In June 2009, swelling of the right salivary glands and elevated serum levels of hepatobiliary enzymes were detected. He was referred to our hospital for further examination.

The physical findings on admission included proptosis and oculomotor deficits of the left eye and painless swelling of bilateral lacrimal glands and the right salivary gland. No superficial lymphadenopathy, hepatomegaly, or splenomegaly was noted. The visual acuity of the right eye was 1.2, left eye was 0.2. The ophthalmologic examinations showed a lower visual field deficit (Humphrey Field Analyzer, Carl Zeiss Meditec, Dublin, CA) (Fig. 1a) and an omnidirectional ocular motility disorder of the left eye.

Laboratory examinations showed elevation of serum hepatobiliary enzyme levels: alanine aminotransferase, 307 (normal range, 5-40) IU/L, aspartate aminotransferase, 319 (5-35) IU/L, alkaline phosphatase, 1191 (80-260) IU/L, γ-glutamyl transpeptidase, 988 (5-70) IU/L, lactic dehydrogenase, 205 (115-245) IU/L, and leucine aminopeptidase 193 (<170) IU/L. Hepatitis B surface antigen and antibody to hepatic C virus were negative. Immunologically, the serum IgG level was 4,135 (<1,700) mg/dL and IgG4 level was 2,490 (<135) mg/dL; antinuclear antibody(ANA) was positive (×80). Anti-Ro antibody (SS-A), anti-La antibody (SS-B), anti-mitochondrial antibody, and anti-smooth muscle antibody were all negative.

Head magnetic resonance imaging (MRI) showed proptosis of the left eye due to an orbital tumor and swelling of bilateral lacrimal glands (Fig. 2a, b). There were no sites of involvement on head MRI. Re-examination of the biopsied piece of the orbital tumor in the previous hospital revealed abundant infiltration of IgG4-positive plasma cells and lymphocytes, and focally storiform-like fibrosis (Fig. 3a-d). There were no findings of obliterative phlebitis or MALT-lymphoma.

Abdominal computed tomography (CT) and MRI revealed diffuse pancreatic enlargement and mild thickening of the gallbladder and bile duct wall (Fig. 4a). An endoscopic retrograde cholangiopancreatography indicated diffusely irregular narrowing of the main pancreatic duct and stenosis of the lower bile duct. The patient was diagnosed as having AIP according to the Japanese Clinical Diagnostic Criteria for Autoimmune Pancreatitis 2006 (6).

Percutaneous liver biopsy performed for liver dysfunction after admission to our hospital revealed dense infiltration of lymphocytes and IgG4-positive plasma cells and mild fibrosis in the periportal area of the liver (Fig. 5).

The patient was diagnosed as having AIP associated with IgG4-related sclerosing dacryoadenitis, orbital pseudotumor, IgG4-related sialadenitis, and IgG4-related sclerosing cholangitis. He was begun on treatment for systemic IgG4-related disease with 30 mg prednisolone daily for 2 weeks. The dose was tapered by 2.5-5 mg every two weeks. Four weeks after starting steroid therapy, findings on abdominal CT/MRI (Fig. 4b) and blood tests improved. The visual acuity, lower visual field deficit, and omnidirectional ocular motility disorder of the left eye also improved significantly (Fig. 1b), along with improvement of proptosis and lacrimal gland swelling (Fig. 2c, d).

Discussion

AIP patients frequently have significantly elevated serum IgG4 levels and various extrapancreatic lesions. AIP and its extrapancreatic lesions show similar histopathological findings and good responsiveness to steroid therapy. Currently, they are recognized as organs clinically involved in IgG4-

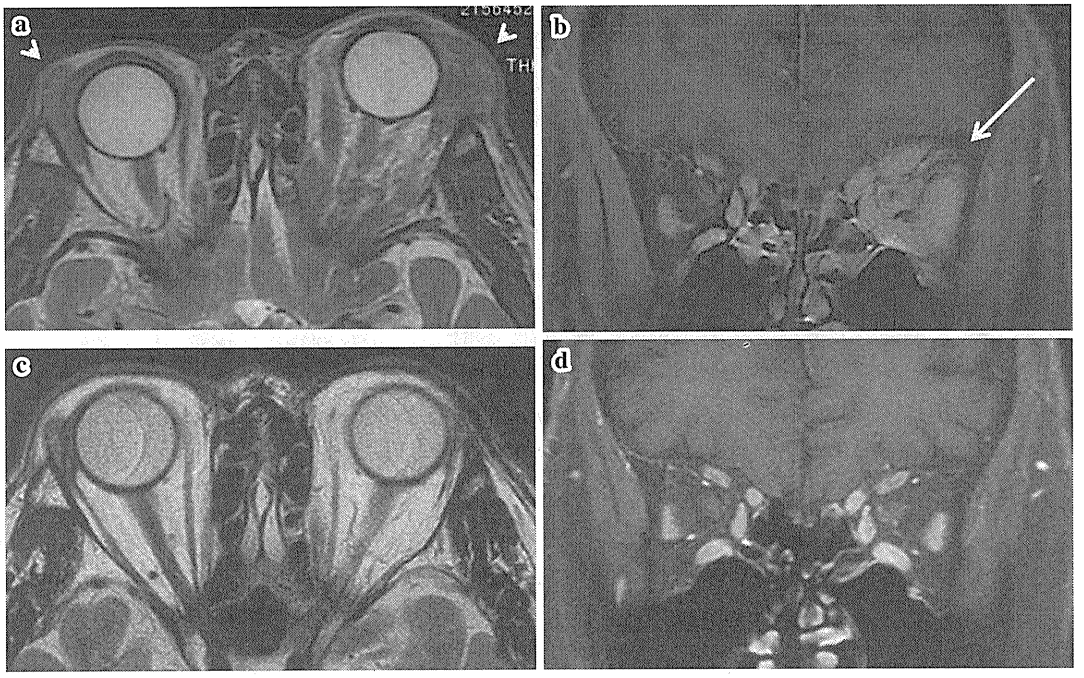

Figure 2. **Magnetic resonance imaging of orbit reveals swelling of bilateral lacrimal grands (short arrow) (a, axial T2-weighted imaging) and an orbital floor pseudotumor (long arrow) (b, coronal T1-weighted fat-suppressed imaging). (c, d) These lesions improved markedly after steroid therapy.**

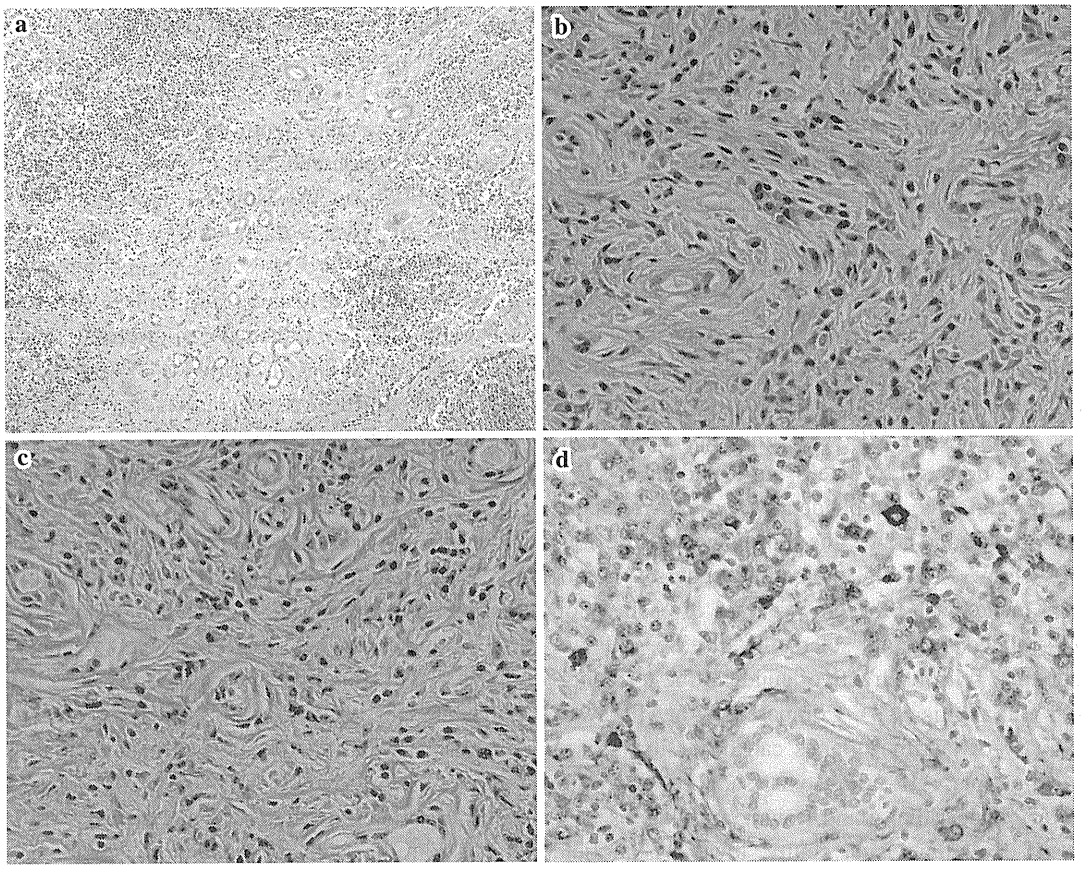

Figure 3. **Histology of the biopsied orbital tumor showing lymphoplasmacytic infiltration and focally storiform-like fibrosis [(a) lower power view, Hematoxylin and Eosin staining; (b) high power view, Hematoxylin and Eosin staining; (c) Elastica Van Gienson staining]. (d) Immunohistochemically, abundant infiltration of IgG4-positive plasma cells was detected (IgG4-immunostaining).**

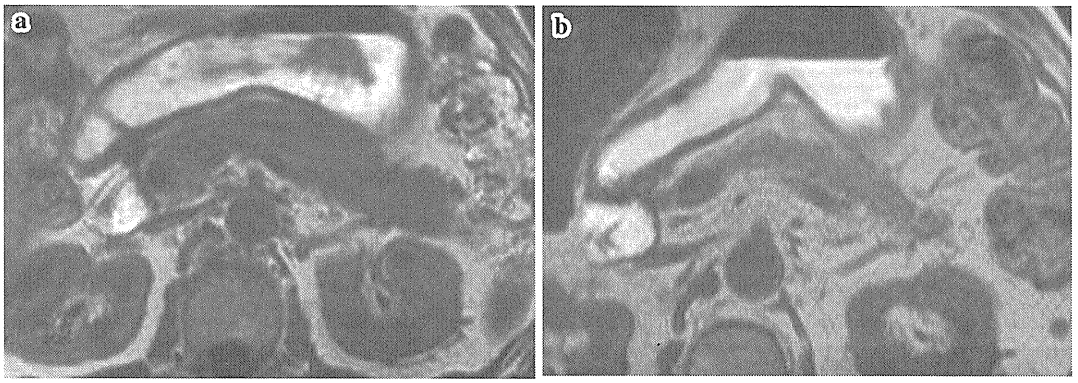

Figure 4. (a) Magnetic resonance imaging showing diffuse enlargement of the pancreas. (b) The pancreatic enlargement improved after steroid therapy.

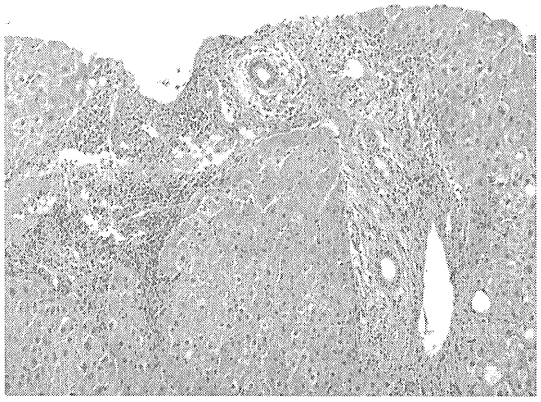

Figure 5. Histology of liver biopsy showing dense lymphoplasmacytic infiltration and mild fibrosis in the periportal area of the liver (Hematoxylin and Eosin staining).

related systemic sclerosing disease. In some cases, only 1 or 2 organs are clinically involved, while in others, 3 or 4 organs are affected (1, 2). Cases with significantly higher serum IgG4 levels, as in this patient, show higher AIP activity and frequently have associated extrapancreatic lesions (7). Extrapancreatic lesions with AIP sometimes appear metachronously. In our previous study, sclerosing sialadenitis, swelling of the lacrimal glands, lymphadenopathy, and retroperitoneal fibrosis were found to be the extrapancreatic lesions preceding AIP, while sclerosing cholangitis occurs synchronously (8). It is unclear why the onset period of each lesion differs in IgG4-related systemic sclerosing disease. AIP occurs most frequently with obstructive jaundice due to associated sclerosing cholangitis (1-3). Compared with AIP, swelling of the salivary or lacrimal glands can be easily noticed even without symptoms. AIP might exist subclinically when preceding salivary or lacrimal gland lesions are diagnosed.

In the present case, the visual acuity, lower visual field deficit, and omnidirectional ocular motility disorder of the left eye improved along with improvement of proptosis. There was no abnormality of the optic nerve and brain on head MRI. Therefore, these ophthalmic symptoms would have been caused by an orbital pseudotumor. Orbital pseudotumor is an idiopathic, benign, inflammatory condition that accounts for approximately 10% of all orbital mass lesions (9, 10). The etiology of orbital pseudotumor is unknown (11). The presentation may be acute or subacute and may occasionally exhibit chronic progression. Orbital pseudotumor may be unifocal or diffuse and may affect any part of the orbit (12). It is usually unilateral, but it may occasionally be bilateral. Chirapapaisan et al. reported that the presenting symptoms included proptosis (80%), oculomotor deficits (61%), pain (51%), lid swelling or a mass (45%), ptosis (25%), and chemosis (18%) in 49 patients with orbital pseudotumor (13). Some patients with orbital pseudotumor may have decreased visual acuity due to optic nerve compression (14). Orbital pseudotumor is sometimes difficult to differentiate from MALT-lymphoma, there was no finding of lymphoma in the biopsy specimen of this case. Multifocal fibrosclerosis is an uncommon fibroproliferative systemic disorder with multiple manifestations, including retroperitoneal fibrosis, sclerosing cholangitis, and salivary gland fibrosis. There are some reports that multifocal fibrosclerosis was complicated by fibrotic orbital pseudotumor (15-17). We have reported a close relationship between AIP and multifocal fibrosclerosis (5). Some orbital pseudotumors including the present case appear to be orbital lesions involved in IgG4-related systemic disease.

Lacrimal gland swelling is also a rare extrapancreatic lesion of AIP. Lacrimal gland swelling was detected in 3.6% of 56 cases in our study (8). Hamano et al (18) reported that lacrimal gland swelling was detected in 8 (12.5%) of 64 AIP patients, and 6 of them had salivary gland swelling. Recently, it was reported that serum IgG4 levels were elevated, and abundant infiltration of IgG4-positive plasma cells with fibrosis was detected in the lacrimal glands in patients with Mikulicz's disease (19, 20), which is a unique condition that refers to bilateral, painless, symmetrical swelling of the lacrimal, parotid, and submandibular glands (21). Mikulicz's disease is currently recognized as the lacrimal and salivary gland lesions of IgG4-related systemic disease (20).

IgG4-related sclerosing cholangitis is frequently associated with AIP, and the stenosis is usually located in the lower part of the common bile duct (1-3). In cases with stenosis of the lower bile duct, thickening of the bile duct

wall consists of fibrosis with infiltration of IgG4-positive plasma cells that sometimes spread extensively to the upper bile duct (1, 2). In the present patient, infiltration of mononuclear cells, including IgG4-positive plasma cells, and mild fibrosis were observed in the periportal area obtained by liver biopsy.

AIP sometimes develops with various symptoms due to associated extrapancreatic lesions. According to the classification (head and neck, thoracic, hepatic and pancreatobiliary, retroperitoneal, and systemic group) of IgG4-related disease by Zen and Nakanuma (22), the present case would be classified into systemic group with multiple lesions not restricted to 1 area. However, a visual field deficit due to orbital pseudotumor appears to be quite rare. An AIP patient who developed a visual field deficit of the left eye and swelling of bilateral salivary glands, which was metachronously associated with sclerosing sialadenitis and sclerosing cholangitis, was reported.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

This work was partly supported by The Research Committee on Intractable Diseases provided by Ministry of Health, Labour, and Welfare of Japan.

References

1. Kamisawa T, Okamoto A. Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease. J Gastroenterol **41**: 613-625, 2006.
2. Kamisawa T, Takuma K, Tabata T, et al. Autoimmune pancreatitis and IgG4-related sclerosing disease. Nat Rev Gastroenterol Hepatol **7**: 401-409, 2010.
3. Ohara H, Nakazawa T, Sano H, et al. Systemic extrapancreatic lesion associated with autoimmune pancreatitis. Pancreas **31**: 232-237, 2005.
4. Kamisawa T, Funata N, Hayashi Y, et al. A new clinicopathological entity of IgG4-related autoimmune disease. J Gastroenterol **38**: 982-984, 2003.
5. Kamisawa T, Funata N, Hayashi Y, et al. Close relationship between autoimmune pancreatitis and multifocal fibrosclerosis. Gut **52**: 683-687, 2003.
6. Okazaki K, Kawa S, Kamisawa T, et al. Clinical diagnostic criteria of autoimmune pancreatitis: revised proposal. J Gastroenterol **41**: 626-631, 2006.
7. Kamisawa T, Imai M, Egawa N, Tsuruta K, Okamoto A. Serum IgG4 levels and extrapancreatic lesions in autoimmune pancreatitis. Eur J Gastroenterol **20**: 1167-1170, 2008.
8. Takuma K, Kamisawa T, Anjiki H, et al. Metachronous extrapancreatic lesions in autoimmune pancreatitis. Intern Med **49**: 529-533, 2010.
9. Swamy BN, McCluskey P, Nemet A, et al. Idiopatic orbital inflammatory syndrome: clinical feature and treatment outcome. Br J Opthalmol **91**: 1667-1670, 2007.
10. Shields JA, Shields CL, Scartozzi R. Survey of 1264 patients with orbital tumors and simulating lesions: the 2002 Montgomery Lecture, part 1. Ophthalmology **111**: 997-1008, 2004.
11. Mombaerts I, Goldschmeding R, Schlingemann RO, et al. What is orbital pseudotumor? Surv Ophthalmol **41**: 66-78, 1996.
12. Smitt MC, Donaldson SS. Radiation therapy for benign disease of orbit. Semin Radiat Oncol **9**: 179-189, 1999.
13. Chirapapaisan N, Chuenkongkaew W, Pornpanich K, Vangveeravong S. Orbital pseudotumor: clinical features and outcomes. Asian Pac J Allergy Immunol **25**: 215-218, 2007.
14. Yan J, Wu Z, Li Y. The differentiation of idiopathic inflammatory pseudotumor from lymphoid tumors of orbit: analysis of 319 cases. Orbit **23**: 245-254, 2004.
15. Aylward GW, Sullivan TJ, Garner A, et al. Orbital involvement in multifocal fibrosclerosis. Br J Ophthalmol **79**: 246-249, 1995.
16. Levine MR, Kaye L, Mair S, et al. Multifocal fibrosclerosis. Report of a case of bilateral idiopathic sclerosing pseudotumor and retroperitoneal fibrosis. Arch Ophthalmol **111**: 841-843, 1993.
17. Rootman J, McCarthy M, White V, et al. Idiopathic sclerosing inflammation of the orbit. A distinct clinicopathologic entity. Ophthalmology **101**: 578-584, 1994.
18. Hamano H, Arakura T, Muraki T, et al. Prevalence and distribution of extrapancreatic lesions complicating autoimmune pancreatitis. J Gastroenterol **41**: 1197-1205, 2006.
19. Takahashi M, Kawano M, Zen Y, et al. IgG4-related chronic sclerosing dacryadenitis. Arch Ophthalmol **125**: 1575-1578, 2007.
20. Yamamoto M, Harada S, Ohara M, et al. Clinical and pathological differences between Mikulicz's disease and Sjögren's syndrome. Rheumatology **44**: 227-234, 2005.
21. Schaffer AJ, Jacobsen AW. Mikulicz's syndrome: a report of ten cases. Am J Dis Child **34**: 327-346, 1927.
22. Zen Y, Nakanuma Y. IgG4-related disease. A cross-sectional study of 114 cases. Am J Surg Pathol **34**: 1812-1819, 2010.

J Gastroenterol (2011) 46:108–116
DOI 10.1007/s00535-010-0317-2

Serum IgG4-negative autoimmune pancreatitis

Terumi Kamisawa · Kensuke Takuma · Taku Tabata · Yoshihiko Inaba · Naoto Egawa · Koji Tsuruta · Tsunekazu Hishima · Tsuneo Sasaki · Takao Itoi

Received: 22 June 2010 / Accepted: 12 July 2010 / Published online: 8 September 2010

Abstract
Background Autoimmune pancreatitis (AIP) is considered to be a pancreatic lesion of IgG4-related systemic disease, but about 20% of AIP patients do not have elevated serum IgG4 levels. This study aimed to clarify the pathophysiology of AIP patients without elevated serum IgG4 levels.
Methods Fifty-eight AIP patients were divided into 2 groups: those with elevated serum IgG4 levels (>135 mg/dl; SIgG4-positive AIP) and those without (SIgG4-negative AIP). The 2 groups' clinical, serological, radiological, and histological findings, as well as salivary and lacrimal gland function, were compared.
Results Serum IgG4 levels were elevated in 45 AIP patients and normal in 13 patients. In SIgG4-negative AIP patients, the female ratio tended to be high; obstructive jaundice was less likely; abdominal pain and acute pancreatitis were more likely; segmental swelling of the pancreatic body and/or tail was more common; sclerosing extrapancreatic lesions, salivary and lacrimal gland dysfunction, and abundant infiltration of IgG4-positive plasma cells in the gastric mucosa were less likely; and conservative follow-up was sometimes implemented. Histological examination of the pancreas of SIgG4-negative AIP showed lymphoplasmacytic sclerosing pancreatitis (LPSP) rather confined to the pancreas ($n = 4$), inadequate material ($n = 2$), and pancreatic fibrosis showing infiltration of lymphocytes without infiltration of IgG4-positive cells or neutrophils ($n = 2$).
Conclusions Clinicopathological features of SIgG4-negative AIP differed from those of SIgG4-positive AIP. Some SIgG4-negative AIP cases are LPSP rather confined to the pancreas. SIgG4-negative AIP may include idiopathic duct-centric pancreatitis (IDCP) or sclerosing pancreatitis other than LPSP or IDCP, but further studies are needed to clarify this issue.

Keywords Autoimmune pancreatitis · IgG4 · Lymphoplasmacytic sclerosing pancreatitis · Idiopathic duct-centric pancreatitis

T. Kamisawa (✉) · K. Takuma · T. Tabata · Y. Inaba · N. Egawa
Department of Internal Medicine, Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8677, Japan
e-mail: kamisawa@cick.jp

K. Tsuruta
Department of Surgery, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

T. Hishima
Department of Pathology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

T. Sasaki
Department of Chemotherapy, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

T. Itoi
Department of Gastroenterology and Hepatology, Tokyo Medical University, Tokyo, Japan

Introduction

Autoimmune pancreatitis (AIP) is a distinct entity that is being recognized with increasing frequency worldwide. Since it responds dramatically to steroids, an autoimmune process has been implicated in its etiology. The histological pattern of AIP is called lymphoplasmacytic sclerosing pancreatitis (LPSP), which is characterized by dense

infiltration of IgG4-positive plasma cells and T lymphocytes, storiform fibrosis and obliterative phlebitis [1–3]. Since AIP is frequently associated with various sclerosing extrapancreatic lesions, such as sclerosing cholangitis, sclerosing sialadenitis, and retroperitoneal fibrosis, showing similar histological findings to the pancreas, AIP can be considered to be a pancreatic lesion of IgG4-related systemic disease, and its extrapancreatic lesions are clinical manifestations of organs involved in this systemic disease [4–6].

Another characteristic feature of AIP is significant elevation of serum IgG4 levels [1–3]. Hamano et al. [7] reported that the serum IgG4 level was >135 mg/dl in 19 of 20 AIP patients and that an IgG4 cutoff value of 135 mg/dl resulted in high accuracy (97%), sensitivity (95%), and specificity (97%) in distinguishing AIP from pancreatic cancer. However, in a recent study, the sensitivity of an elevated serum IgG4 level was 68% [8] to 81% [1].

From retrospective, histological examination of the resected pancreases of patients with mass-forming chronic pancreatitis, American and European pathologists described another unique histological pattern, which they called idiopathic duct-centric pancreatitis (IDCP) [9] or AIP with granulocyte epithelial lesions (GEL) [10]. It is histologically characterized by ductal epithelial granulocytic infiltration leading to ductal damage and obstruction, a feature not seen in LPSP. The lobular infiltrate contains neutrophils. Obliterative phlebitis is uncommon in IDCP, and the tissue does not generally stain for IgG4-positive cells [3, 9–11]. Although the typical clinical features of IDCP have not yet been clarified, typical serological abnormalities seen in AIP are not seen in IDCP [3]. IDCP is sometimes detected in Western countries, but it is uncommon in Japan and Korea [2].

In this study, to clarify the pathophysiology of AIP patients without elevated serum IgG4 levels (SIgG4-negative AIP), clinicopathological differences detected between AIP patients exhibiting elevated serum IgG4 levels (SIgG4-positive AIP) and SIgG4-negative AIP patients were examined.

Patients and methods

Study patients

Serum IgG4 levels were measured by nephelometry using IgG subclass kits (BS-NIA IgG4, Medical & Biological Laboratories, Nagoya, Japan) in 58 AIP patients [43 males and 15 females; average age 63.2 ± 12.9 (mean ± SD) years] before steroid therapy or surgical resection. They were diagnosed as having AIP according to the Asian diagnostic criteria [12] based on radiological, serological and histological findings, and responsiveness to steroid. The serum IgG4 cutoff value was 135 mg/dl, which has been used widely [7]. Serum IgG4 levels were measured at least more than twice in patients without an elevated serum IgG4 level on the first examination.

Pancreatic resection and bypass operation were performed on suspicion of pancreatic cancer in 7 and 4 patients, respectively; steroid therapy was performed finally in 42 patients; and 6 patients have been followed conservatively. Steroid therapy was started at 0.6 mg/kg/day of prednisolone and gradually tapered to a maintenance dose over a period of 3–6 months. To prevent relapse, steroid maintenance therapy (2.5 mg–5 mg/day) was performed for 1–3 years. Relapse of AIP was defined as reappearance of symptoms with the development or reappearance of pancreatic and/or extrapancreatic abnormalities on imaging studies [13, 14].

Clinical, serological, and radiological analysis

The following clinical factors were retrospectively assessed: age at the time of diagnosis; sex ratio; drinking and smoking habits; present or past history of allergic diseases such as acute allergic rhinitis, atopic dermatitis, and bronchial asthma; and treatment. Drinking habit was defined as drinking more than 80 g of alcohol/day for more than 7 years, and smoking habit was defined as smoking more than 20 pack-years (the number of packages of cigarette per day times years of smoking). Acute pancreatitis was diagnosed when both severe upper abdominal pain and elevated serum amylase levels (>3 times normal) were met. Furthermore, SIgG4-negative AIP patients were subdivided into 2 groups according to the degree of serum IgG4 levels: slightly lower SIgG4 patients (IgG4 70–135 mg/dl) and extremely lower SIgG4 patients (IgG4 <70 mg/dl), and clinical features were compared between the 2 groups.

Serologically, serum IgG levels ($n = 58$), autoantibodies ($n = 56$) including antinuclear antigen and rheumatoid factor, serum IgE levels ($n = 30$), peripheral eosinophil count ($n = 49$), and serum amylase levels ($n = 58$) were reviewed.

Radiologically analyzed findings were as follows: enlargement of the pancreas (diffuse/segmental) and extrapancreatic lesions on CT, fluorine-18 fluorodeoxyglucose (FDG) uptake and maximum standardized uptake value (SUV) on FDG positron emission tomography (PET) ($n = 13$) [15, 16], and high signal intensity and apparent diffusion coefficient (ADC) value on diffusion-weighted magnetic resonance imaging (DWI) ($n = 13$) [17]. The presence of 4 extrapancreatic lesions that were detected with a relatively high frequency in AIP patients (sclerosing cholangitis of the hilar or intrahepatic bile duct, sclerosing