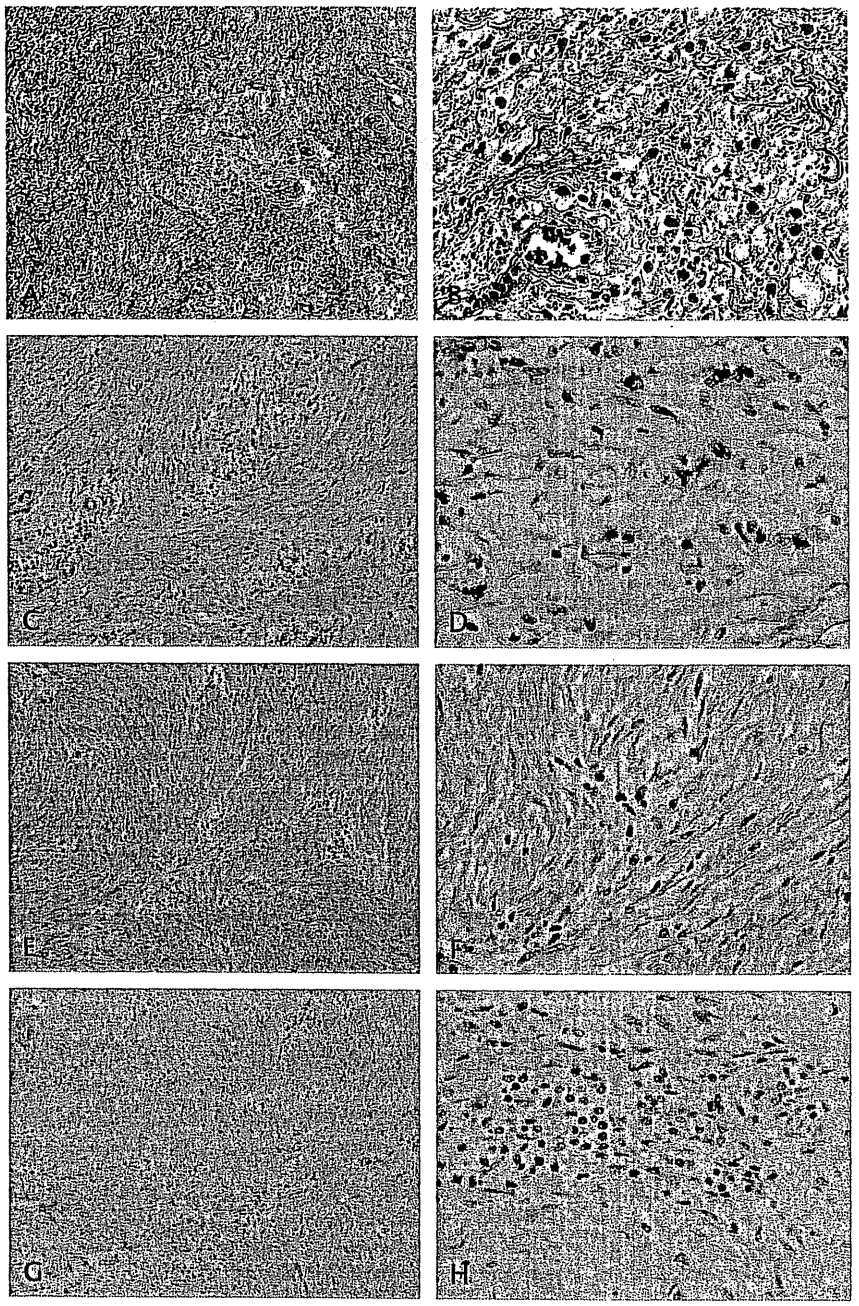

FIGURE 2. Histopathologic and immunohistochemical findings of the pancreas in alcoholic CP. Hematoxylin-eosin staining (original magnification ×100 [A], ×400 [B]). Photomicrograph of alcoholic CP showed dense fibrosis that surrounded atrophic or normal-appearing lobules. Some lobules were divided or replaced by dense fibrous tissue that extended from perilobular fibrosis bundles. Immunoglobulin 4–positive (original magnification ×100 [E], ×400 [F]) and Foxp3-positive cells (original magnification × 100 [G], ×400 [H]) were scattered, whereas IgG1-positive cells (original magnification ×100 [C], ×400 [D]) were abundant.

Circulating $CD4^+CD25^{high}$Tregs Were Higher in Patients With AIP

$CD4^+CD25^{high}$ Tregs (%$CD4^+CD25^{high}$ Tregs of $CD4^+$) were significantly higher in AIP patients (n = 31, 4.99% [SD, 2.70%]) compared with those with alcoholic CP (n = 11, 3.49% [SD, 1.43%]), those with idiopathic CP (n = 17, 2.24% [SD, 1.01%]), and the healthy control group (n = 16, 2.60% [SD, 1.05%], $P < 0.05$) (Figs. 5 and 6). There was no difference in these Tregs between AIP patients who underwent steroid therapy (n = 22, 5.12% [SD, 2.84%]) and those who did not (n = 9, 4.52% [SD, 2.40%]).

Circulating $ICOS^+$ Tregs Were Higher in Patients With AIP

There was no difference in these $ICOS^+$ lymphocytes (% $ICOS^+$ cells of lymphocytes) between patients with AIP

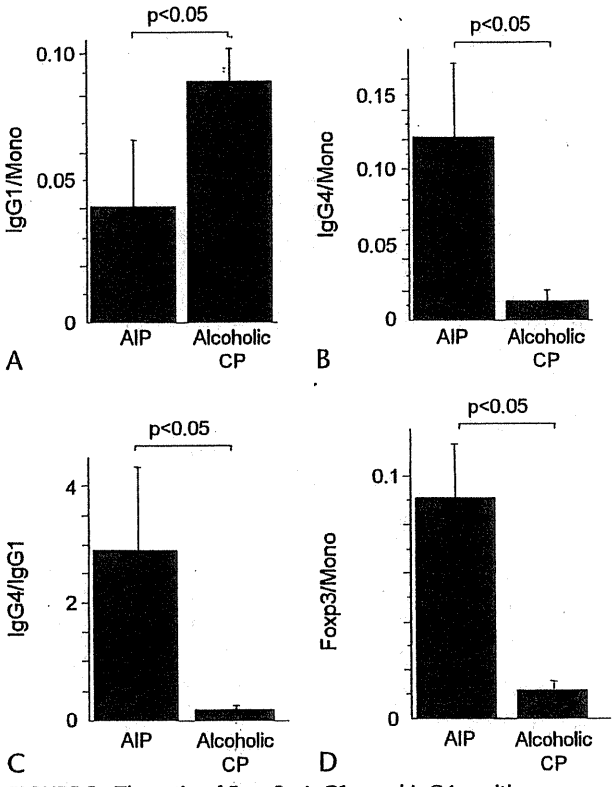

FIGURE 3. The ratio of Foxp3-, IgG1-, and IgG4-positive cells to infiltrated mononuclear cells (Foxp3/Mono, IgG1/Mono, IgG4/Mono) in patients with AIP (n = 9) and alcoholic CP (n = 9). Foxp3/Mono and IgG4/Mono in patients with AIP were significantly increased compared with alcoholic CP. Immunoglobulin 1/Mono in patients with AIP was significantly increased compared with alcoholic CP.

(n = 31, 0.40% [SD, 0.36%]), alcoholic CP (n = 11, 0.43% [SD, 0.26%]), and idiopathic CP (n = 17, 0.49% [SD, 0.30%]) and the healthy control group (n = 16, 0.35% [SD, 0.21%]) (Fig. 7A). However, the ratio of $ICOS^+$ Tregs was significantly higher in the AIP patients. Inducible costimulator–positive Tregs (% $ICOS^+$ Tregs of lymphocytes) were significantly increased in patients with AIP (n = 31, 0.08% [SD, 0.01%]) compared with those with alcoholic CP (n = 11, 0.03% [SD, 0.02%]), those with idiopathic CP (n = 17, 0.02% [SD, 0.01%]), and the healthy control group (n = 16, 0.02% [SD, 0.01%], $P < 0.05$) (Fig. 7B). Inducible costimulator–positive Tregs (% $ICOS^+$ Tregs of $CD4^+$) were significantly increased in patients with AIP (n = 31, 0.18% [SD, 0.15%]) compared with those with alcoholic CP (n = 11, 0.07% [SD, 0.06%]), those with idiopathic CP (n = 17, 0.04% [SD, 0.04%]), and the healthy control (n = 16, 0.04% [SD, 0.03%], $P < 0.05$) (Fig. 7C). Inducible costimulator–positive Tregs (% $ICOS^+$ Tregs of total Tregs) were significantly increased in patients with AIP (n = 31, 3.45% [SD, 1.58%]) compared with those with alcoholic CP (n = 11, 1.71% [SD, 0.98%]), those with idiopathic CP (n = 17, 1.80% [SD, 0.86%]), and the healthy control group (n = 16, 1.57% [SD, 0.61%], $P < 0.05$) (Figs. 5, 7D). There was no difference in these $ICOS^+$ Tregs between AIP patients who underwent steroid therapy (n = 22, 3.51% [SD, 1.57%]) and those who did not (n = 9, 3.31% [SD, 1.68%]).

A Comparison of the IL-10–Producing Ability Between AIP Patients and the Healthy Control Group

$IL\text{-}10^+$ Tregs (% $IL\text{-}10^+$ Tregs of total Tregs) were significantly higher in AIP patients (n = 16, 3.81% [SD, 1.52%]), compared with the healthy control group (n = 11, 1.38% [SD, 0.64%], $P < 0.05$) (Figs. 8 and 9). The ratio of $IL\text{-}10^+$ Tregs and $ICOS^+$ Tregs of total Tregs was significantly higher in AIP patients compared with the healthy control group (Figs. 5 and 8). The ratio that $ICOS^+$ Tregs express IL-10 (n = 16, 11.92% [SD, 3.73%]) in the AIP group is higher in significance than the ratio that $ICOS^-$ Tregs express IL-10 (n = 16, 1.61% [SD, 3.73%]) in the AIP group (Fig. 10).

DISCUSSION

Autoimmune pancreatitis is accepted worldwide as a distinctive type of pancreatitis, which contains 2 forms, IgG4-related AIP (type 1 AIP, LPSP type) and AIP with GEL (type 2 AIP, IDCP type).[14] Although the precise mechanism remains unclear, autoimmune mechanisms are suspected to be involved in the development of both types of AIP. In addition to pancreatitis, patients with IgG4-related AIP often manifest extrapancreatic lesions such as biliary lesions, sialadenitis, retroperitoneal fibrosis, enlarged celiac and hilar lymph nodes, chronic thyroiditis, and interstitial nephritis,[10] suggesting that IgG4-related AIP may be the pancreatic manifestation of a systemic disorder, IgG4-related disease.[5] On the other hand, ulcerative colitis is sometimes associated with AIP with GEL (IDCP or type 2 AIP).[12]

Patients with IgG4-related AIP are known to show higher levels of serum IgG4 than patients with other pancreatic diseases or healthy control subjects.[4,5] However, the mechanism of increased IgG4 production is still unclear. Previous reports showed that both activated $CD4^+$ and $CD8^+$ T cells increased significantly in the peripheral blood and infiltrated into the pancreas more predominantly than B cells, in addition to abundant infiltration of IgG4-positive plasma cells. $CD4^+$ T cells differentiate from naive T cells (T_H0) to T-helper 1 (T_H1), T_H2, T_H17, and Tregs.[36] In the peripheral blood of AIP patients, T_H1 cells are predominant over T_H2 cells,[35] whereas T_H2 dominates in the

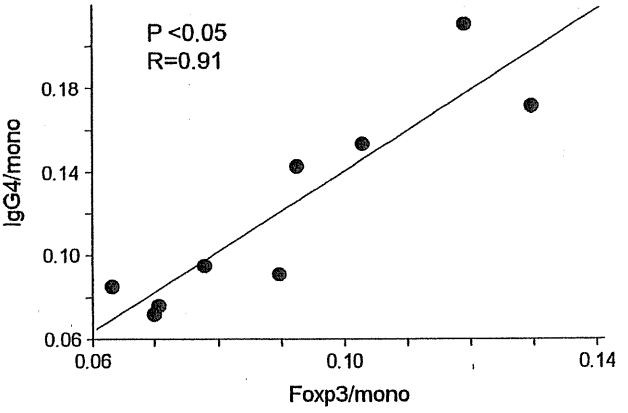

FIGURE 4. Correlation between the ratio of Foxp3-positive cells to infiltrated mononuclear cells (Foxp3/Mono) and the ratio of IgG4-positive cells to infiltrated mononuclear cells (IgG4/Mono) in patients with AIP. Foxp3/Mono and IgG4/Mono are positively correlated (n = 9, $P < 0.05$; $R = 0.91$).

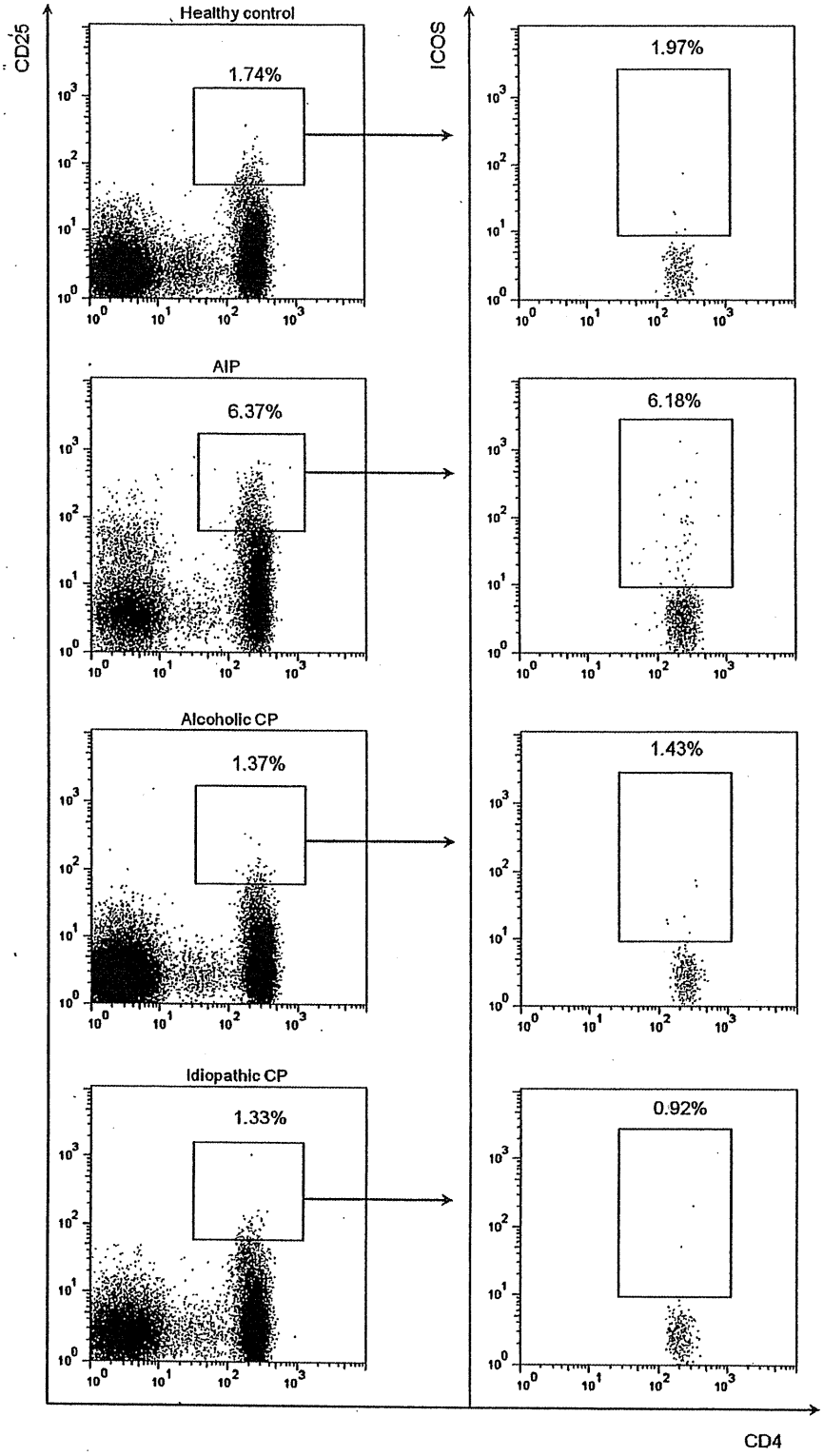

FIGURE 5. Flow cytometric analysis of $CD4^+CD25^{high}$ Tregs and $ICOS^+$ Tregs. Human peripheral blood $CD4^+CD25^{high}$ Tregs separated into $ICOS^+$ subpopulations. In AIP patients (n = 31), the number of $CD4^+CD25^{high}$ Tregs and $ICOS^+$ Tregs increased compared with healthy control (n = 16), alcoholic CP (n = 11), and idiopathic CP (n = 17) group.

involved organs.[37] This discrepancy is possibly caused by the shift of T_H2 cells from the periphery to local tissues, or different disease stages.

Different from T_H1/T_H2 immune balances, the role of Tregs in AIP is still unclear. Animal models have demonstrated that decreased Tregs induce various autoimmune diseases including

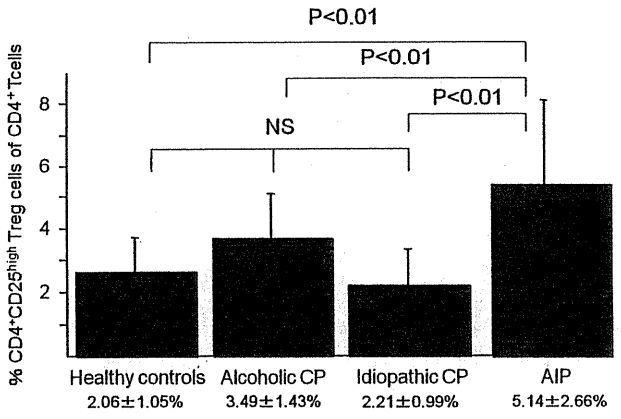

FIGURE 6. Percentage of $CD4^+$ $CD25^{high}$ Tregs. $CD4^+CD25^{high}$ Tregs were significantly increased in patients with AIP (n = 31, 4.99% ± 2.70%) compared with alcoholic CP (n = 11, 3.49% ± 1.43%), idiopathic CP (n = 17, 2.24% ± 1.01%), and healthy control groups (n = 16, 2.60% ± 1.05%, $P < 0.05$).

pancreatitis models.[38] Recent studies of immune tolerance and allergy showed that high doses of antigen exposure cause both immune deviation of T_H2 response in favor of a T_H0/T_H1, and the generation of IL-10– and transforming growth factor β (TGF-β)–producing Tregs.[39] High-dose antigen exposure inhibits the usual T_H2 T-cell response activation and/or maintenance. In addition, interferon γ and IL-10 induce preferential switching of B-cell response in favor of producing IgG and IgG4 antibodies, respectively, and possibly IgA antibodies under the influence of TGF-β.[40] Recent reports showed that $CD4^+$ $CD25^{high}Foxp3^+$ Tregs also produce IL-10.[41–43] In general, high amounts of IL-10–producing Tregs are well known as type 1 regulatory (Tr1) cells. These Tregs also produce TGF-β. There is some evidence in adult humans that constitutive $CD4^+CD25^{high}$ Tregs and inducible IL-10– and TGF-β–secreting Tr1 cells represent overlapping populations, based on CD25 expression on $CD4^+$ Tr1 cells.[39] $CD4^+CD25^{high}$ Tregs also produce IL-10 to educate the antigen-presenting cells.[44] Akitake et al[45] reported that peripheral blood mononuclear cells (PBMCs) isolated from a patient with AIP exhibited enhanced production of IgG4 and IL-10 upon stimulation with Toll-like receptor ligands, compared with those from a healthy control. We did not examine immunological relations with Toll-like receptor in AIP patients, but this will need examination in the future. Our previous findings showed increasing inducible memory Tregs but decreasing naive Tregs in the peripheral blood of patients with IgG4-related AIP.[25,46] In another study of IgG4-related sclerosing cholangitis, prominent Tregs infiltration was observed in the liver biopsy specimens.[25,34,37]

In 1999, Hutloff et al[47] reported that ICOS is a costimulatory molecule in the CD28 family, whose expression is induced during the activation of CD4 T cells. Most notably, it is expressed on the T-cell subset found in B-cell follicles.[47] It is also associated with the production of IL-10 by T cells.[47] Inducible costimulator ligand, also known as B7RP-1,[48] B7-H2,[49] or B7h,[50,51] is expressed on resting B cells, dendritic cells, and activated monocytes. Inducible costimulator–positive expression is low on naive human and murine cells and is up-regulated within hours after T-cell receptor engagement.[47,48,52] Inducible costimulator expression appears to be higher on T_H2 $CD4^+$ T cells than on T_H1-$CD4^+$ T cells.[52,53] After activation, ICOS expression persists on recently activated as well as memory T_H1 and T_H2 $CD4^+$ T cells.[53,54]

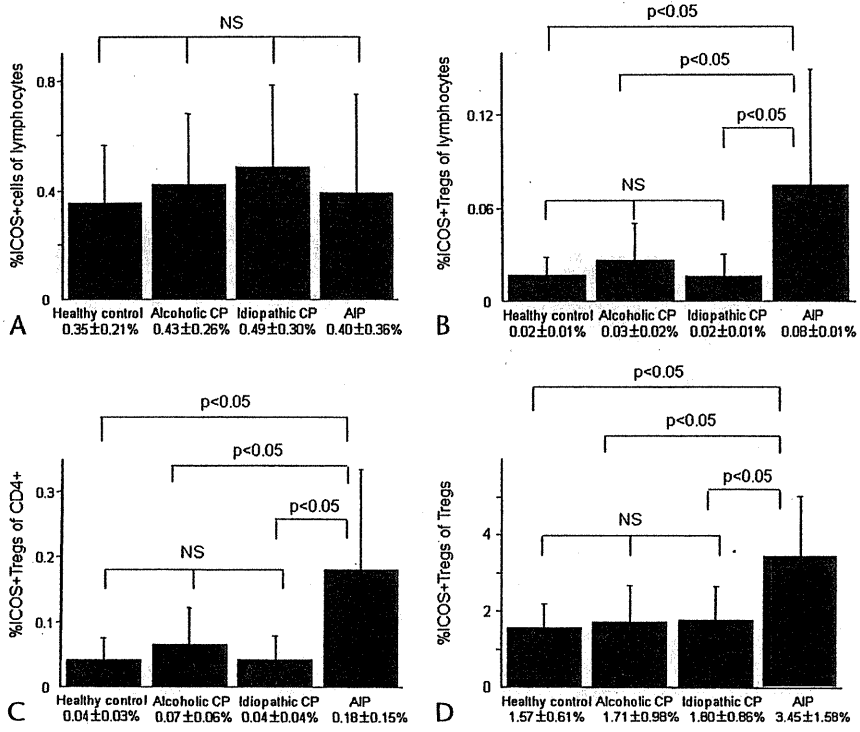

FIGURE 7. Percentage of $ICOS^+$ cells and $ICOS^+$ Tregs. There is no difference in these $ICOS^+$ lymphocytes (%$ICOS^+$ cells of lymphocytes) between patients with AIP (n = 31), alcoholic CP (n = 11), idiopathic CP (n = 17), and healthy control (n = 16). Inducible costimulator–positive Tregs (%$ICOS^+$ Tregs of lymphocytes, $CD4^+$ cells, and Tregs) were significantly increased in patients with AIP compared with those with alcoholic CP, those with idiopathic CP, and healthy control ($P < 0.05$).

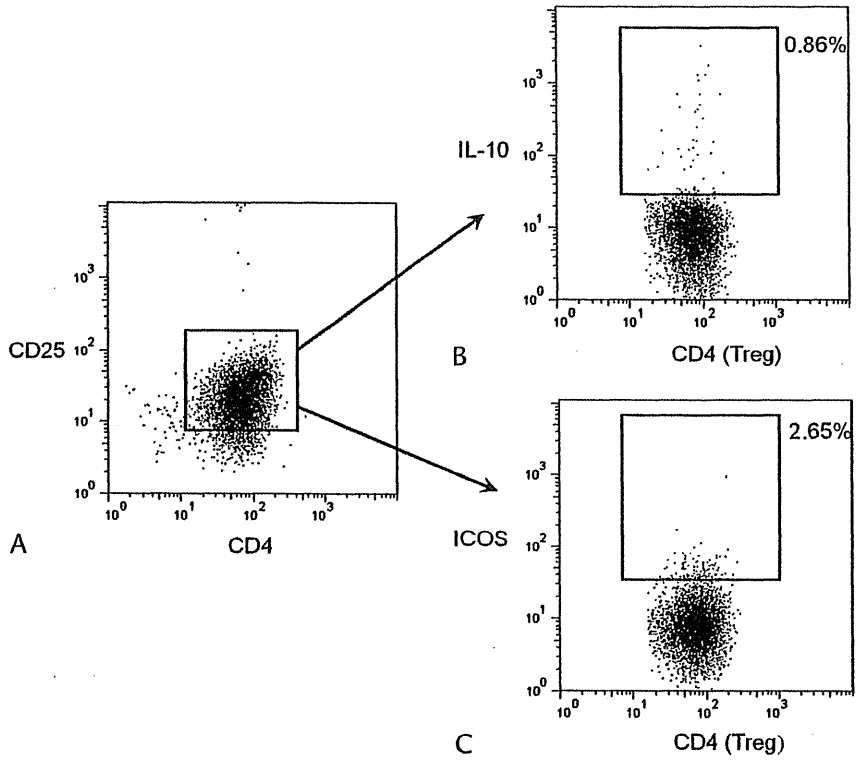

FIGURE 8. Flow cytometric analysis of Tregs (A) that express IL-10 (B) and ICOS (C) in a healthy control. The ratio of IL-10$^+$ Tregs in total Tregs (%IL-10$^+$ Tregs) was 0.86% (B). The ratio of ICOS$^+$ Tregs in total Tregs (%ICOS$^+$ Tregs) was 2.65% (C).

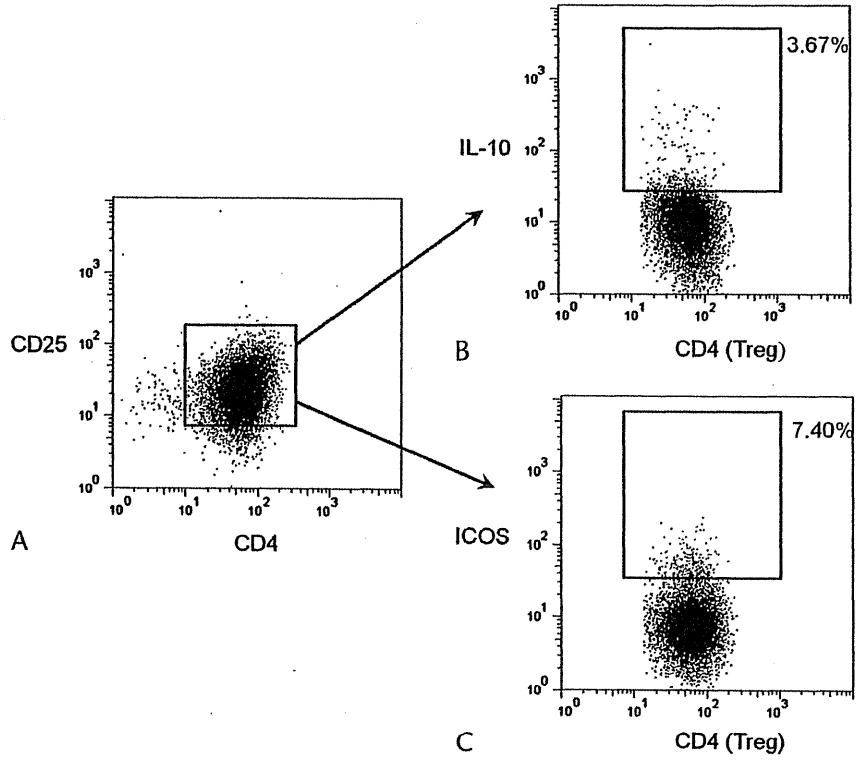

FIGURE 9. Flow cytometric analysis of Tregs (A) that express IL-10 (B) and ICOS (C) in an AIP patient. %IL-10$^+$ Tregs of total Tregs was 3.67% (B). %ICOS$^+$ Tregs was 7.40% (C).

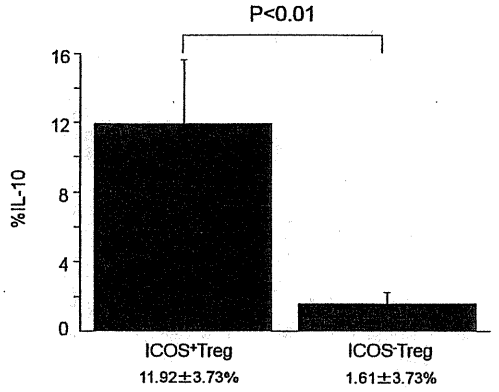

FIGURE 10. The ratio of IL-10$^+$ Tregs in ICOS$^+$ Tregs and ICOS$^-$ Tregs with AIP patients (% IL-10$^+$) (n = 16). Interleukin 10–positve cells were significantly increased in ICOS$^+$ Tregs compared with ICOS$^-$ Tregs ($P < 0.05$).

Ex vivo, the levels of ICOS expression correlate with a cytokine production pattern; peripheral T cells are either IL-10–producing (high ICOS); IL-4–, IL-5– and IL-13–producing (medium ICOS); or IL-2–, IL-3–, IL-6–, and interferon γ–producing cells (low ICOS).[55] Moreover, ICOS expression is highly expressed on Tregs, and blocking ICOS function on these cells abrogates their regulatory capacity in diabetic lesions[56] and also in an allergic asthma model,[28] possibly through lack of inhibitory IL-10 production. Some studies also suggest that intermediate ICOS expression is associated with high production of T_H2 cytokines, whereas high levels of ICOS predominantly translate into high IL-10 production.[55] This is in concordance with the recent finding that ICOS expression is high on IL-10–producing Tregs, which require ICOS presence for optimal regulatory function.[28,56] Recently, Ito et al[30] reported that expression of ICOS by human Foxp3$^+$ Tregs was shown to distinguish 2 subsets: ICOS$^+$Foxp3$^+$ Tregs inhibited dendritic cell function via IL-10, and T cells via TGF-β, whereas ICOS$^-$Foxp3$^+$ Tregs used TGF-β only.

In the present study, we identified that circulating ICOS$^+$ CD4$^+$CD25^{high} Tregs expressing IL-10 were significantly increased in the peripheral blood of the patients with IgG4-related AIP. We also confirmed abundant infiltration of CD4$^+$CD25^{high} Tregs in the pancreas tissues of IgG4-related AIP in addition to the liver of IgG4-related sclerosing cholangitis. In general, it has been reported that CD4$^+$CD25^{high}Foxp3$^+$ Tregs[41–43] producing IL-10 are regulated by the ICOS molecule. However, the involvement of the ICOS molecule on Tregs in AIP is still unknown. Therefore, to clarify the role of IgG4 in IgG4-related AIP, in the present study, we focused on the relations among serum levels of IgG4, ICOS molecules, IL-10, and Tregs in the peripheral blood and the pancreas. In addition to increasing numbers of ICOS$^+$ Tregs expressing IL-10, the ratio of ICOS$^+$ Tregs to ICOS$^-$ Tregs in AIP was similar to control subjects, which suggested unpaired function (data not shown).

Taken together, our findings suggested that increasing IL-10 secreted from ICOS$^+$ Tregs switched B cells to produce IgG4 in the periphery and abundant infiltration of IgG4-positive cells in the pancreas, which consequently may function to inhibit immune responses against inflammation (Fig. 11). According to this theory, AIP patients may be exposed to high doses of unknown disease-related antigens such as lactoferrin, carbonic anhydrase II,[8,38] or pancreatic secretory trypsin inhibitor,[57] resulting in activation of both T_H1 type immune cells and Tregs suppressing T_H2-type immune cells. However, the actual mechanism of infiltration of ICOS$^+$ Tregs from peripheral blood into

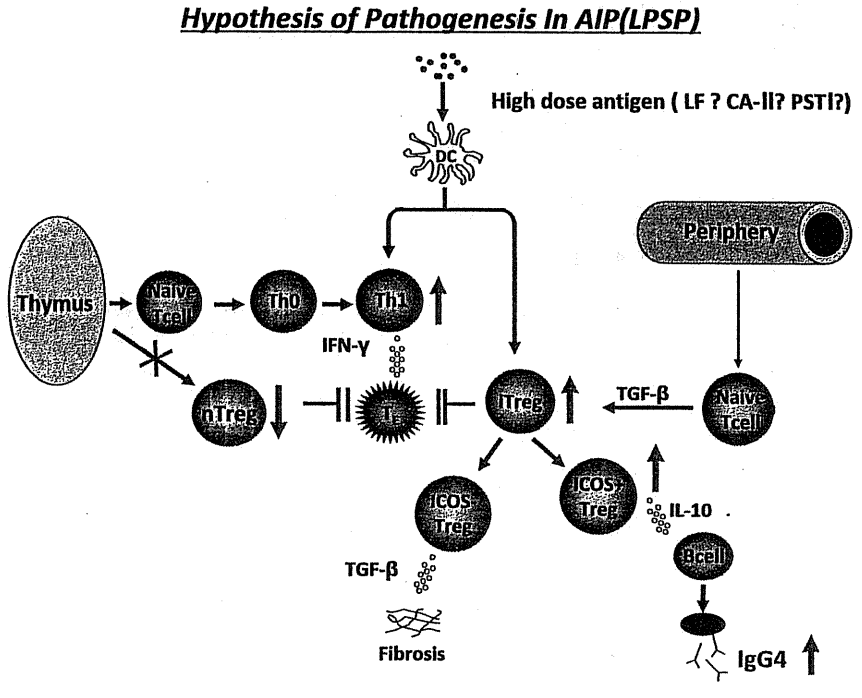

FIGURE 11. Hypothesis of AIP. High-dose antigens (carbonic anhydrase II, lactoferrin, or PST1) induce Tregs from periphery. Increased ICOS$^+$ Tregs correlate with the production of IL-10, which may influence the switching of B cells to IgG4-producing plasmacytes and the production of serum IgG4. On the other hand, ICOS$^-$ Tregs, which produce TGF-β, may influence fibrosis in AIP. Moreover, decreased naive Tregs may be involved in the pathogenesis of AIP.

the pancreatic tissues and fibrosis in AIP remains unclear. Future studies should be addressed to clarify this point. In conclusion, increased quantities of $ICOS^+CD4^+CD25^{high}$ Tregs may influence IgG4 production in AIP.

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

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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

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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.

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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

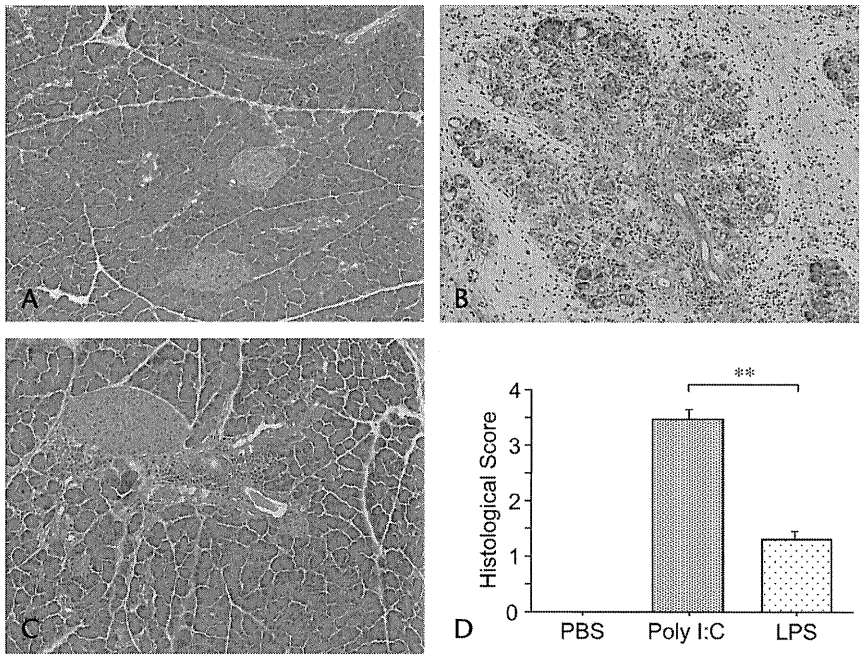

FIGURE 1. Histopathological examination of the pancreas and histological scoring of pancreatitis in MRL/Mp mice. Representative pancreatic sections stained with hematoxylin and eosin: 12-week treatment with PBS (A), 12-week treatment with poly I:C (B), and 12-week treatment with LPS (C). After the mice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

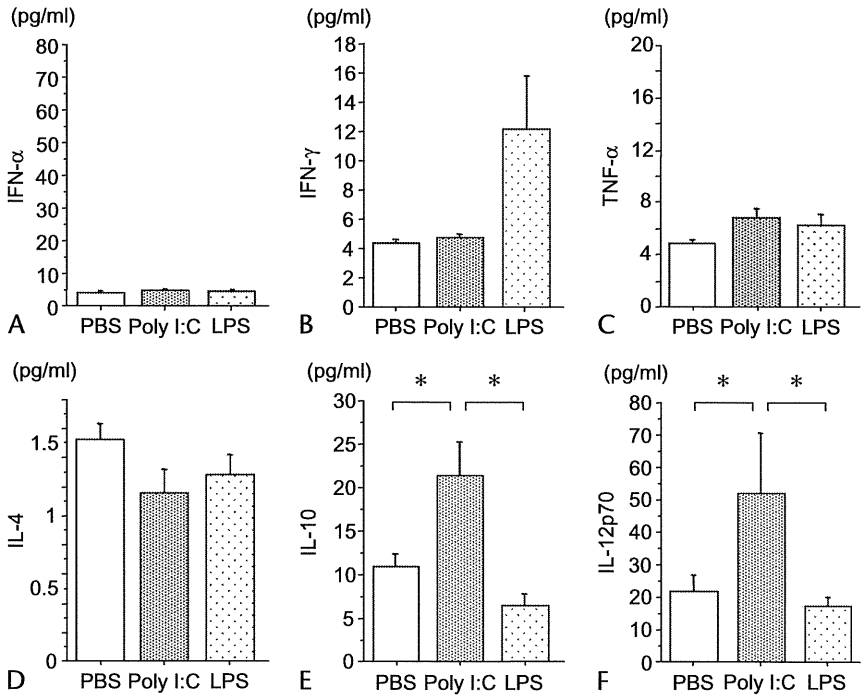

FIGURE 2. Serum cytokine levels in MRL/Mp mice treated with PBS, poly I:C, or LPS for 12 weeks: IFN-α (A), IFN-γ (B), TNF-α (C), IL-4 (D), IL-10 (E), and IL-12p70 (F). There were no significant differences in IFN-α, TNF-α, or IL-4 levels between PBS-treated, poly I:C-treated, and LPS-treated mice. Interferon-γ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

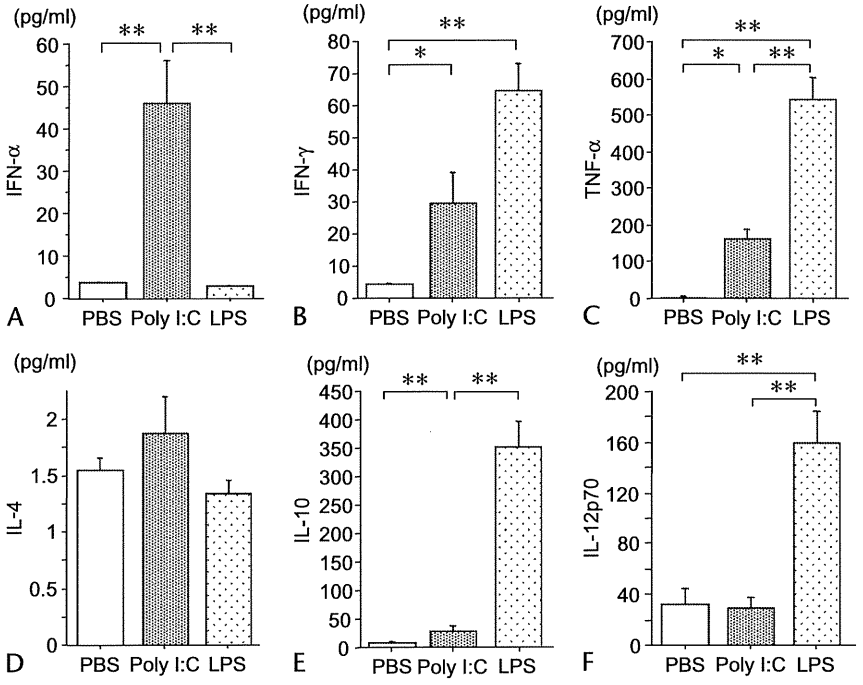

FIGURE 3. Serum cytokine levels in MRL/Mp mice treated with a single injection of PBS, poly I:C, or LPS: IFN-α (A), IFN-γ (B), TNF-α (C), IL-4 (D), IL-10 (E), and IL-12p70 (F). Interferon-α levels were increased in mice treated with poly I:C. Interferon-γ and TNF-α levels were elevated in poly I:C-treated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

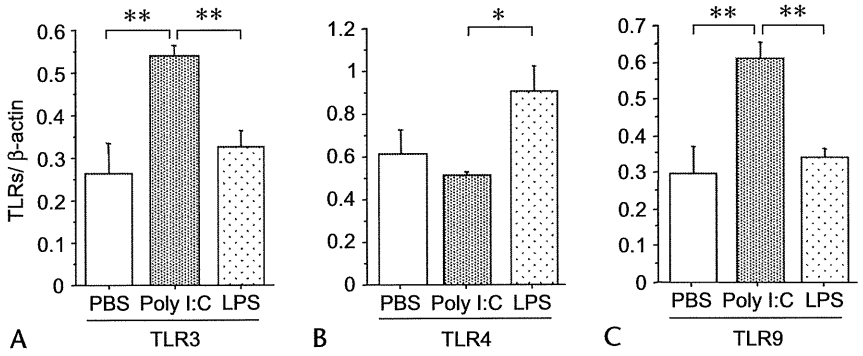

FIGURE 4. Toll-like receptor mRNA expression in pancreatic tissues of MRL/Mp mice treated with single injections of PBS, poly I:C, or LPS: TLR3 (A), TLR4 (B), and TLR9 (C). Toll-like receptor 3 and TLR9 mRNA expression were significantly increased in mice treated with poly I:C. Lipopolysaccharide injection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

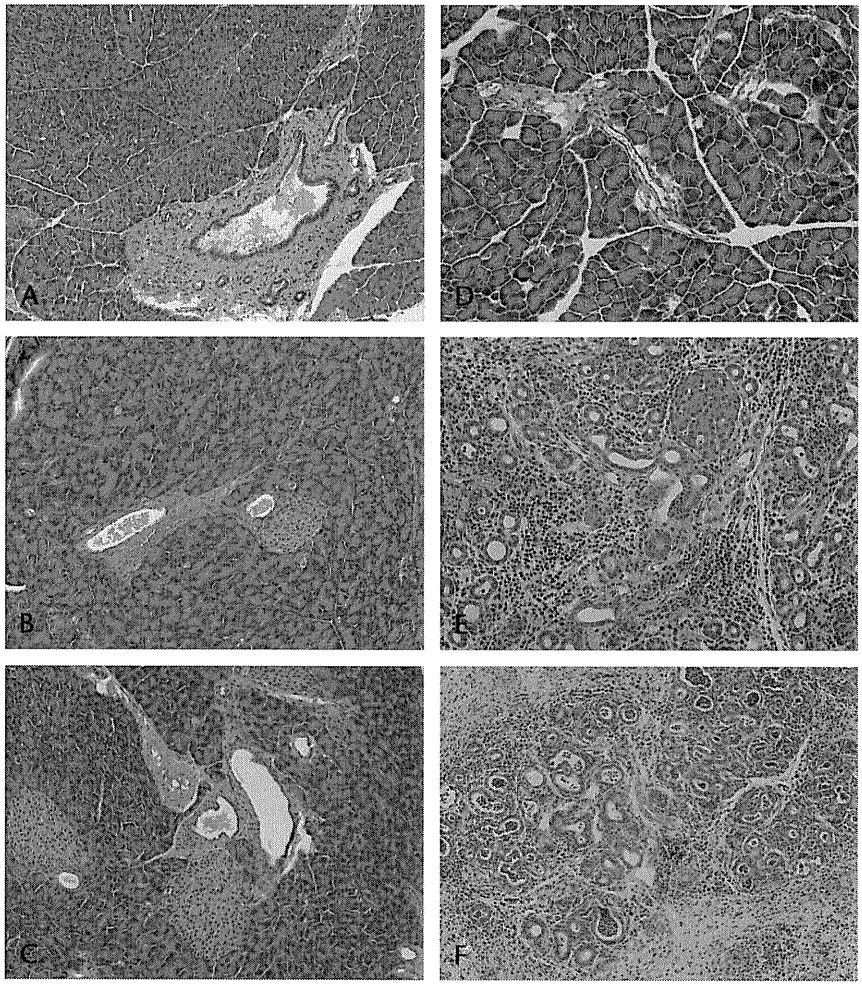

FIGURE 5. Histopathological examination of the pancreas of C57BL/6 WT and IL-10KO mice. Representative pancreatic sections stained with hematoxylin and eosin: 8-week treatment of WT mice with PBS (A), poly I:C (B), or LPS (C); 8-week treatment of IL-10KO mice with PBS (D), poly I:C (E), or LPS (F). Eight-week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

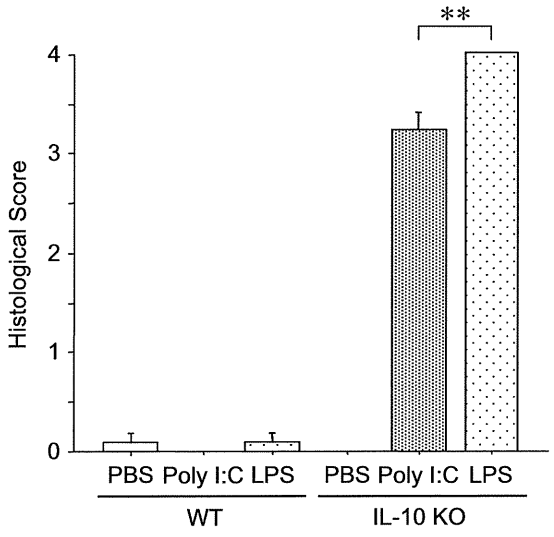

FIGURE 6. Histological pancreatitis scores for WT and IL-10KO mice. The severity of pancreatitis was scored using a scale of 0 to 4, which was based on a histological scoring system. Histological scores for pancreatitis were highest in LPS-treated mice (4.0 ± 0) and were higher than in IL-KO mice (3.3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

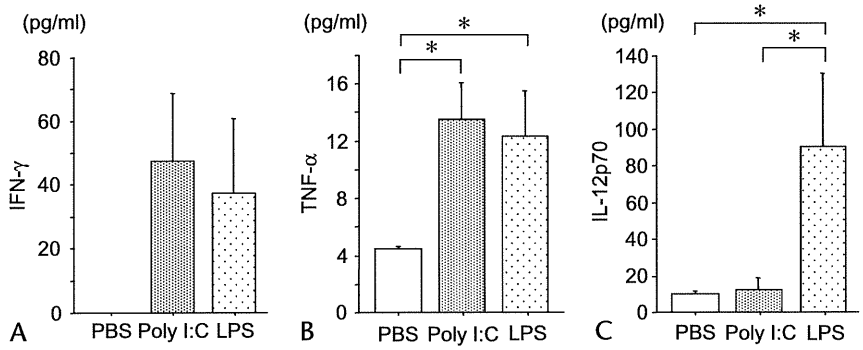

FIGURE 7. Serum cytokine levels in IL-10KO mice treated with PBS, poly I:C, or LPS for 8 weeks: IFN-γ (A), TNF-α (B), and IL-12p70 (C). Although the increase in IFN-γ levels was not significant, TNF-α levels increased in mice treated with poly I:C or LPS ($^{*}P < 0.05$). Interleukin 12p70 levels were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

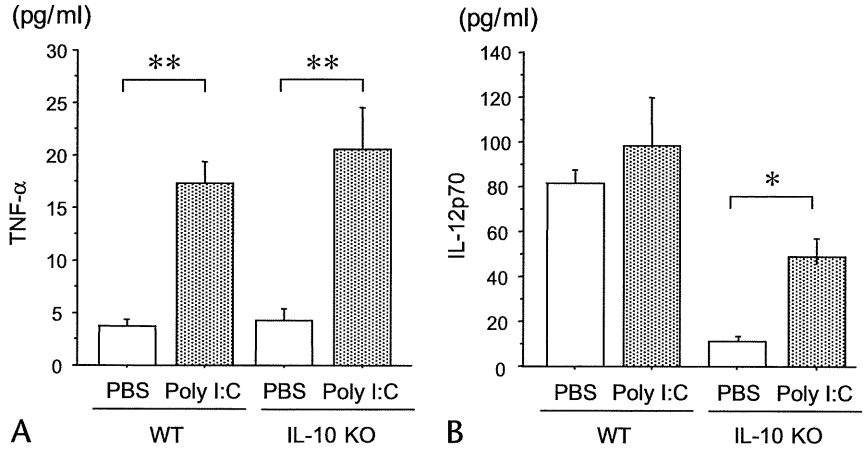

FIGURE 8. Serum cytokine levels in IL-10KO mice treated with single injections of PBS, poly I:C, or LPS: TNF-α (A) and IL-12p70 (B). Tumor necrosis factor-α levels were elevated in WT and IL-KO mice after a single injection of poly I:C. However, an elevated IL-12p70 level was observed only in IL-10KO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

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Autoimmune Pancreatitis and Diagnostic Criteria

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Abstract: Autoimmune pancreatitis is a unique form of chronic pancreatitis with autoimmune phenomena, including hypergammaglobulinemia, lymphoplasmacytic infiltration, and responsiveness to corticosteroid therapy. Autoimmune pancreatitis tends to affect elderly males and it presents with pancreatic swelling and irregular narrowing of the pancreatic duct. The symptoms of autoimmune pancreatitis mimic the clinical features of pancreatic cancer; thus, it is important to differentiate between the two conditions. Autoimmune pancreatitis is also characterized by high serum IgG4 concentrations and infiltration of IgG4-bearing plasma cells into the pancreatic tissue. Although these are considered serological and histological hallmarks of autoimmune pancreatitis, the role of IgG4 in the pathogenesis of the disease remains unclear. Furthermore, many cases are complicated by extra-pancreatic manifestations with pathological findings similar to those observed in the pancreatic lesions; these extra-pancreatic manifestations tend to respond favorably to corticosteroid therapy. Autoimmune pancreatitis is now regarded as a member of a new class of IgG4-related disease. Due to inconsistencies in the diagnostic criteria for autoimmune pancreatitis, there is a need for an international consensus on this disease.

Keywords: Autoimmune pancreatitis, IgG4, IgG4-related disease.

INTRODUCTION

Autoimmune pancreatitis (AIP) is a unique form of chronic pancreatitis [1] characterized by high serum IgG4 concentrations [2] and infiltration of large numbers of IgG4 bearing plasma cells into pancreatic tissue [3]. AIP is associated with hypergammaglobulinemia, histological evidence of lymphoplasmacytic inflammation (i.e., lymphoplasmacytic sclerosing pancreatitis [LPSP]), and positive responsive to glucocorticoid treatment, features suggesting that an autoimmune mechanism is involved in its pathogenesis [1, 4-7]. Most patients are elderly males who exhibit clinical features such as obstructive jaundice, pancreatic swelling, and irregular narrowing of the pancreatic ducts [8], all of which mimic the features indicative of pancreatic cancer [4-6]. Thus, during diagnostic workup, it is important to differentiate AIP from pancreatic cancer and biliary malignancy [9]. AIP may also be complicated by a variety of extra-pancreatic lesions [10-12], which have pathological characteristics similar to those of the pancreatic lesions in AIP, including lymphoplasmacytic infiltration, infiltration of large numbers of IgG4-bearing plasma cells, storiform fibrosis, and obstructive phlebitis; together, these lesions are indicative of a comprehensive pathological condition known as IgG4-related disease [3, 13-15]. Recently, AIP was recognized as a member of a new class of IgG4-related disease.

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A second type of AIP has also been described, based pathologically on granulocyte infiltration into the pancreatic duct epithelium [16]. These pathological findings have been termed idiopathic duct-centric chronic pancreatitis (IDCP) [17] or AIP with granulocytic epithelial lesions (AIP with GEL) [18]. Although these forms of AIP seem to be prevalent in Europe [18, 19], their clinical features remain obscure [16]. Accordingly, this article will focus on the LPSP form of AIP [7].

EPIDEMIOLOGY

AIP is a rare disease. A nationwide survey in Japan in 2002 showed a prevalence of 0.82/100,000 inhabitants, corresponding roughly to 2% of patients with chronic pancreatitis [20, 21]. Due to the formulation of diagnostic criteria in 2002, resulting in more consistent recognition of this disease, the number of patients with AIP in Japan has increased [22]. This disease primarily affects elderly males [1, 5, 6]. The 2002 nationwide survey in Japan showed that the male: female ratio of AIP patients was 2.85:1, and that the age of disease onset was over 45 years in 95% of patients [20]. In accordance with this survey, we found that 83% of patients with AIP were males, and the mean age of onset was 62.2 years [6, 23].

CLINICAL FINDINGS

Obstructive jaundice is a major symptom at AIP onset, observed in 60~70% of patients [1, 5, 6, 8, 23]. This is mainly due to stenosis in the lower bile duct caused by swelling of the pancreatic head, though concentric bile duct

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wall thickening may also contribute to the stenosis [24, 25]. Obstructive jaundice responds well to corticosteroid therapy and sometimes subsides spontaneously. In contrast to patients with ordinary chronic pancreatitis (e.g., the alcoholic type), patients with AIP rarely complain of severe abdominal pain [1, 5, 6, 8]. Diabetes mellitus (DM), primarily type II, is observed in about half of these patients, and glucose intolerance is sometimes ameliorated by corticosteroid therapy [26-28]. However, older age is associated with higher rates of new development or exacerbation of diabetes mellitus [26]. Diarrhea or steatorrhea due to severe exocrine dysfunction is rare, but exocrine function is somewhat reduced, as determined by the bentiromide and secretin tests [28]; however, function may be restored by corticosteroid therapy [29].

AIP is sometimes complicated by a variety of extra-pancreatic involvements [10-12, 30], including lachrymal and salivary gland lesions [31], respiratory lesions [32-34], sclerosing cholangitis [7, 35, 36], and retroperitoneal fibrosis [3]. These complications are associated, respectively, with lachrymal or salivary gland swelling; cough, shortness of breath, or dyspnea, and lumbago due to hydronephrosis. Further details are provided below, in the section on 'Extra-pancreatic lesions'.

LABORATORY TESTS

Blood Chemistry

In 70~80% of patients, blood chemistry tests have shown abnormal findings related to obstructive jaundice, including elevated serum concentrations of bilirubin, biliary enzymes, and transaminase [6, 23]. Pancreatic enzymes were mildly or moderately elevated in 60% of patients [6]. The tumor-associated carbohydrate antigen, CA19-9, was elevated in 50% of patients, probably due to cholestasis rather than to a malignant mechanism [6, 23].

Immunological Tests

Gamma-globulin, IgG, and IgE were found to be elevated in 60%, 70%, and 33% of AIP patients, respectively [6, 23], with significant elevations of IgG4 observed in 90% of patients [2, 6, 23]. Interestingly, decreased IgA and IgM concentrations were observed in patients with increased IgG4 [37]. Positivity for anti-nuclear antibody and rheumatoid factor was observed in 40% and 30% of patients, respectively; with positivity for other antibodies, including anti-thyroglobulin and anti-thyroid peroxidase antibodies, observed in 10~20% of patients [6, 23]. Anti-SS-A/Ro, anti-SS-B/La, and anti-mitochondrial antibodies specific to Sjogren's syndrome or primary biliary cirrhosis (PBC) are rarely observed [6, 23]. Taken together, these findings suggest that patients with AIP tend to produce various autoantibodies that are not disease-specific.

Complement

Serum concentrations of complement proteins C3 and C4 were found to be reduced in 36% of patients, suggesting that the complement activation system may contribute to the pathogenesis of AIP [38]. Reduced complement and IgG1, but not IgG4, concentrations have been closely associated with high serum immune complex (IC) concentrations [38]. The complement activation system consists of classical, alternative, and mannose-binding lectin (MBL) pathways. Reduced C4 concentrations suggest that the classical, not the alternative, pathway is involved in the pathogenesis of AIP; the contribution of the MBL pathway has not yet been determined [38]. Although overproduction of as yet undetermined IgG1 autoantibodies and immune complexes in patients with AIP may activate the classical pathway, complement deposits have never been detected in pancreatic lesions of AIP. Nevertheless, tubulointerstitial nephritis found in AIP is sometimes accompanied by hypocomplementemia [39, 40] and C3 deposits in tubular basement membranes [39].

Pancreatic Function

High HbA1c levels indicative of reduced endocrine function have been observed in 50~70% of patients with AIP [6, 26]. Although corticosteroid therapy has been shown to have a beneficial effect on the clinical course of DM in approximately 50% of patients with AIP [26, 27], corticosteroid therapy also had a negative effect in some, particularly older, patients [26]. Bentiromide tests have indicated that 66% of patients with AIP have reduced exocrine function [6]. Moreover, secretin tests have shown that 8% of AIP patients have 1-factor abnormalities, indicating reductions in volume, and 42% have 2-factor abnormalities, indicating reductions in volume and amylase output. These results suggested that duct cells possessing functional bicarbonate secretion may have been intact [28]. Another study, however, found evidence of decreased bicarbonate secretion by ductal cells, a decrease that was closely associated with the aberrant localization of cystic fibrosis transmembrane conductance regulator (CFTR) in these cells [29]. Histologically, AIP has been associated with lymphoplasmacytic cell infiltration into tissue surrounding the pancreatic ducts, but basement membranes were intact. Corticosteroid therapy can result in regeneration of acinar cells, restoring the secretion of digestive enzymes [29].

IMAGE FINDINGS (REFER TO DR. FUJINAGA'S REVIEW)

US, EUS and IDUS

Abdominal ultrasonography (US) of patients with AIP typically shows a characteristic sonolucent swelling of the pancreas (diffuse enlarged low-echo pancreas with a scattered high echo spot), giving it a so-called "sausage-like" appearance (Fig. **1**) [4]. Usually, the main pancreatic duct (MPD) is not visible, but dilatation of the common bile duct is frequently observed. Bile duct dilatation is likely due to stenosis of the lower bile duct caused by pancreatic head swelling and/or inflammatory thickening of the bile duct wall [1, 24]. Endoscopic US (EUS) typically shows a relatively diffuse homogeneous hypo-echoic pattern and linear or reticular hyper-echoic inclusions [41]. EUS and intraductal US (IDUS) imaging show concentric wall thickening of the distal common bile duct [24]. This wall thickening may extend from the extra-hepatic to the intra-hepatic bile duct system, even when cholangiography shows

normal findings. Furthermore, thickening of the gallbladder wall usually occurs [42].

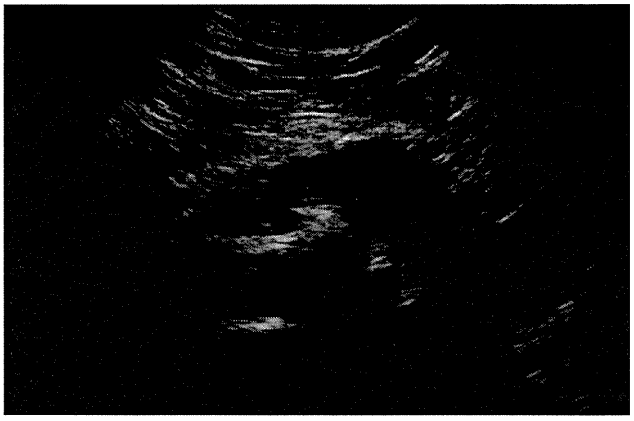

Fig. (1). Abdominal ultrasonography (US) of a patient with autoimmune pancreatitis. Sonolucent swelling is observed in the body and tail of the pancreas.

CT

Similar to US, abdominal computed tomography (CT) of patients with AIP typically shows a diffuse or focal enlargement of the pancreas [1, 4, 43, 44]. Contrast-enhanced CT has shown delayed homogeneous enhancement in pancreatic lesions, representing widespread loss of the parenchyma and severe fibrosis [1, 43]. A characteristic finding of contrast-enhanced CT is a capsule-like, low-density rim surrounding the pancreas, which is prominent at the body and tail regions and is indicative of severe fibrotic changes [43]. The presence of a capsule-like rim is highly indicative of AIP. Another characteristic feature is a straight margin with a sharp outline and an absence of the pancreatic cleft; however, aged pancreases generally show lobulated margins [44].

MRI

Magnetic resonance imaging (MRI) also typically shows a diffuse or focal enlargement of the pancreas, a delayed enhancement, and/or a capsule-like rim [43, 44]. Fat-suppressed T1-weighted images of patients with AIP show reduced signal intensity in the pancreas compared with the liver [43]; in contrast, the signal intensity of a healthy pancreas is higher than that of the liver. T2-weighted MR images generally show high signal intensity [43, 44], reflecting severe lymphoplasmacytic infiltration. T2-weighted MR images with MR cholangiopancreatography (MRCP) sometimes show the MPD clearly penetrating through the lesion mass (duct penetrating sign), a finding useful for differentiating between AIP and pancreatic cancer [45]. Dynamic T2-weighted MR images sometimes show a capsule-like rim [43, 44]. Although MRCP may reveal a diffuse narrowing of the MPD [44], current MRI resolution makes it difficult to evaluate MPD findings accurately, and MRCP is not recommended for diagnosing AIP in Japan [5, 9].

ERCP

Endoscopic retrograde cholangiopancreatography (ERCP) typically shows a characteristic irregular narrowing with 1) a narrow caliber, 2) wall irregularity, and 3) diffuse or local distribution (Fig. **2**) [1, 4, 46-50]. In a typical case, the narrowing extends over one third of the entire pancreatic duct, with diffuse change observed in over half of these patients. Imaging in patients with focal AIP typically shows a characteristic change in the MPD over a restricted area, a finding similar to those in pancreatic cancer, thus requiring differentiation [49]. Even when the MPD change is restricted, the absence of upstream dilatation and an interrupted distribution may be useful for differentiating AIP from pancreatic cancer [46]. During follow-up, restricted changes can become diffuse changes [47]. Most patients also exhibit stenosis in the lower bile duct, which can be caused by pancreatic head swelling and/or inflammatory thickening of the bile duct wall [1, 24, 51]. Bile duct stenosis sometimes extends into the extra- or intra-bile duct systems [36, 48, 51]; in these patients, it is necessary to differentiate between AIP and primary sclerosing cholangitis [48, 51-53] or cholangiocarcinoma [53].

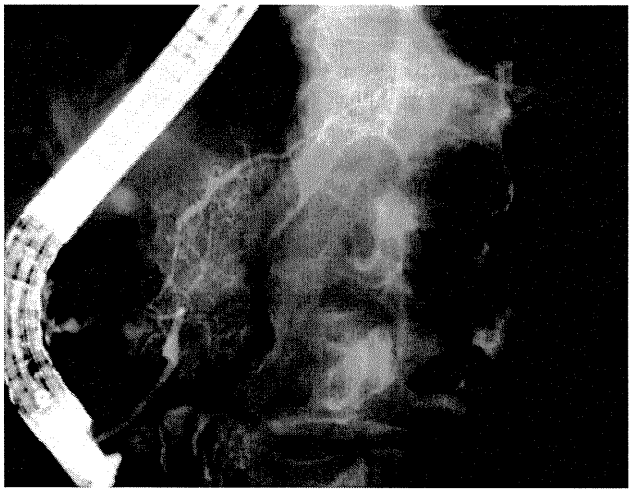

Fig. (2). ERP in a patient with autoimmune pancreatitis, showing diffuse irregular narrowing of the main pancreatic duct.

Gallium Scintigraphy and FDG-PET

Gallium scintigraphy [30, 33, 54-56] and positron emission tomography with a F-18 2-fluoro-2-deoxy-D-glucose tracer (FDG-PET) [57-71] are imaging methods useful for detecting both AIP and extra-pancreatic lesions. Since AIP is characterized by severe lymphocytic inflammation and since gallium-67 (Ga-67) accumulates in lymphoid cells, Ga-67 scintigraphy is a useful tool in examining patients with AIP. Before corticosteroid treatment, marked Ga-67 accumulation was observed in 67% of the pancreatic lesions in AIP patients, whereas, after 4 weeks of corticosteroid therapy, all patients were negative for Ga-67 [33]. Patients with Ga-67 positive images had significantly higher serum IgG4 concentrations than those with Ga-67 negative images, indicating that pancreatic lesions that accumulated Ga-67 were highly active [33]. Increased uptake of Ga-67 has also been observed in a variety of extra-pancreatic lesions [30], including lachrymal and salivary glands [30], hilar and mediastinal lymph nodes [33, 54, 56], retroperitoneal fibrosis [30], and renal lesions [55].

Although FDG uptake is commonly regarded as a diagnostic hallmark of pancreatic cancer, intense FDG uptake has also been observed in the pancreatic lesions of patients with AIP [57-60, 62-71]. To differentiate between AIP and pancreatic cancer, it is important to note that accumulation patterns characterized by nodular shapes is significantly more frequent in pancreatic cancer, whereas patterns of longitudinal or diffuse shape are usually indicative of AIP [67, 69]. Furthermore, heterogeneous accumulation is observed in almost all patients with AIP, whereas homogeneous accumulation is characteristic of pancreatic cancer [67]. Solitary localization in the pancreas is significantly more frequent in pancreatic cancer, whereas multiple localization suggests the presence of AIP [66, 67]. In addition, reduced FDG uptake after a short course of steroid treatment may distinguish AIP from pancreatic cancer [71], although Japanese diagnostic criteria discourages facile therapeutic diagnosis based on steroid administration [9]. FDG-PET also can detect a variety of extra-pancreatic lesions, including sclerosing sialadenitis [62, 67-70], hilar and mediastinal lymph nodes [67], sclerosing cholangitis [61, 62, 67, 70], retroperitoneal fibrosis [62, 67, 70], interstitial nephritis [62, 64, 69], prostate hypertrophy [63, 67, 70], and inflammatory pseudotumor [65]. Concomitant FDG uptake by these extra-pancreatic organs may support the diagnosis of AIP [67, 69].

PATHOLOGICAL FINDINGS

Gross examination of the involved pancreas typically shows a glistening white, firm or hard, enlarged mass [7, 72, 73]. The lesion may involve the whole pancreas, or be limited to one portion of the pancreas, most often the head, but also the body or tail. Lymphoplasmacytic infiltration and fibrosis are characteristic features of pancreatic lesions, a condition known as lymphoplasmacytic sclerosing pancreatitis (LPSP) [7]. Infiltrating lymphocytes are predominantly T cells, and plasma cells characteristically bear IgG4 [3, 73-75], a diagnostic hallmark of AIP. Pancreatic infiltration of IgG4-bearing plasma cells, however, may also be observed in other conditions, including pancreatic cancer and chronic pancreatitis [75, 76]. The inflammatory changes that accompany cell infiltration are most prominent around the pancreatic duct, usually resulting in stenosis or obstruction of the duct [77]. Severe fibrosis typically displays a loose texture with stromal edema [78] or a storiform arrangement. Another characteristic feature is obliterating phlebitis, consisting of marked cell infiltration into the venous wall and venous thrombosis [7, 17, 77]. Similar histological features have also been observed in extra-pancreatic lesions, including those in the salivary glands [31] and retroperitoneal regions [3]. The diagnostic criteria proposed by researchers from Japan [9], Korea [79-81], and the Mayo Clinic [75, 82] include LPSP as a typical histological feature of AIP. In contrast, researchers in Europe and the United States regard the infiltration of granulocytes into the duct epithelium, known as "idiopathic duct-centric chronic pancreatitis" (IDCP) [17] or AIP with GEL [18], as a histological feature of AIP. In Europe, the clinical features of AIP differ from those of LPSP, with the former characterized by frequent severe abdominal pain in younger patients and no gender bias, a close association with inflammatory bowel disease, and a low frequency of serum IgG4 elevation [19, 83]. These findings suggest that LPSP has distinct clinical features from IDCP and AIP with GEL, making it a distinct disease [16, 21].

DIFFERENTIAL DIAGNOSIS

Pancreatic Cancer

The clinical features of AIP mimic those of pancreatic cancer, including a preponderance in older individuals, obstructive jaundice, and a swelling or mass-forming lesion of the pancreas [1, 4-6, 23]. Pathological analysis of resected pancreatic specimens from individuals who underwent surgery for pancreatic cancer revealed that 2-3% of these patients had LPSP [84, 85]. Because AIP responds favorably to corticosteroid treatment, differentiation between the two conditions is mandatory.

Among the clinical findings differentiating these two conditions are abdominal pain, body weight loss, obstructive jaundice, and extra-pancreatic lesions [86]. Severe and persistent abdominal pain that may require narcotics is frequently present in the advanced stages of pancreatic cancer; in contrast, abdominal pain is mild in AIP [1, 5, 6]. Body weight loss is common in pancreatic cancer, but rare in AIP. Jaundice is progressive in pancreatic cancer, but fluctuates or may subside spontaneously in AIP [1, 5, 6, 21, 23]. AIP is often accompanied by a variety of extra-pancreatic lesions, including sialadenitis [31], thyroiditis [87], hilar or mediastinal lymphadenopathy [33], sclerosing cholangitis [36], and retroperitoneal fibrosis [3]; in contrast, extra-pancreatic lesions observed in patients with pancreatic cancer were found to be limited to lower bile duct stenosis, metastatic lesions, and direct invasion. Furthermore, the presentation of extra-pancreatic lesions is considered diagnostic for AIP [11, 88].

Among various serum markers, IgG4 has the highest sensitivity, specificity, and accuracy in differentiating between AIP and pancreatic cancer [2], though elevated serum IgG4 may also be observed in some patients with pancreatic cancer [89]. Accordingly, serum IgG4 elevation provides a useful tool for differentiation, but it cannot rule out the possibility of pancreatic cancer.

Various imaging methods are useful for differentiating between AIP and pancreatic cancer. Abdominal US, EUS, or T2-weighted MR images showing evidence of duct penetration indicates the presence of a benign pancreatic mass, including AIP [45]. CT or MRI showing a capsule-like rim is also considered a diagnostic hallmark of AIP [43]. FDG-PET showing diffuse, multiple, and heterogeneous uptake is indicative of AIP, whereas solitary, homogeneous uptake indicates pancreatic cancer [67]. Decreased FDG uptake after a course of steroid treatment can be useful for discriminating between AIP and pancreatic cancer [71].

Although AIP has several pathological characteristics, it is associated with inflammatory changes that are sometimes observed in pancreatic cancer [86]. Infiltration into pancreatic tissue of large numbers of IgG4-bearing plasma cells is a diagnostic hallmark of AIP [3], but has also been observed in patients with pancreatic cancer [75]. In addition, the synchronous occurrence of AIP and pancreatic cancer