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

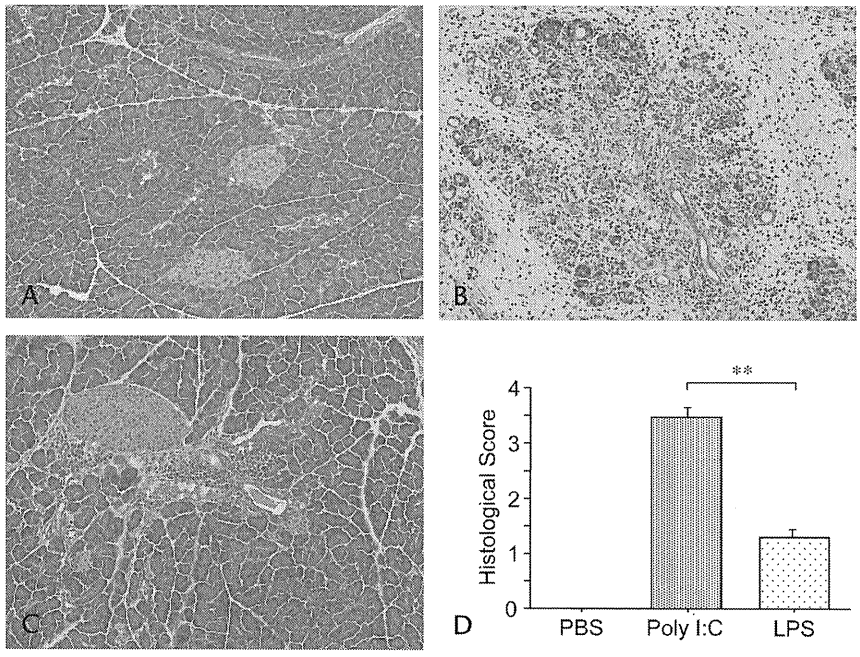


FIGURE 1. Histopathological examination of the pancreas and histological scoring of pancreatitis in MRL/Mp mice. Representative pancreatic sections stained with hematoxylin and eosin: 12-week treatment with PBS (A), 12-week treatment with poly I:C (B), and 12-week treatment with LPS (C). After the mice 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).

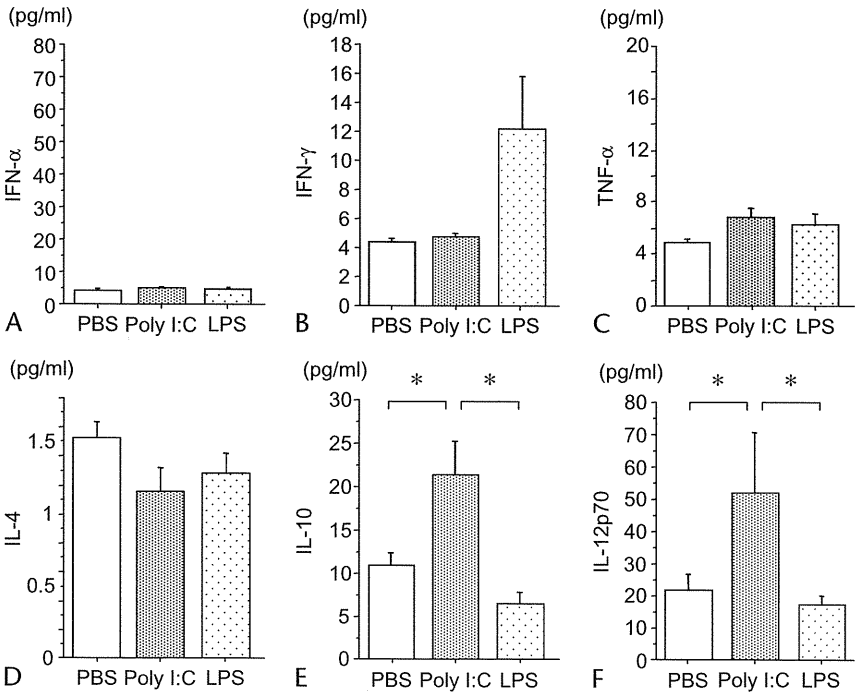


FIGURE 2. Serum cytokine levels in MRL/Mp mice treated with PBS, poly I:C, or LPS for 12 weeks: IFN-α (A), IFN-γ (B), TNF-α (C), IL-4 (D), IL-10 (E), and IL-12p70 (F). There were no significant differences in IFN-α, TNF-α, or IL-4 levels between PBS-treated, poly I:C-treated, and LPS-treated mice. Interferon-γ 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 ($^{*}P < 0.05$).

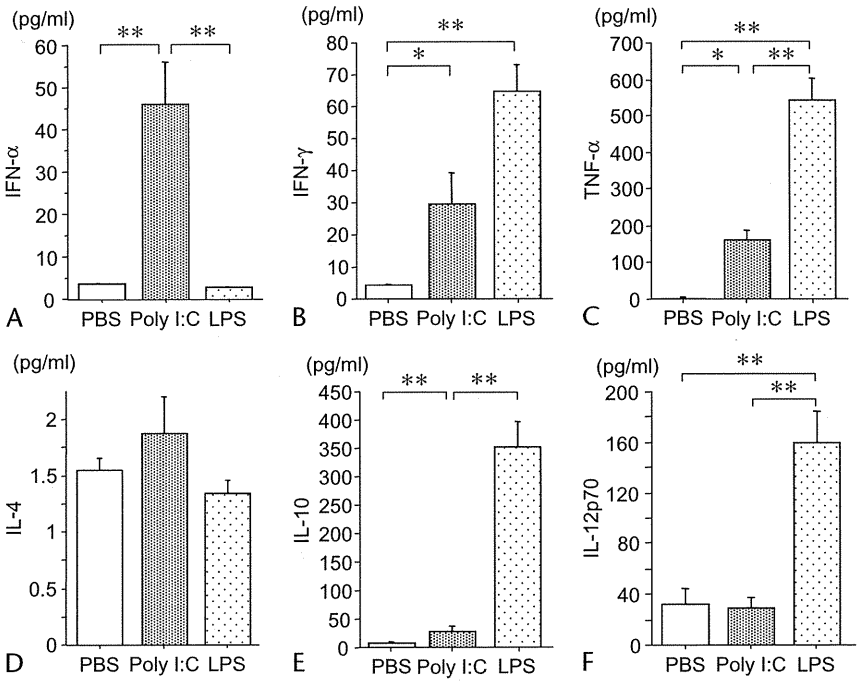


FIGURE 3. Serum cytokine levels in MRL/Mp mice treated with a single injection of PBS, poly I:C, or LPS: IFN-α (A), IFN-γ (B), TNF-α (C), IL-4 (D), IL-10 (E), and IL-12p70 (F). Interferon-α levels were increased in mice treated with poly I:C. Interferon-γ and TNF-α levels were elevated in poly I:C-treated 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

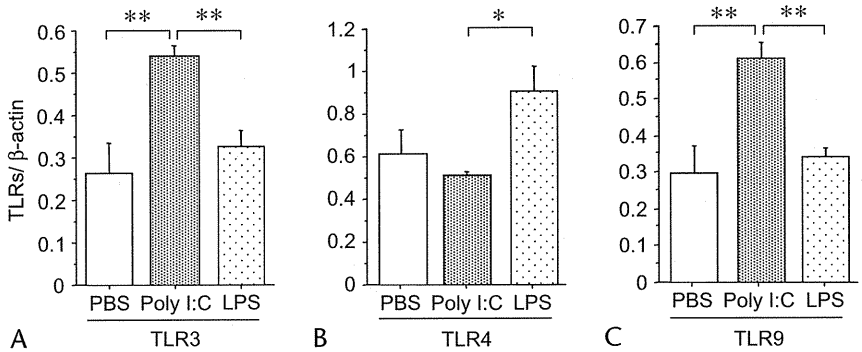


FIGURE 4. Toll-like receptor mRNA expression in pancreatic tissues of MRL/Mp mice treated with single injections of PBS, poly I:C, or LPS: TLR3 (A), TLR4 (B), and TLR9 (C). Toll-like receptor 3 and TLR9 mRNA expression were significantly increased in mice treated with poly I:C. Lipopolysaccharide injection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

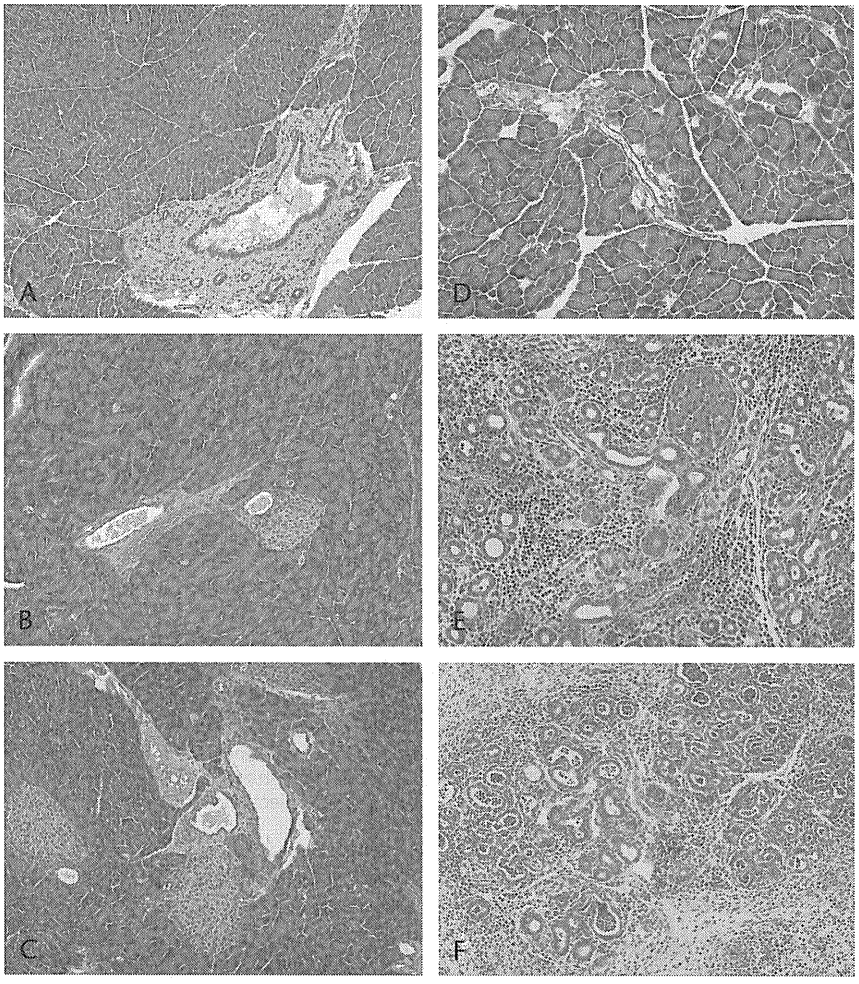


FIGURE 5. Histopathological examination of the pancreas of C57BL/6 WT and IL-10KO mice. Representative pancreatic sections stained with hematoxylin and eosin: 8-week treatment of WT mice with PBS (A), poly I:C (B), or LPS (C); 8-week treatment of IL-10KO mice with PBS (D), poly I:C (E), or LPS (F). Eight-week 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

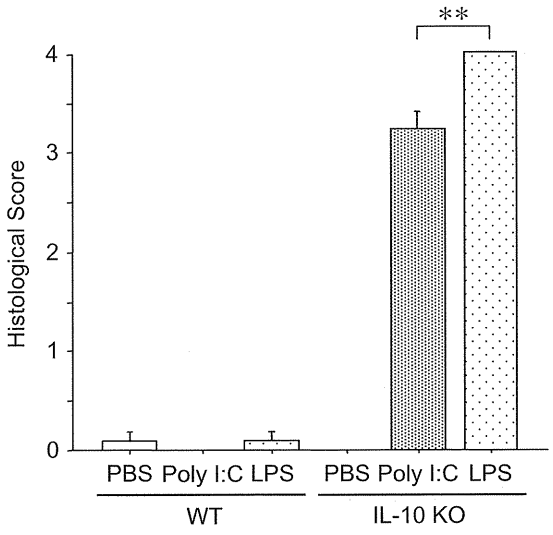


FIGURE 6. Histological pancreatitis scores for WT and IL-10KO mice. The severity of pancreatitis was scored using a scale of 0 to 4, which was based on a histological scoring system. Histological scores for pancreatitis were highest in LPS-treated mice (4.0 ± 0) and were higher than in IL-KO mice (3.3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

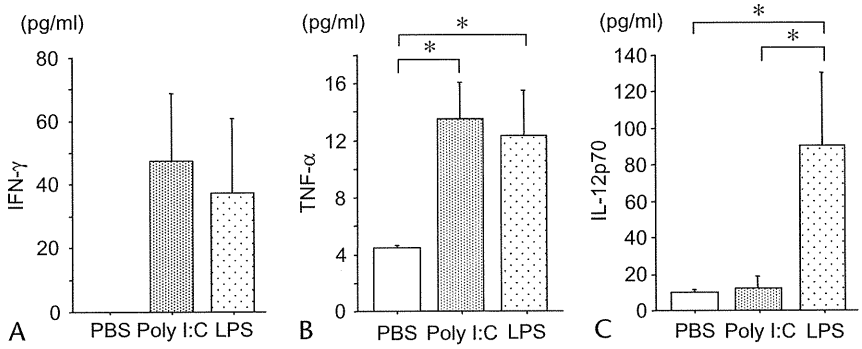


FIGURE 7. Serum cytokine levels in IL-10KO mice treated with PBS, poly I:C, or LPS for 8 weeks: IFN-γ (A), TNF-α (B), and IL-12p70 (C). Although the increase in IFN-γ levels was not significant, TNF-α levels increased in mice treated with poly I:C or LPS ($^{*}P < 0.05$). Interleukin 12p70 levels were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

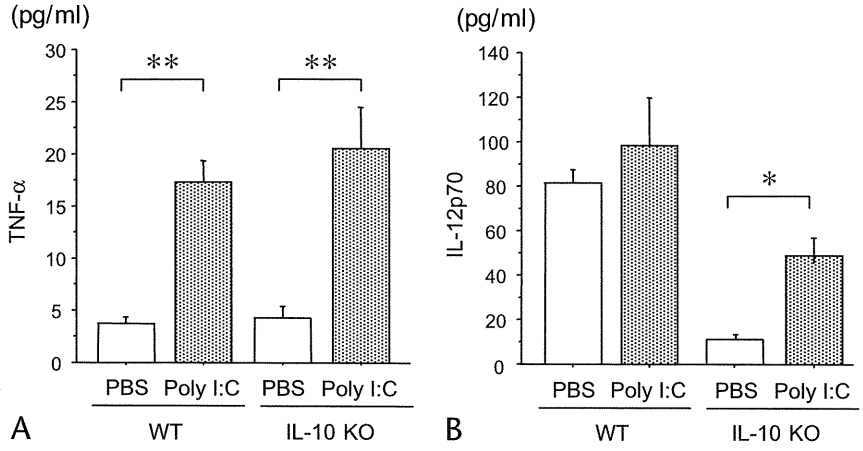


FIGURE 8. Serum cytokine levels in IL-10KO mice treated with single injections of PBS, poly I:C, or LPS: TNF-α (A) and IL-12p70 (B). Tumor necrosis factor-α levels were elevated in WT and IL-KO mice after a single injection of poly I:C. However, an elevated IL-12p70 level was observed only in IL-10KO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

□ CASE REPORT □

Idiopathic Duct-Centric Pancreatitis (IDCP) with Immunological Studies

Takeo Kusuda[1], Kazushige Uchida[1], Sohei Satoi[2], Masanori Koyabu[1], Norimasa Fukata[1], Hideaki Miyoshi[1], Tsukasa Ikeura[1], Yutaku Sakaguchi[1], Katsunori Yoshida[1], Toshiro Fukui[1], Masaaki Shimatani[1], Mitsunobu Matsushita[1], Makoto Takaoka[1], Akiyoshi Nishio[1], Yoshiko Uemura[3], A-Hon Kwon[2] and Kazuichi Okazaki[1]

Abstract

A 65-year-old woman with elevated serum levels of pancreatic enzymes was referred to our hospital for further examinations. Abdominal US and contrast-enhanced CT demonstrated swelling of the pancreas body and tail. MRCP and ERCP revealed abrupt ending of the MPD in the pancreas body. Under the suspicion of malignancy, distal pancreatectomy and splenectomy were performed. The histopathological findings showed idiopathic duct-centric pancreatitis (IDCP) with granulocytic epithelial lesions (GEL). As most cases of Japanese autoimmune pancreatitis (AIP) are lymphoplasmacytic sclerosing pancreatitis (LPSP), the present case seems to be helpful to clarify the clinical findings of IDCP in Japan.

Key words: autoimmune pancreatitis (AIP), Idiopathic duct-centric pancreatitis (IDCP), lymphoplasmacytic sclerosing pancreatitis (LPSP), granulocytic epithelial lesion (GEL), IgG4

(Intern Med 49: 2569-2575, 2010)
(DOI: 10.2169/internalmedicine.49.4165)

Introduction

Since Sarles et al reported a case of idiopathic pancreatitis with hypergammaglobulinemia in 1961 (1), several investigators have reported that autoimmune mechanisms may be involved in the etiology of chronic pancreatitis. Yoshida et al first proposed the concept of "autoimmune pancreatitis" (AIP) in 1995 (2). Thereafter, many cases of AIP have been reported mainly from Japan until the disease concept was accepted worldwide. As previously reported, the characteristic features (3, 4) of the Japanese patients with AIP show (i) diffuse enlargement of the pancreas on US, CT and MRI, (ii) irregular narrowing of the pancreatic duct (sclerosing pancreatitis) on endoscopic retrograde cholangiopancreatographic (ERCP) images, (iii) histologically termed lymphoplasmacytic sclerosing pancreatitis (LPSP) with fibrosis, abundant infiltration of lymphocyte and IgG4-positive plasmacytes and obliterative phlebitis, and (iv) it is often associated with extrapancreatic lesions, such as sclerosing cholangitis similar to primary sclerosing cholangitis (PSC), sclerosing cholecystitis, sclerosing sialoadenitis, retroperitoneal fibrosis, interstitial renal tubular disorders, enlarged celiac and hilar lymph nodes, chronic thyroiditis, and pseudotumor of the liver (5-7). On the other hand, in Western countries, another type of AIP different from the AIP commonly observed in Japan has been reported. In a study performed by a group at the Mayo Clinic, it was demonstrated that there may be two histological types of AIP, LPSP and idiopathic duct-centric pancreatitis (IDCP) (6, 8). IDCP was characterized by lobular fibrosis and pancreatic duct damage mainly caused by infiltration of neutrophils without obliterative phlebitis (8). Zamboni et al also recognized a subtype of AIP occurring in a subset of patients who are younger and more commonly have ulcerative colitis and Crohn's disease, which is characterized by the presence of granulocytic epithelial lesions (GEL) (9). There are a number of similarities in the clinical and histopathological findings between AIP

[1]The Third Department of Internal Medicine, Division of Gastroenterology and Hepatology, Kansai Medical University, Moriguchi, [2]Department of Surgery, Kansai Medical University, Moriguchi and [3]Department of Pathology, Kansai Medical University, Moriguchi
Received for publication July 3, 2010; Accepted for publication August 2, 2010
Correspondence to Dr. Kazuichi Okazaki, okazaki@hirakata.kmu.ac.jp

with GEL and IDCP, but not between AIP with LPSP and AIP with GEL or IDCP. Although Japanese AIP cases are almost all LPSP (4, 7), those concerning IDCP have been rarely reported from Japan (10). Therefore, it still remains unclear whether the clinical manifestations of the Japanese patients with IDCP are similar to those of Western countries or not. Herein, we report the first case of IDCP in Japan with full radiological and histopathological findings.

Case Report

A 65-year-old woman with elevated serum levels of pancreatic enzymes, as discovered by an annual health check, was referred to our hospital for further examination in the beginning of December 2004. She had no history of other illness or alcohol abuse. Furthermore, the symptom of inflammatory bowel disease including diarrhea was absent. Physical examination at the time of admission revealed no significant findings. Laboratory examinations showed the following values (normal range): peripheral white cell count, 4,600/μL; peripheral eosinocyte count, 690/μL; C-reactive protein, 0.04 mg/dL (<0.3 mg/dL); total bilirubin, 0.7 mg/dL; alkaline phosphatase, 313 IU/L (107-323 IU/L); γ-glutamyl transpeptidase, 13 IU/L (8-45 IU/L); asparate aminotransferase, 23 IU/L (12-31 IU/L); alanine aminotransferase, 18 IU/L (6-24 IU/L). Pancreatic enzymes were elevated: amylase 292 IU/L (32-112 IU/L), lipase 473 IU/L (16-60 IU/L), and elastase-1 950 ng/dL (100-400 ng/dL). Hepatitis B surface antigen and antibody to hepatitis C virus were negative. Serum γ-globulin, IgG levels were 1.43 g/dL (0.7-1.6 g/dL), 1,523 mg/dL (870-1,700 mg/dL), respectively. Serum autoantibodies were all negative, including antinuclear antibody, rheumatoid factor, anti-Ro antibody (SS-A), anti-La antibody (SS-B), and anti-mitochondrial antibody. Among tumor markers, CEA was 1.1 ng/dL (<5.0); DUPAN-2, 25 U/mL (<150); and CA19-9, 25.3 U/mL (<37). Abdominal US showed the partially enlarged pancreas body and tail with homogenous hypoechoic pattern (Fig. 1). Contrast-enhanced CT demonstrated moderate swelling in the body and tail of the pancreas with homogenous enhancement, but not capsular-like low density rim or swelling of peripancreatic lymph nodes (Fig. 2). MRI demonstrated the enlarged pancreas body and tail with no obvious intensity of change(Fig. 3A, B). MRCP revealed obstruction of the main pancreatic duct (MPD) in the body concordant with pancreas cancer tumors (Fig. 3C). ERCP demonstrated abrupt ending of the MPD in the pancreas body and irregular strictures of the pancreatic ducts in the pancreas head (Fig. 4). Transpapillary biopsy of the obstructive pancreatic duct and cytology of the pancreatic duct did not show malignancy. We were not able to identify a mass in the pancreas in the image, but also were not able to deny the possibility of the pancreatic cancer because we showed the disruption of the pancreatic duct. Therefore, we performed distal pancreatectomy and splenectomy. The postoperative course was uneventful and the patient was discharged after eight days. After hospital discharge, the patient had no recurrence to date.

The cut surface of the resected specimen showed swelling

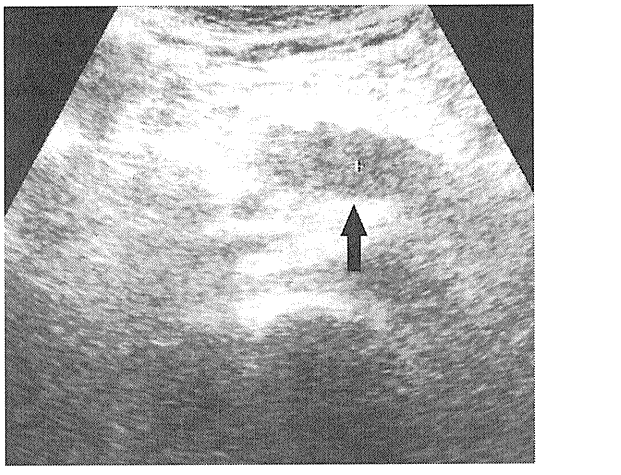


Figure 1. Abdominal ultrasonography (US) of the pancreas. US showed the partially enlarged pancreas body and tail with homogenous hypoechoic pattern (arrow).

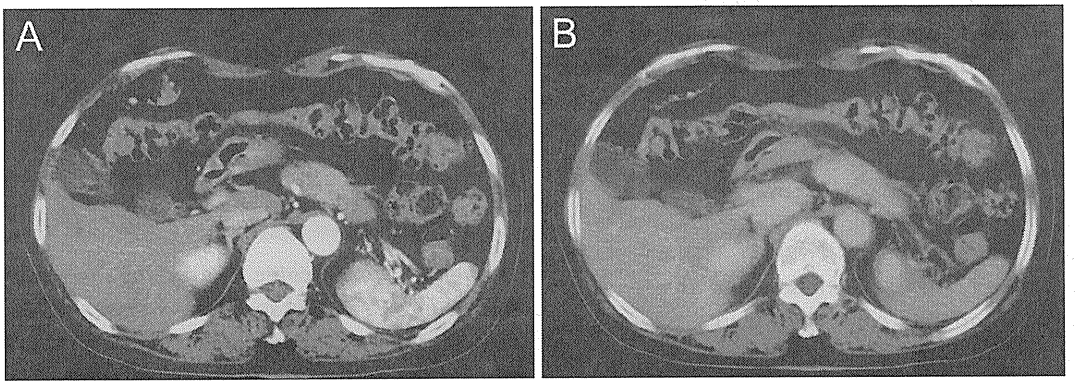


Figure 2. Contrast-enhanced computed tomography (CT) of the pancreas. Contrast-enhanced CT demonstrated moderate swelling in the body and tail of the pancreas with homogenous enhancement, but not capsular-like low density rim or swelling of peripancreatic nodes. (A) early phase (B) delayed phase.

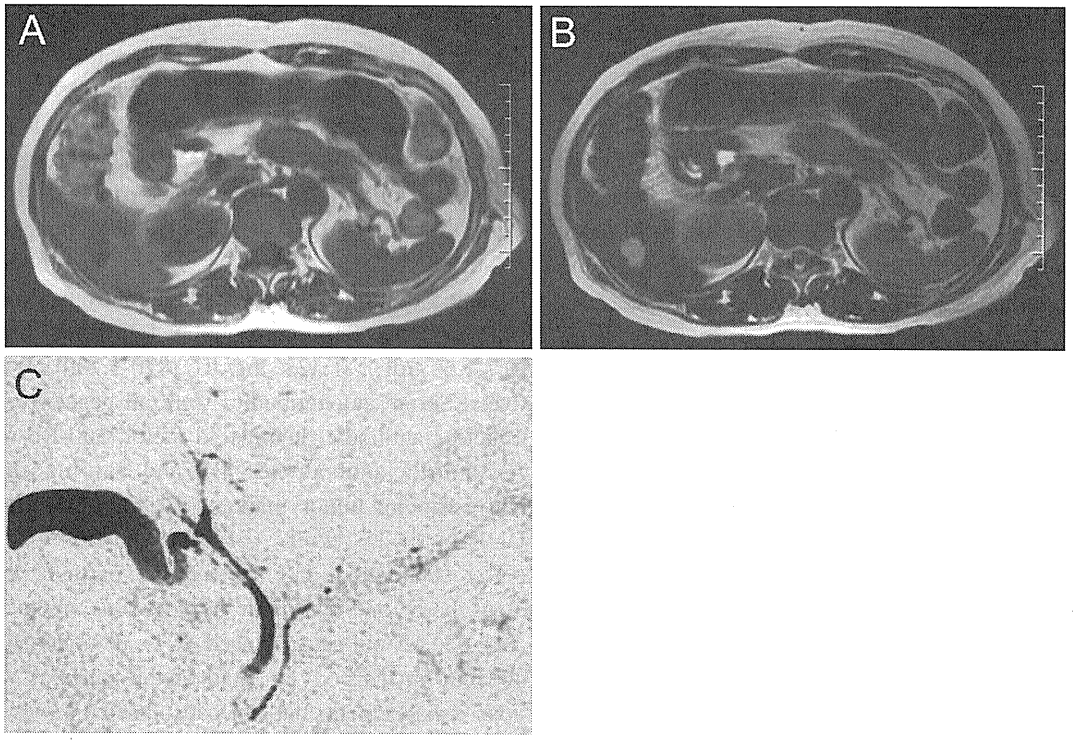


Figure 3. Magnetic resonance imaging (MRI) of the pancreas. MRI demonstrated swelling in the body and tail of the pancreas with no obvious intensity of change (A; T1 intensive image, B; T2 intensive image). Magnetic resonance cholangiopancreatography (MRCP) revealed obstruction of the main pancreatic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

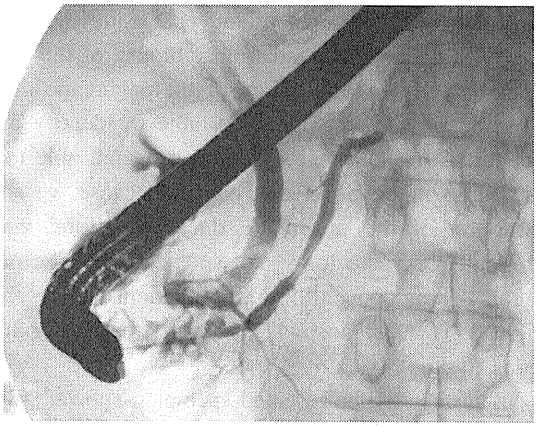


Figure 4. Endoscopic retrograde cholangiopancreatography (ERCP). ERCP image demonstrated abrupt ending of the main pancreatic duct in the body compatible with pancreatic cancer.

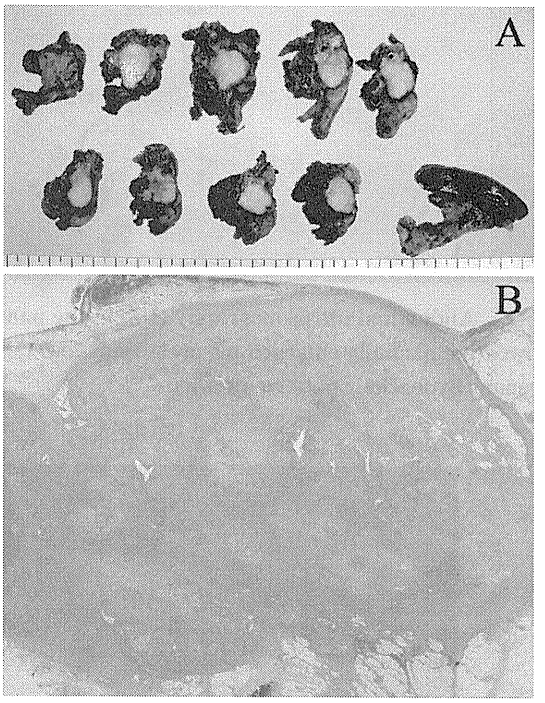


Figure 5. Macroscopic findings of the pancreas. The cut surface of the resected specimen showed swelling of parenchyma with the whitish indurated tissue (Fig. 5A). The picture of loupe of the greatest surface of cut specimen (Fig. 5B).

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

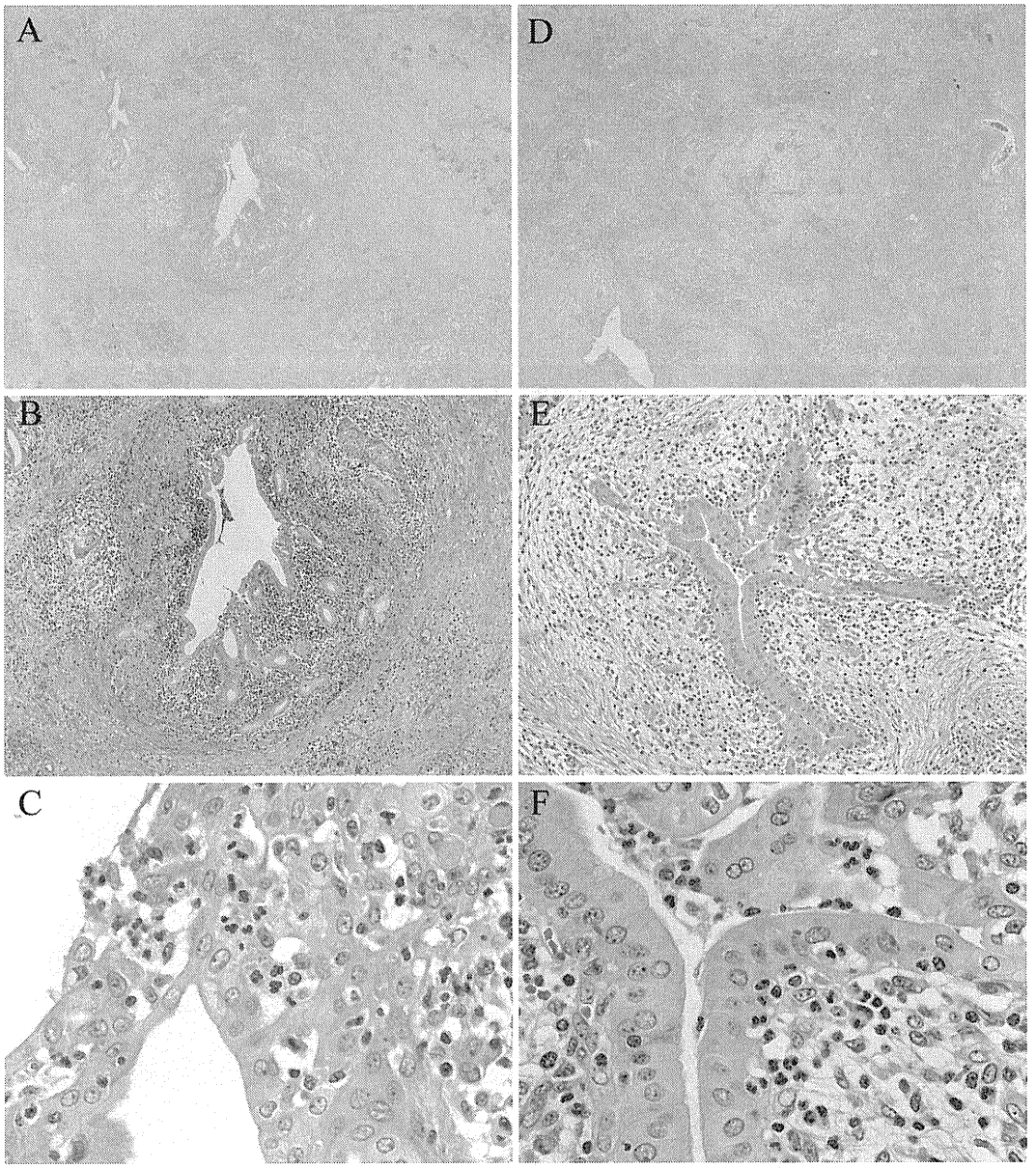


Figure 6. **Histological findings of the pancreas (Hematoxylin and Eosin staining). Although fibroblastic proliferation and fibrosis are seen, inflammatory infiltrates are scarce between the lobules (A, ×20; D, ×20). Periductal inflammation with destruction of pancreatic epithelia by abundant neutrophils 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/G1 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

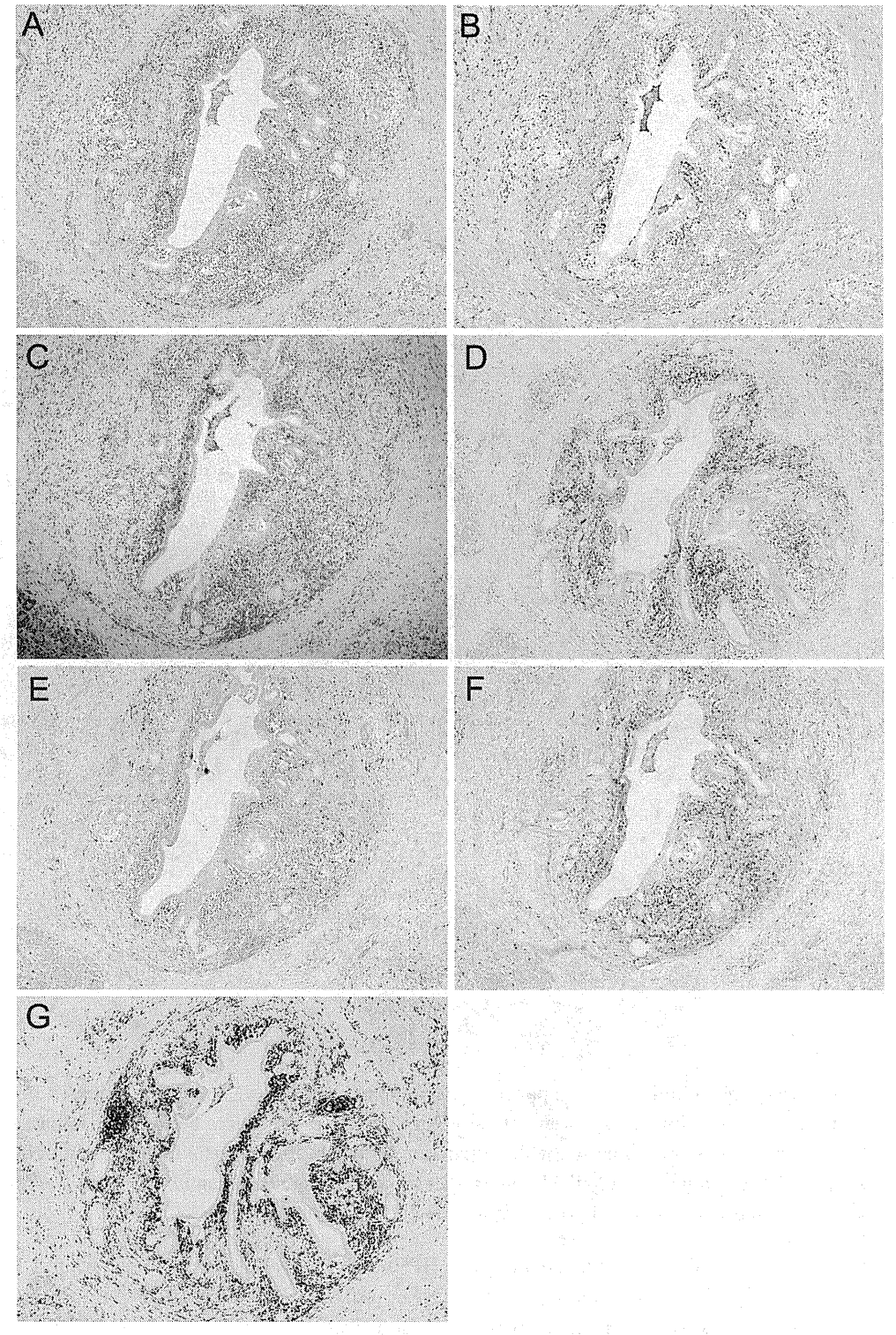


Figure 7. Immunohistochemistry of the pancreas (A, IgG4; B, IgG1; C, Foxp3; D, CD3; E, CD4; F, CD8; G, CD79a). IgG4-positive plasma cells were scattered, whereas IgG1-positive plasma cells were abundant (A, B). Foxp3-positive T lymphocytes were observed around the intralobular duct (C). A predominance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

J Gastroenterol (2010) 45:122–129
DOI 10.1007/s00535-009-0130-y

CASE REPORT

Primary sclerosing cholangitis with elevated serum IgG4 levels and/or infiltration of abundant IgG4-positive plasma cells

Masanori Koyabu · Kazushige Uchida · Norimasa Fukata · Takeo Kusuda · Tsukasa Ikeura · Yutaku Sakaguchi · Katsunori Yoshida · Masaaki Shimatani · Toshiro Fukui · Mitsunobu Matsushita · Yoshiko Uemura · Masaki Kaibori · Makoto Takaoka · Akiyoshi Nishio · Kazuichi Okazaki

Received: 14 July 2009 / Accepted: 19 August 2009 / Published online: 18 September 2009

Abstract Immunoglobin G4-related sclerosing cholangitis (IgG4-SC) is recognized as one of the systemic sclerosing diseases characterized by abundant IgG4-positive plasma cells with effective steroid therapy. On the other hand, primary sclerosing cholangitis (PSC), recognized as a sclerosing cholangitis of unknown origin without steroid efficacy, has been often clinically confused with IgG4-SC. To date, the prognosis of IgG4-SC is unclear, while the prognosis of PSC is well known to be poor. Therefore, it is clinically very important to be able to distinguish IgG4-SC from PSC. However, at the present time it still remains unclear whether PSC may sometimes be misdiagnosed as IgG4-SC or not. Herein, we report three rare cases of PSC with elevated serum IgG4 levels and/or an infiltration of abundant IgG4-positive plasma cells in the liver: a young male with ulcerative colitis (UC), and elderly female and a young female, each with elevated serum IgG4 levels. The first two patients showed infiltration of abundant IgG4-positive plasma cells in the portal area of the liver without response to steroid therapy. From our experiences, we emphasize that some patients with PSC, who do not respond to steroid therapy, show elevated serum IgG4 levels and/or infiltration of abundant IgG4-positive plasma cells, although the mechanism still remains unclear.

Keywords Primary sclerosing cholangitis · IgG4 · Autoimmune pancreatitis

M. Koyabu · K. Uchida · N. Fukata · T. Kusuda · T. Ikeura · Y. Sakaguchi · K. Yoshida · M. Shimatani · T. Fukui · M. Matsushita · M. Takaoka · A. Nishio · K. Okazaki (✉)
The Third Department of Internal Medicine, Division of Gastroenterology and Hepatology, Kansai Medical University, Moriguchi, Japan
e-mail: okazaki@hirakata.kmu.ac.jp

Y. Uemura
Department of Clinical Sciences and Laboratory Medicine, Kansai Medical University, Moriguchi, Japan

M. Kaibori
Department of Surgery, Kansai Medical University, Moriguchi, Japan

Introduction

Recently, autoimmune pancreatitis (AIP) has been accepted worldwide as a unique, distinctive disease, in which histopathological findings show abundant infiltration of IgG4-positive plasma cells and fibrosis, known as lymphoplasmacytic sclerosing pancreatitis (LPSP), and clinical manifestations that dramatically respond to steroid therapy. In addition to pancreatic lesions, patients with AIP have occasional extrapancreatic lesions such as sclerosing cholangitis (SC), sclerosing sialoadenitis, and retroperitoneal fibrosis similar to LPSP. Among the extrapancreatic lesions, the bile duct is the most commonly involved organ, manifesting as a sclerosing cholangitis which results in obstructive jaundice. AIP is recognized as the pancreatic manifestation of a novel systemic disease referred to as IgG4-related sclerosing disease [1].

IgG4-related sclerosing cholangitis (IgG4-SC) is a recently recognized disease entity characterized by microscopic findings of sclerosing inflammation with an infiltration of abundant IgG4-positive plasma cells, and AIP is associated in most cases. Before establishing the concept of AIP, IgG4-SC used to be misdiagnosed as primary sclerosing cholangitis (PSC) complicating chronic pancreatitis. Therefore, differential diagnosis between IgG4-SC and PSC is important. The cholangiographic findings in IgG4-SC and PSC are similar [2, 3]. Elevation

of serum IgG4 is frequently observed in patients with IgG4-SC, which responds dramatically to steroid therapy [4]. In contrast, even if the patients with PSC are medicated, it remains a progressive disease that involves the intra- and extra-hepatic bile ducts and leads to biliary cirrhosis. The effects of steroid therapy for PSC have been reported to be skeptical [5, 6] and liver transplantation is the only effective therapy. Histopathologically, lymphoplasmacytic and eosinophilic infiltration with mild fibrosis are seen in both IgG4-SC and PSC; and recent studies based on immunohistochemical findings of liver biopsy specimens report that IgG4-positive plasma cell infiltration is significantly more severe in IgG4-SC than in PSC [4, 7–11]. However, herein, we report 3 cases of PSC with an infiltration of abundant IgG4-positive plasma cells and ineffective steroid therapy.

Case report

Case 1

A 32-year-old man with elevated serum levels of hepatobiliary enzymes was admitted to our hospital. At the age of 22 years, the patient was diagnosed as PSC in other hospitals, and he had been treated with ursodeoxycholic acid. At the age of 24 years, he was found to have ulcerative colitis (UC). Physical examination at the time of admission revealed no significant findings except for jaundice. Laboratory examinations showed the following values (normal range): peripheral white cell count, 5700/μl (3500–8500); peripheral eosinocyte count, 251/μl (18–510); C-reactive protein, 1.1 mg/dl (<0.3); total bilirubin, 10.9 mg/dl (0.2–1.2); alkaline phosphatase, 2929 U/l (107–340); γ-glutamyl transpeptidase, 413 U/l (11–64); asparate aminotransferase, 133 U/l (13–35); alanine aminotransferase, 192 U/l (5–35); amylase, 127 U/l (37–125). Hepatitis B surface antigen and antibody to hepatitis C virus were negative. Serum IgG, IgG4, IgA, and IgM levels were 2104 mg/dl (870–1700), 96 mg/dl (4.8–105), 291 mg/dl (110–410), and 157 mg/dl (33–190), respectively. Rheumatoid factor was negative. Antinuclear antibody was positive. Among tumor markers, CEA was 1.8 ng/ml (<5.0) and CA19-9 was 237.2 U/ml (<37). Magnetic resonance cholangiopancreatography (MRCP) and endoscopic retrograde cholangiopancreatography (ERCP) revealed strictures of both the hepatic hilar region and the distal common bile duct and no narrowing of the main pancreatic duct (Fig. 1a, b). Cytology of bile juice was negative for malignancy. Histopathological examination by liver biopsy showed moderate lymphoplasmacytic and eosinophil infiltration with fibrosis in the enlarged portal area (Fig. 1c). Duct and ductular proliferation was conspicuous. Fibrous cholangitis (onion-skin fibrosis) was observed. These findings were compatible with PSC. The numbers of immunohistochemically identified IgG4-positive plasma cells were counted under five different high-power fields (hpf). Immunostaining study showed typical inflammation with abundant IgG4-positive plasma cells (126 cells/hpf) (Fig. 1d), a characteristic finding in IgG4-SC. His liver dysfunction was serious, with progressive ascites and jaundice, therefore it was determined that liver transplantation might be necessary.

Although oral steroid therapy requires a long period for drug tapering, steroid pulse therapy is a well-recognized alternative for refractory autoimmune pancreatitis without steroid tapering, as previously reported [12]. Therefore, we twice administrated steroid pulse therapy with 500 mg/day of methylprednisolone for 3 days/week. The hepato-biliary enzymes improved a little after steroid therapy, but MRCP revealed no improvements of strictures of the hilar and distal common bile ducts. Therefore, we strongly suspected PSC. Two months later, we decided on liver transplantation with consent of the patient and his family. Histopathological findings of the liver after transplantation showed severe lymphoplasmacytic and eosinophil infiltration with fibrosis in the enlarged portal area (Fig. 2a, b). Duct and ductular proliferation was conspicuous, and onion-skin fibrosis was observed, which suggested typical advanced PSC findings. Histopathological findings of the pancreas biopsy during the operation showed infiltration of mononuclear cells around the pancreatic duct (Fig. 2c) with an infiltration of abundant IgG4-positive plasma cells (Fig. 2d), but did not show LPSP.

Case 2

A 74-year-old woman was admitted to our hospital with liver dysfunction. Laboratory examinations showed the following values (normal range): peripheral white cell count, 8900/μl (3500–8500); C-reactive protein, 2.19 mg/dl (<0.3); total bilirubin, 0.6 mg/dl (0.2–1.2); alkaline phosphatase, 1544 U/l (107–340); γ-glutamyl transpeptidase, 1030 U/l (11–64); asparate aminotransferase, 130 U/l (13–35); alanine aminotransferase, 140 U/l (5–35); amylase, 44 U/l (37–125). Hepatitis B surface antigen and antibody to hepatitis C virus were negative. Serum IgG, IgM, and IgE levels were 1960 mg/dl (870–1700), 77 mg/dl (33–190), and 370 (0–320), respectively. Antinuclear antibody was positive. Antimitochondrial antibody was negative. Among tumor markers, CEA, CA19-9, and soluble interleukin 2 receptor (sIL-2R) were 2.1 ng/ml (<5.0), 18.5 U/ml (<37), and 611 U/ml (<650), respectively. Abdominal computed tomography (CT) showed dilatation of common bile duct (Fig. 3a) and no significant pancreatic lesions (Fig. 3b). ERCP revealed irregular narrowing of the

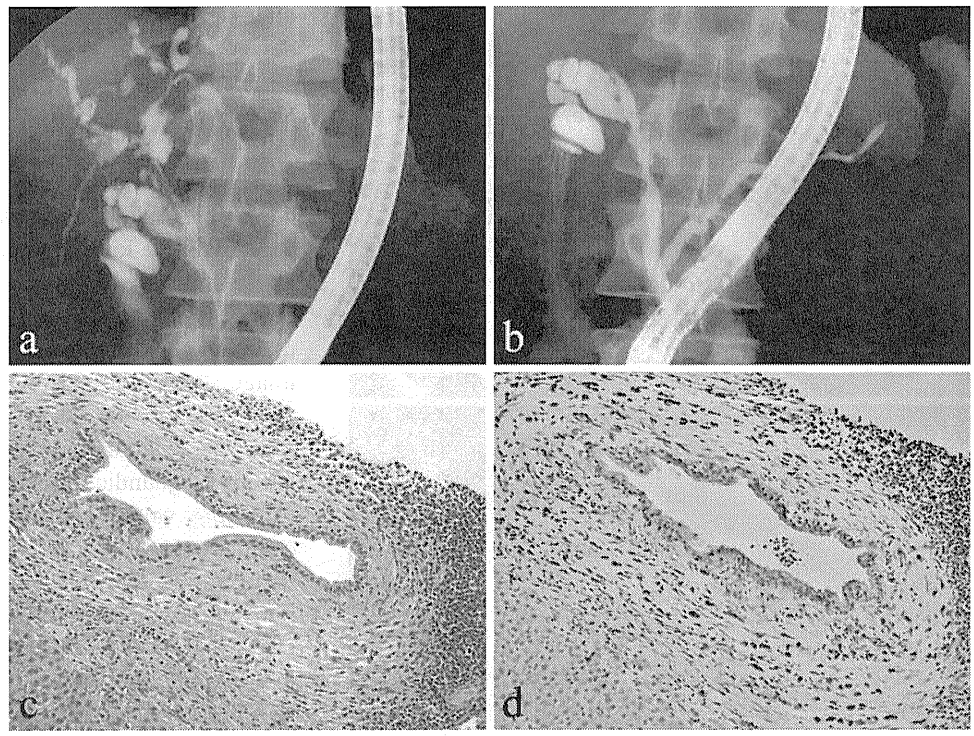


Fig. 1 ERCP images and histopathological findings of case 1 on clinical onset. ERCP revealed strictures of the hepatic hilar area (**a**) and the distal common bile duct, and no narrowing of the main pancreatic duct (**b**). Histopathological findings of the liver biopsy showed a moderate lymphoplasmacytic and eosinophil infiltration with fibrosis, and fibrous cholangitis (onion-skin fibrosis) (H&E staining ×200, **c**). IgG4-immunostaining of the liver biopsy showed infiltration of abundant IgG4-positive plasma cells (×200, **d**)

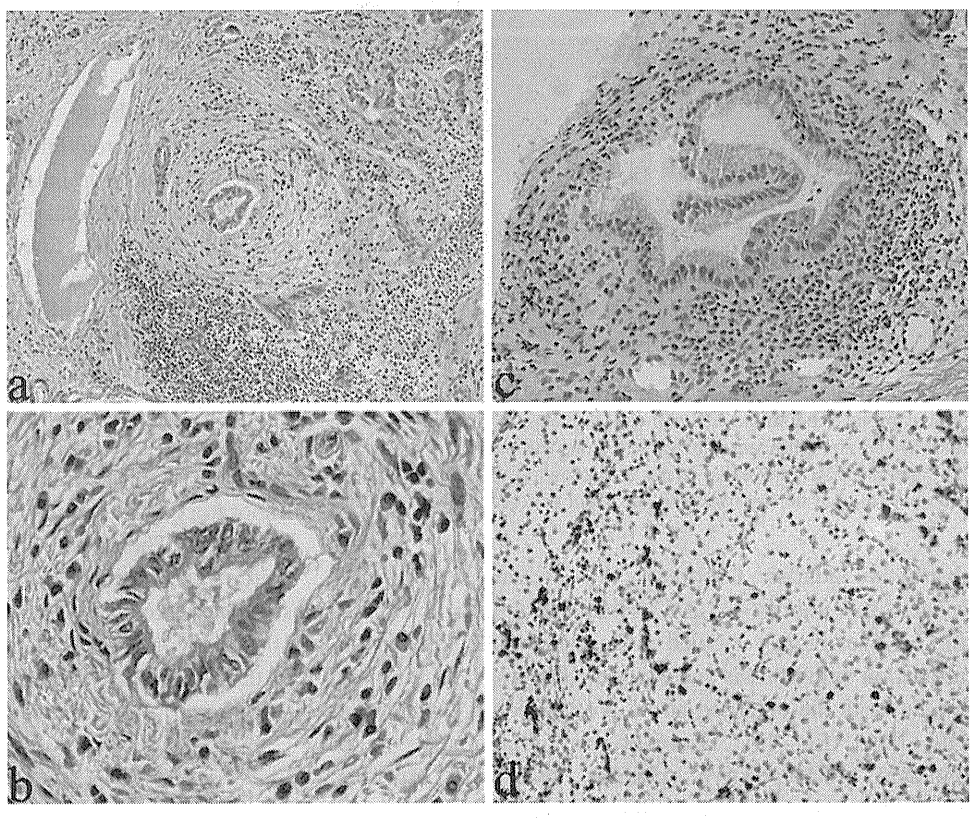


Fig. 2 Histopathological findings of case 1 on transplantation. Histopathological findings of liver after transplantation showed severe lymphoplasmacytic and eosinophil infiltration with fibrosis in the enlarged portal area (H&E staining ×100, **a**; ×400, **b**). Duct and ductular proliferation was conspicuous, and onion-skin fibrosis was observed. Histopathological findings of the pancreas biopsy during operation showed that infiltration of mononuclear cells around pancreatic duct (H&E staining ×200, **c**). IgG4-immunostaining of the pancreas biopsy during operation showed infiltration of abundant IgG4-positive plasma cells (×200, **d**)

intrahepatic bile ducts (Fig. 3c) and no narrowing of the main pancreatic duct (Fig. 3d). Intraductal ultrasonography (IDUS) detected wall thickness of the intrahepatic and common bile ducts. Cytology of bile juice was negative for malignancy. She was diagnosed with PSC and treated with ursodeoxycholic acid.

Four months after clinical onset, the patient was referred to our hospital for further evaluation of recurrent

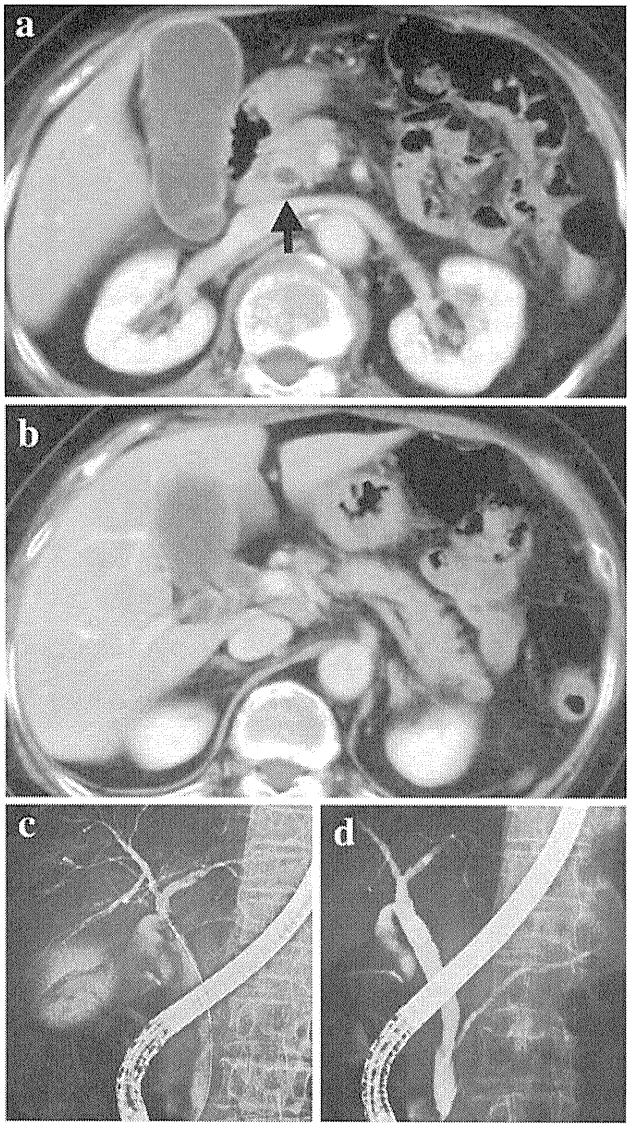


Fig. 3 Abdominal computed tomography (CT) images of case 2 on clinical onset. Abdominal CT showed a dilatation of common bile duct (**a**) and no significant pancreatic lesions (**b**), such as swelling of the pancreas. ERCP revealed irregular narrowing of the intrahepatic bile duct (**c**), and no narrowing of the main pancreatic duct (**d**)

obstructive jaundice. Laboratory tests showed elevations of IgG4 to 206 mg/dl (4.8–105). Histopathological examination by liver biopsy showed moderate lymphoplasmacytic infiltration with fibrosis and fibrotic change surrounding the bile ducts in the enlarged portal area, which is compatible with PSC (Fig. 4a). However, an inflammation with abundant IgG4-positive plasma cells (16 cells/hpf) (Fig. 1d), a characteristic finding in IgG4-SC, was also found. Then, we suspected IgG4-SC, and steroid therapy was initiated at a dose of 30 mg/day. The dose of steroid was reduced by 5 mg/week until it reached 10 mg/day. MRCP revealed no improvements of the irregular narrowing of the intrahepatic lesion and the common bile duct after steroid therapy. One year later, her liver dysfunction developed into liver cirrhosis.

Case 3

The patient was a 23-year-old woman who was admitted to our hospital with the complaint of jaundice. ERCP revealed stricture of the lower common bile duct, irregular dilatation after confluent strictures, and many small defects in intrahepatic bile ducts (Fig. 5a). Endoscopic naso-biliary drainage (ENBD) was performed. The pancreatic-duct image showed no narrowing of the main pancreatic duct. Cytology of bile juice was negative for malignancy. Physical examination revealed no significant findings except for jaundice. Laboratory examinations showed the following values (normal range): peripheral white cell count, 10100/μl (3000–8500); peripheral eosinocyte count, 91/μl; C-reactive protein, 0.09 mg/dl (<0.3); total bilirubin, 5.0 mg/dl (0.2–1.2); alkaline phosphatase, 1750 U/l (107–323); γ-glutamyl transpeptidase, 211 U/l (8–45); asparate aminotransferase, 90 U/l (12–31); alanine aminotransferase, 101 U/l (6–24); amylase, 37 U/l (32–112). Hepatitis B surface antigen and antibody to hepatitis C virus were negative. Serum IgG, IgA, and IgM levels were 2570 mg/dl (1092–1577), 208 mg/dl (134–287), and 363 mg/dl (60–161), respectively. Rheumatoid factor, antinuclear antibody, and antimitochondrial antibody were negative. The irregular dilatation of bile ducts improved 5 months after a drainage procedure with a biliary plastic stent, but irregular narrowing of the intrahepatic bile ducts persisted (Fig. 5b). She was diagnosed with PSC and treated with ursodeoxycholic acid.

Three years after clinical onset, the patient was referred to our hospital for further evaluation of recurrent obstructive jaundice. Histopathological examination by liver biopsy showed an infiltration of lymphocytes and ductular proliferation in the portal area (Fig. 6a), and a few IgG4-positive plasma cells (1 cell/hpf) were detected (Fig. 6b). Laboratory tests showed elevations of IgG4 to 313 mg/dl (4.8–105). Therefore, we suspected IgG4-SC and steroid therapy was initiated at the dose of 30 mg/day. The dose of steroid was reduced by 5 mg/day biweekly until it reached 10 mg/day. MRCP revealed no improvements of strictures of the intrahepatic and the common bile ducts after steroid therapy.

Discussion

Sarles et al. [13] observed the first case of pancreatitis with hypergammaglobulinemia, and Yoshida et al. first proposed the concept of autoimmune pancreatitis (AIP) in 1995 [14], in which patients show diffusely enlarged

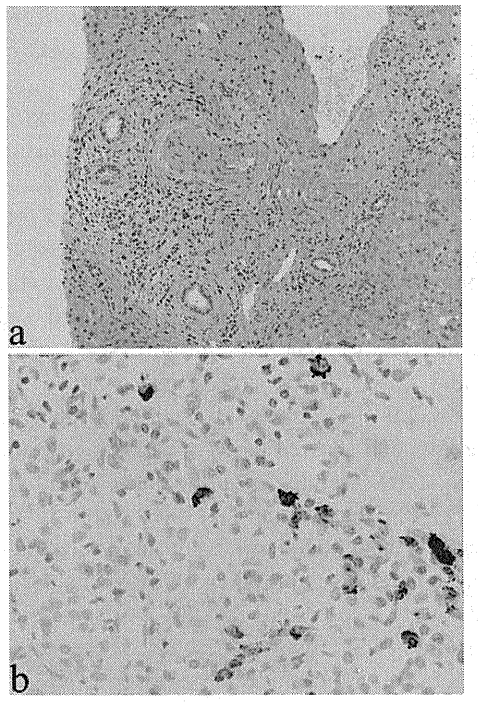
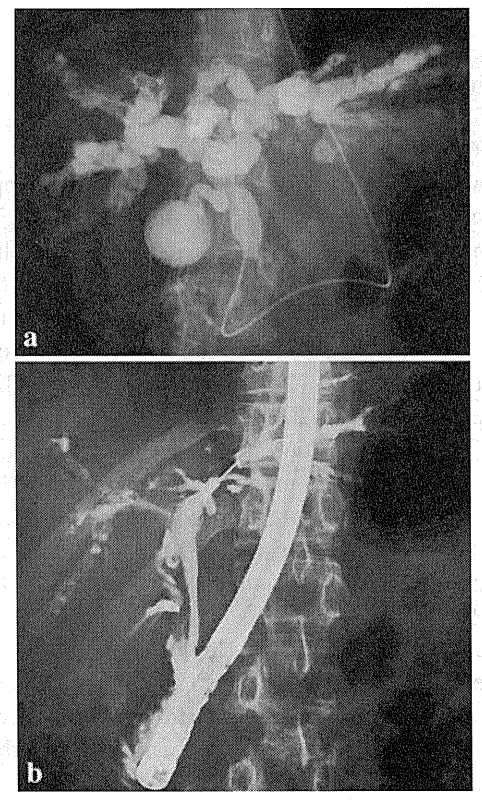


Fig. 4 Histopathological findings of the liver (case 2). Histopathological examination by liver biopsy showed moderate lymphoplasmacytic infiltration with fibrosis and fibrotic change surrounding the bile ducts in the enlarged portal area (H&E staining ×100, **a**). IgG4-immunostaining of the liver specimens showed infiltration of abundant IgG4-positive plasma cells (×400, **b**)



◄ **Fig. 5** Endoscopic nasobiliary drainage (ENBD) cholangiogram and ERCP image of case 3 on clinical onset. Cholangiography through an ENBD tube revealed a stricture of the lower common bile duct, irregular dilatation after confluent strictures, and many small defects in intrahepatic bile ducts (**a**). ERCP after a drainage procedure with a biliary plastic stent revealed improvement of bile duct dilatation, but irregular narrowing of the intrahepatic bile ducts persisted (**b**)

pancreas, narrowing pancreatogram, increased serum IgG, presence of autoantibodies, fibrotic changes with lymphocytic infiltration, and steroidal efficacy. Thereafter, many AIP cases have been reported by Japanese investigators, and AIP has been accepted as a new clinical entity [15–17]. Patients with AIP often show discomfort in the epigastrium, obstructive jaundice due to bile-duct stricture, and diabetes mellitus. AIP is more common in middle-aged and elderly men. Patients with AIP often also have extrapancreatic lesions such as biliary lesions, sialoadenitis, retroperitoneal fibrosis, enlarged celiac and hilar lymph nodes, chronic thyroiditis, and interstitial nephritis [18–23], which suggests that AIP may be a systemic disorder. In 2001, Hamano et al. [24] reported that patients with AIP have high serum IgG4 concentrations. Kamisawa et al. [1] proposed IgG4-related sclerosing disease. Recently, IgG4-SC was recognized as a disease entity characterized by

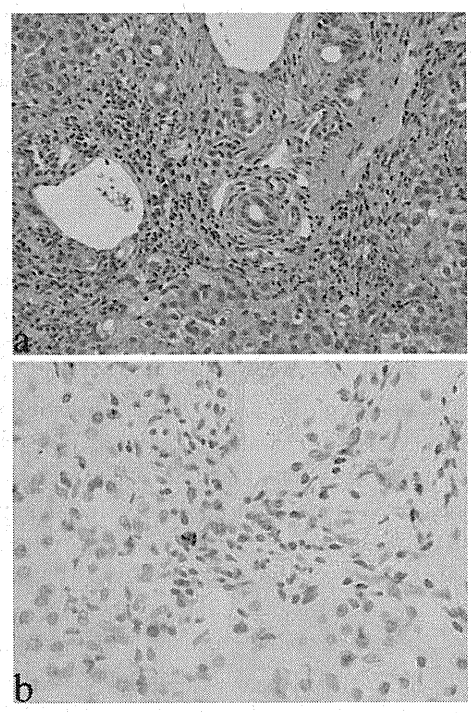


Fig. 6 Histopathological findings of the liver (case 3). Histopathological examination by liver biopsy showed moderate lymphoplasmacytic infiltration and ductular proliferation in the enlarged portal area (H&E staining ×200, **a**). IgG4-immunostaining of the liver specimens showed infiltration of few IgG4-positive plasma cells (×400, **b**)