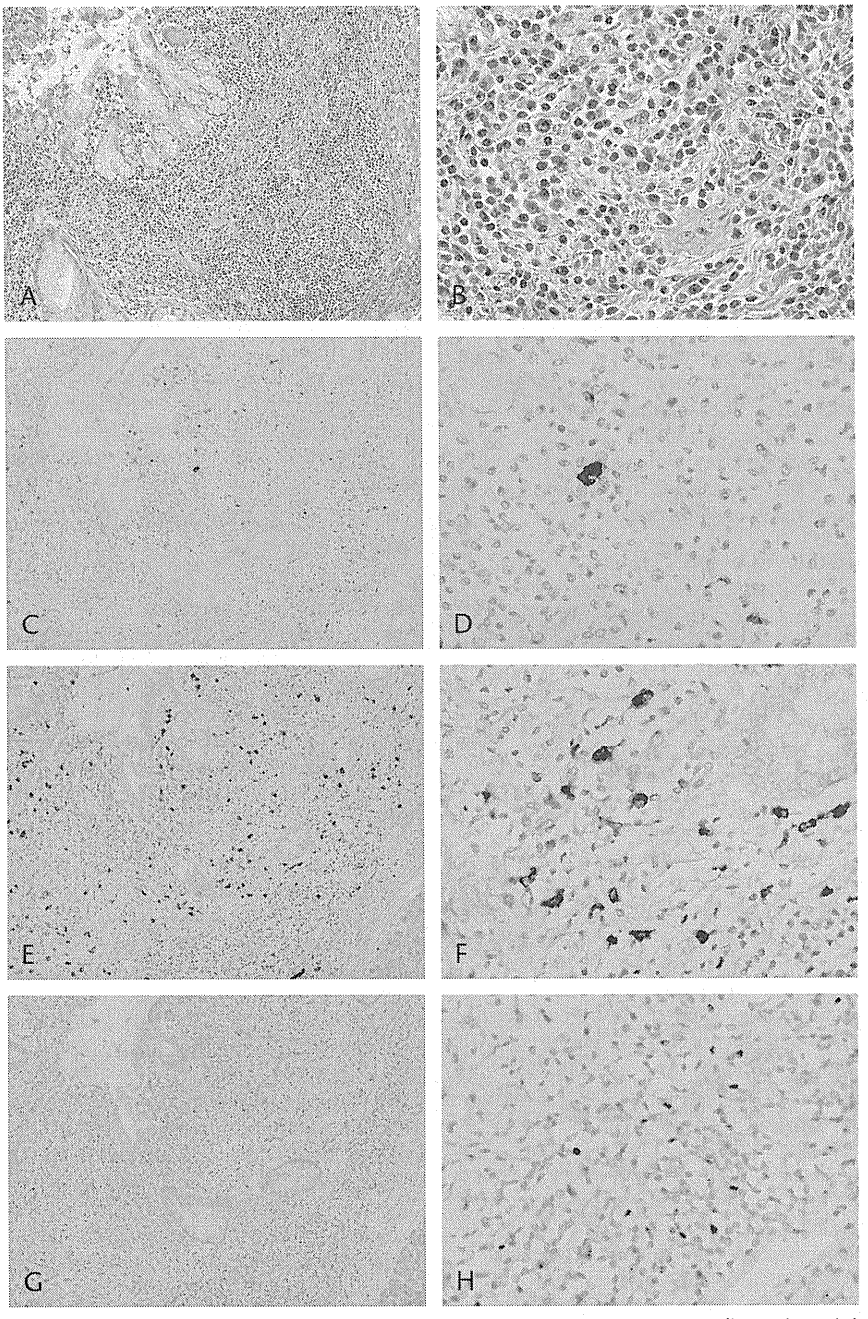

FIGURE 1. Histopathologic and immunohistochemical findings of the pancreas in LPSP. Hematoxylin-eosin staining (original magnification ×100 [A], ×400 [B]). Lymphoplasmacytic sclerosing pancreatitis showed interlobular fibrosis, atrophic pancreatic lobules, and infiltration of lymphoplasmacytes. Immunoglobulin 1–positive cells (original magnification ×100 [C], ×400 [D]) were scattered, whereas IgG4-positive (original magnification ×100 [E], ×400 [F]) and Foxp3-positive cells (original magnification ×100 [G], ×400 [H]) were abundant.

alcoholic CP (n = 9, 0.085 [SD, 0.011]; $P < 0.05$; Figs. 1–3). The ratio of IgG4/Mono to IgG1/Mono (IgG4/G1) was significantly higher in AIP (n = 9, 2.92 [SD, 1.42]) than in alcoholic CP (n = 9, 0.16 [SD, 0.08]; $P < 0.05$; Figs. 1–3A–C).

Immunohistochemical Findings of Foxp3

The ratio of Foxp3-positive cells to infiltrated mononuclear cells (Foxp3/Mono) in patients with AIP (n = 9, 0.091 [SD, 0.023]) was significantly higher than alcoholic CP (n = 9, 0.012 [SD, 0.003]; $P < 0.05$; Figs. 1–3D).

Correlation Between Foxp3/Mono and IgG4/Mono in Patients With AIP

In the group of patients with AIP, Foxp3/Mono and IgG4/Mono were positively correlated (n = 9, $P < 0.05$; $R = 0.91$) (Fig. 4).

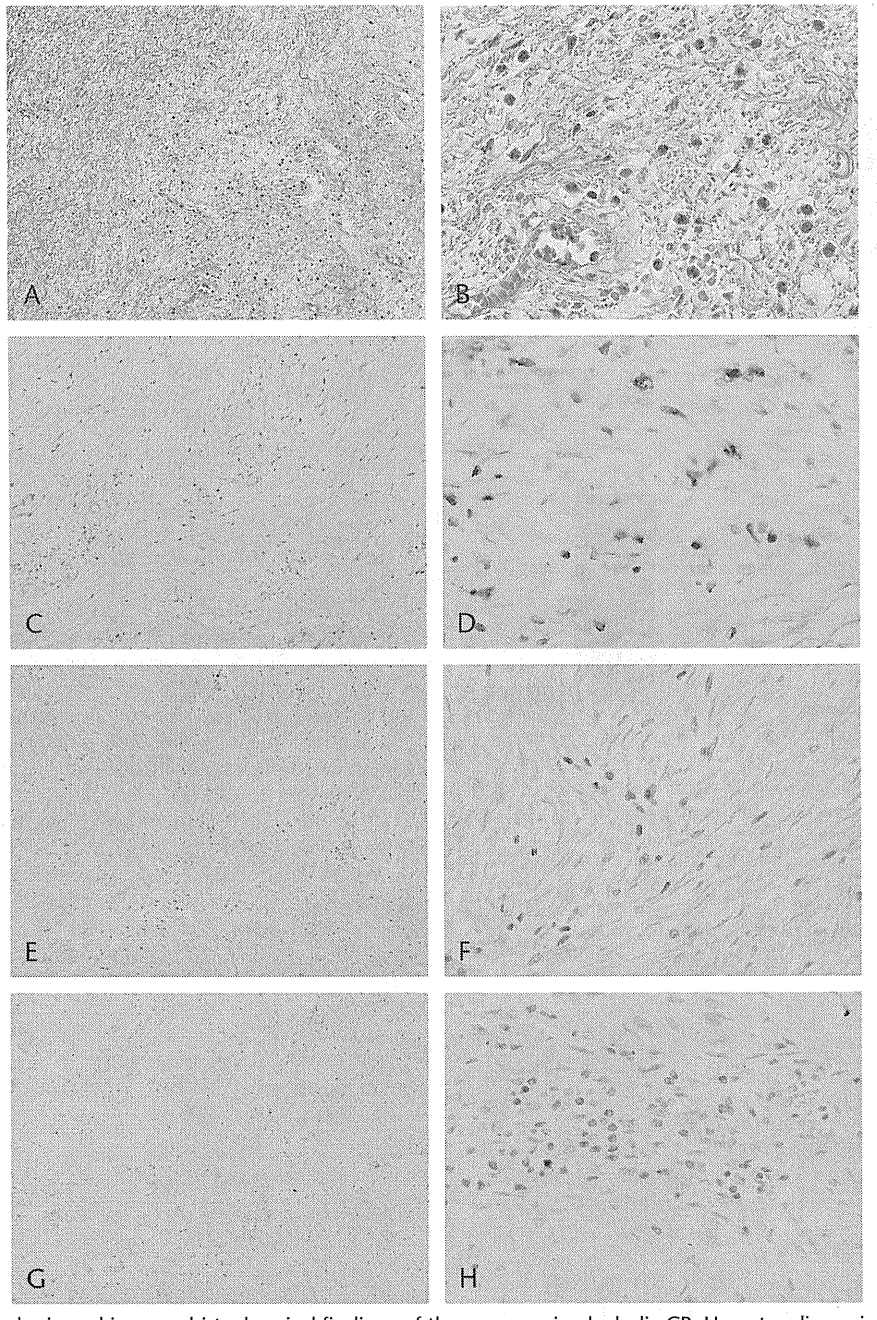

FIGURE 2. Histopathologic and immunohistochemical findings of the pancreas in alcoholic CP. Hematoxylin-eosin staining (original magnification ×100 [A], ×400 [B]). Photomicrograph of alcoholic CP showed dense fibrosis that surrounded atrophic or normal-appearing lobules. Some lobules were divided or 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

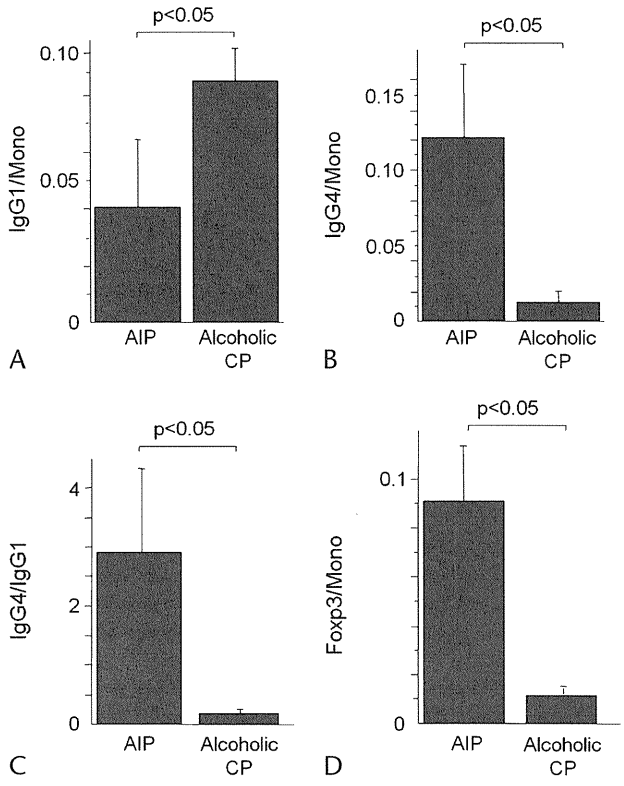

FIGURE 3. The ratio of Foxp3-, IgG1-, and IgG4-positive cells to infiltrated mononuclear cells (Foxp3/Mono, IgG1/Mono, IgG4/Mono) in patients with AIP (n = 9) and alcoholic CP (n = 9). Foxp3/Mono and IgG4/Mono in patients with AIP were significantly increased compared with alcoholic CP. Immunoglobulin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

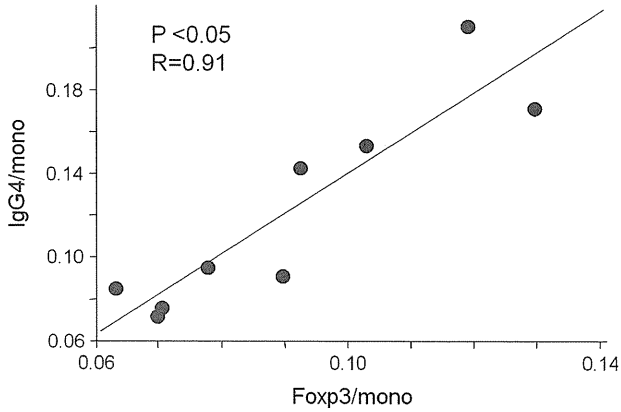

FIGURE 4. Correlation between the ratio of Foxp3-positive cells to infiltrated mononuclear cells (Foxp3/Mono) and the ratio of IgG4-positive cells to infiltrated mononuclear cells (IgG4/Mono) in patients with AIP. Foxp3/Mono and IgG4/Mono are positively correlated (n = 9, $P < 0.05$; $R = 0.91$).

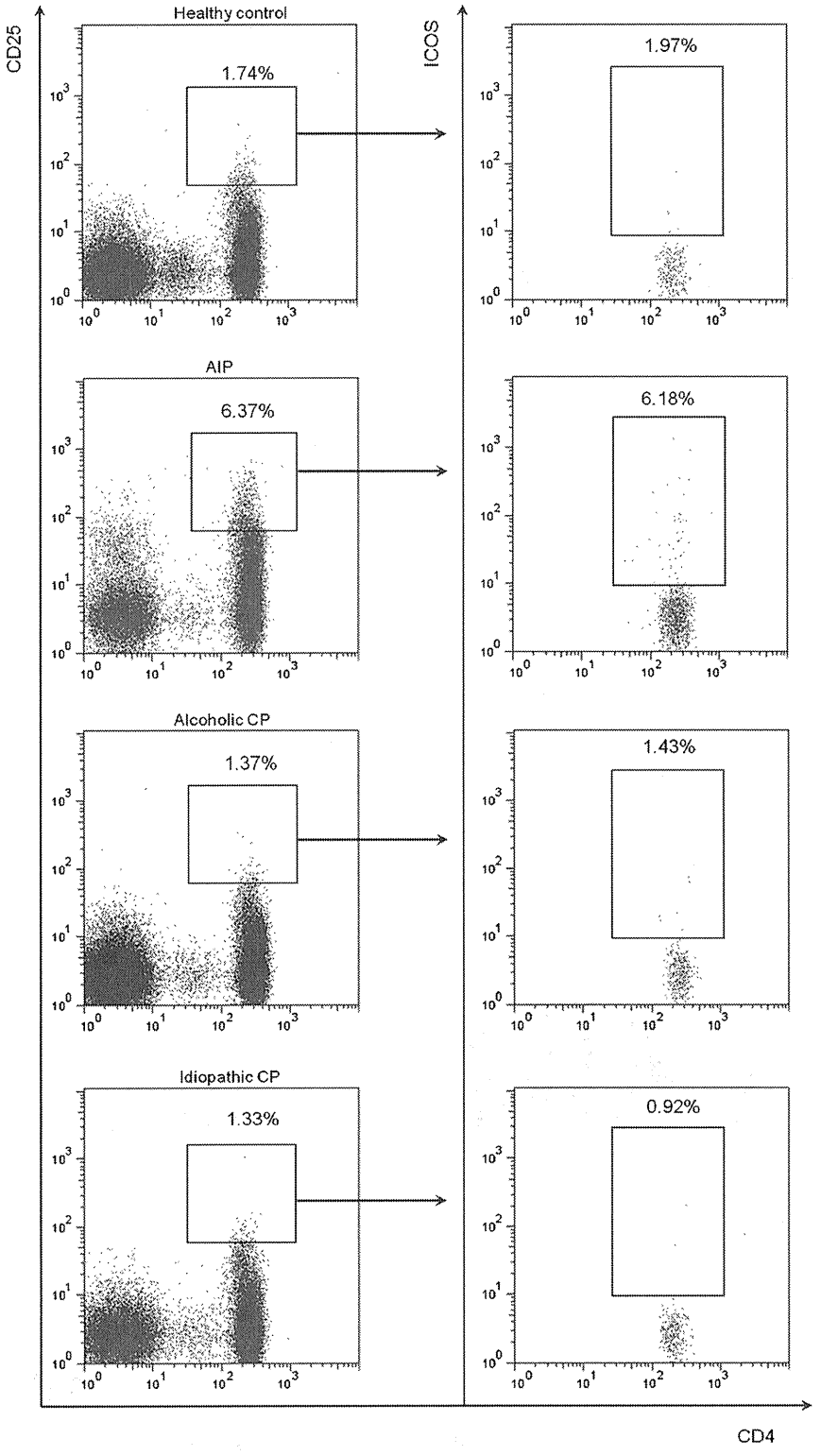

FIGURE 5. Flow cytometric analysis of $CD4^+CD25^{high}$ Tregs and $ICOS^+$ Tregs. Human peripheral blood $CD4^+CD25^{high}$ Tregs separated into $ICOS^+$ subpopulations. In AIP patients (n = 31), the number of $CD4^+CD25^{high}$ Tregs and $ICOS^+$ Tregs increased compared with healthy control (n = 16),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

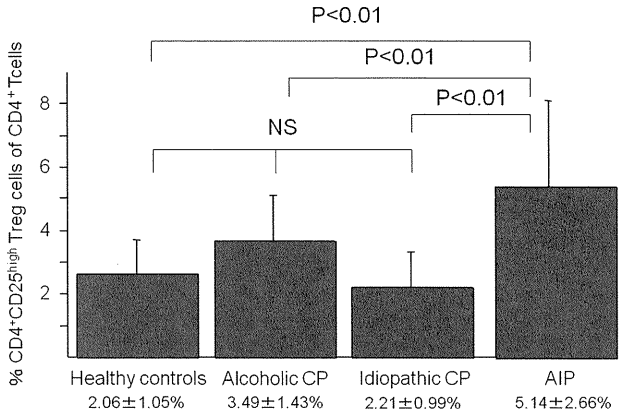

FIGURE 6. Percentage of $CD4^+$ $CD25^{high}$ Tregs. $CD4^+CD25^{high}$ Tregs were significantly increased in patients with AIP (n = 31, 4.99% ± 2.70%) compared with alcoholic CP (n = 11, 3.49% ± 1.43%), idiopathic CP (n = 17, 2.24% ± 1.01%), and healthy control groups (n = 16, 2.60% ± 1.05%, $P < 0.05$).

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]

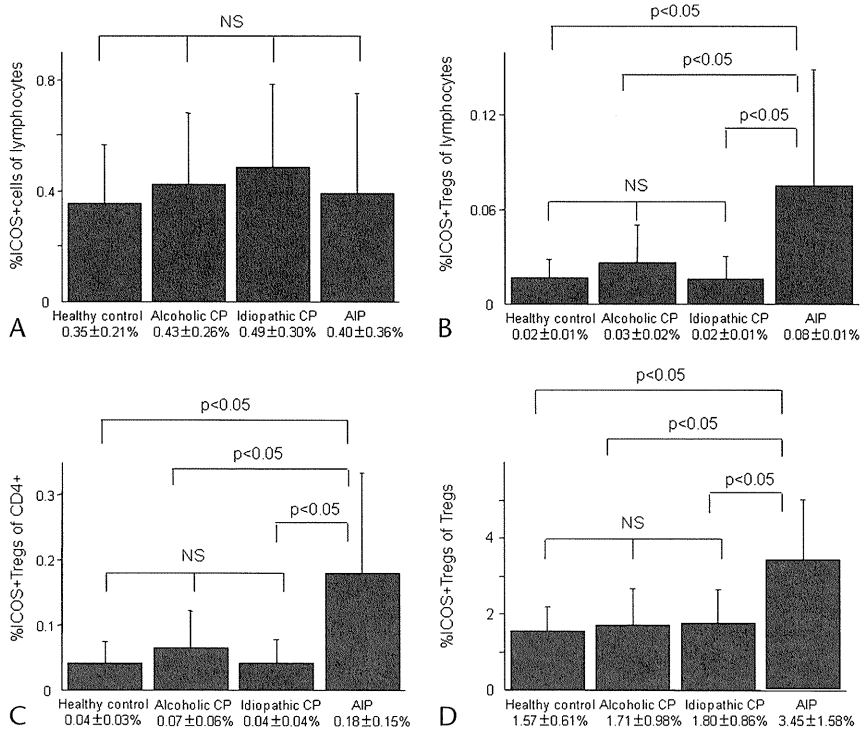

FIGURE 7. Percentage of $ICOS^+$ cells and $ICOS^+$ Tregs. There is no difference in these $ICOS^+$ lymphocytes (%$ICOS^+$ cells of lymphocytes) between patients with AIP (n = 31), alcoholic CP (n = 11), idiopathic CP (n = 17), and healthy control (n = 16). Inducible costimulator–positive Tregs (%$ICOS^+$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

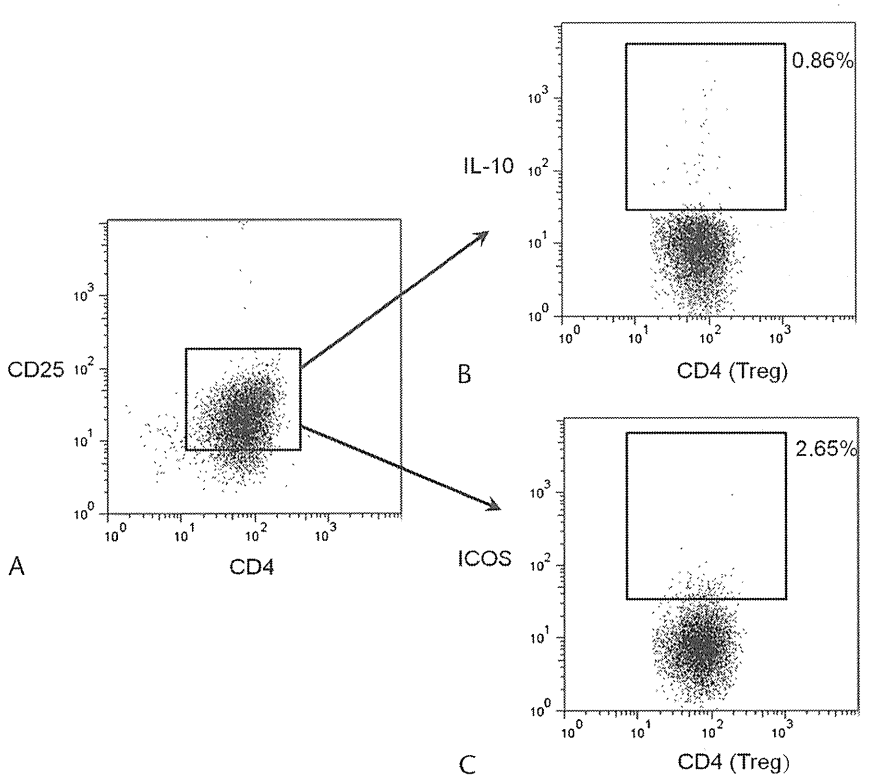

FIGURE 8. Flow cytometric analysis of Tregs (A) that express IL-10 (B) and ICOS (C) in a healthy control. The ratio of IL-10$^+$ Tregs in total Tregs (%IL-10$^+$ Tregs) was 0.86% (B). The ratio of ICOS$^+$ Tregs in total Tregs (%ICOS$^+$ Tregs) was 2.65% (C).

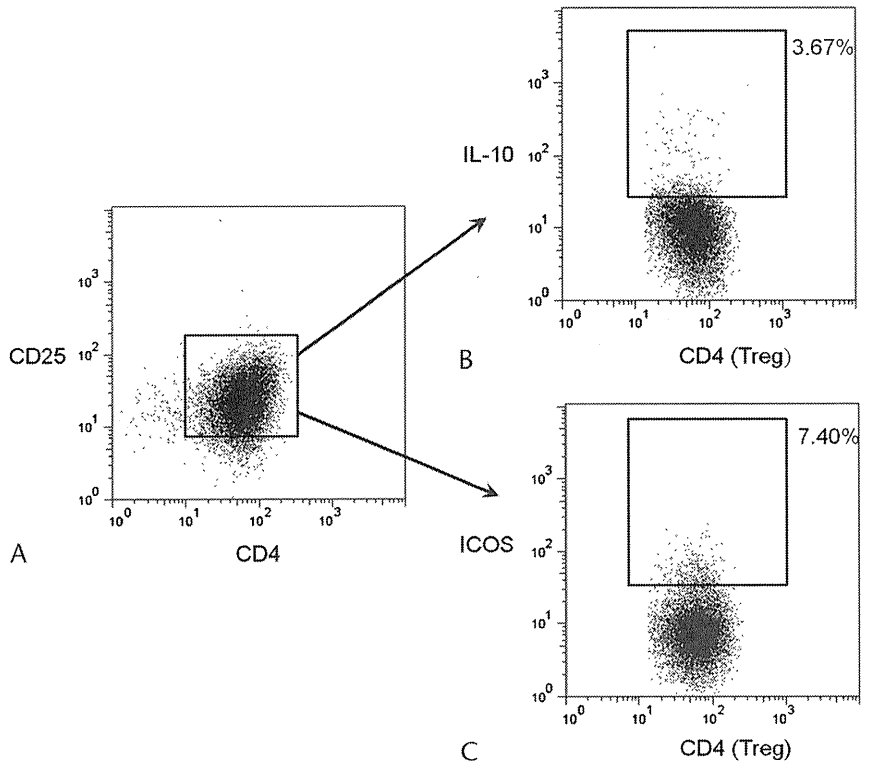

FIGURE 9. Flow cytometric analysis of Tregs (A) that express IL-10 (B) and ICOS (C) in an AIP patient. %IL-10$^+$ Tregs of total Tregs was 3.67% (B). %ICOS$^+$ Tregs was 7.40% (C).

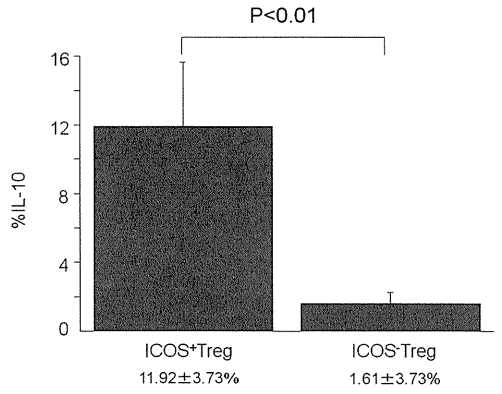

FIGURE 10. The ratio of IL-10$^+$ Tregs in ICOS$^+$ Tregs and ICOS$^-$ Tregs with AIP patients (% IL-10$^+$) (n = 16). Interleukin 10–positve cells were significantly increased in ICOS$^+$ Tregs compared with ICOS$^-$ Tregs ($P < 0.05$).

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

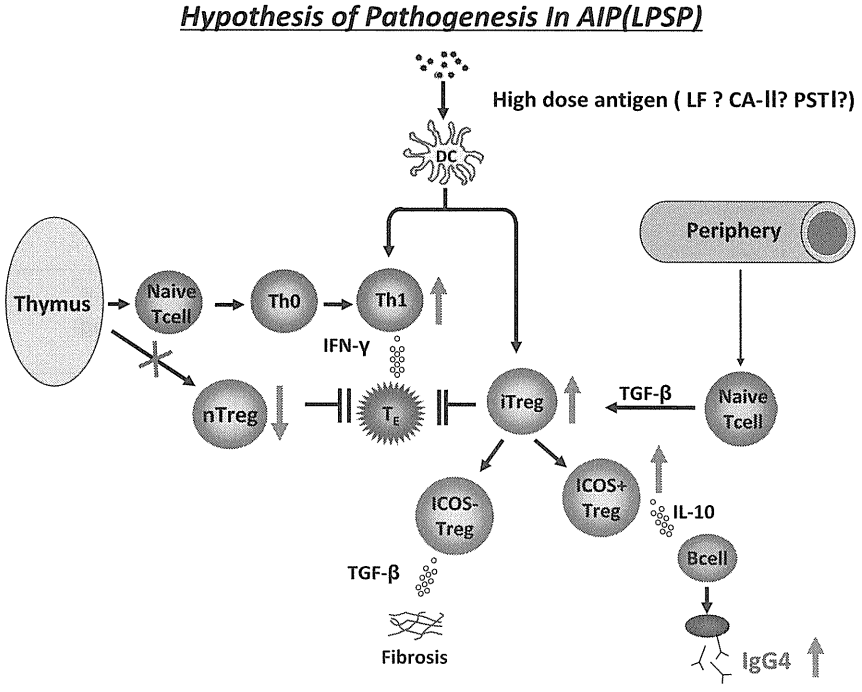

FIGURE 11. Hypothesis of AIP. High-dose antigens (carbonic anhydrase II, lactoferrin, or PST1) induce Tregs from periphery. Increased ICOS$^+$ Tregs correlate with the production of IL-10, which may influence the switching of B cells to IgG4-producing plasmacytes and the production of serum IgG4. On 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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Comparison of steroid pulse therapy and conventional oral steroid therapy as initial treatment for autoimmune pancreatitis

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Abstract
Background The efficacy of oral steroid therapy for autoimmune pancreatitis (AIP) is well known, and oral prednisolone treatment is most usually commenced at 30–40 mg/day, but there have been few reports about comparative studies of oral steroid therapy and steroid pulse therapy as the initial treatment for AIP. We studied the clinical course and image findings to estimate the utility of steroid pulse therapy for AIP, comparing it with oral steroid therapy.
Methods Laboratory and image findings were assessed retrospectively in 11 patients who received steroid pulse therapy, and the findings were compared to those in 10 patients who received conventional oral steroid therapy.
Results Change in pancreatic size showed no significant difference between the therapies after 2 weeks of treatment. Significant improvement of lower bile duct strictures after 2 weeks of treatment and that of immunoglobulin values within 6 months were shown with both therapies. However, steroid pulse therapy showed significant improvement of γ-guanosine triphosphate (GTP) in 2 weeks and of alanine aminotransferase (ALT) in 2 and 8 weeks, compared with oral steroid therapy. Moreover, there was one patient in whom the lower bile duct stricture was not improved by oral steroid therapy, but it did show improvement with steroid pulse therapy.
Conclusions Initial steroid pulse therapy is a beneficial alternative to oral steroid therapy for the improvement of bile duct lesions. In future, the accumulation of a larger number of patients receiving steroid pulse therapy is needed, and prospective studies will be required.

Keywords Autoimmune pancreatitis (AIP) · Steroid pulse therapy · Bile duct stricture · Diabetes mellitus · Pancreatic cancer

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Introduction

Sarles et al. [1] reported a case of chronic pancreatitis with hypergammaglobulinemia, but the clinical entity was not confirmed thereafter. Autoimmune pancreatitis (AIP), which was first proposed as a clinical entity by Yoshida et al. [2] from Japan, is now generally accepted as a distinctive type of pancreatitis [1]. AIP is characterized by diffuse irregular narrowing of the main pancreatic duct, sausage-like diffuse swelling of the pancreas, high serum levels of IgG or IgG4, and steroid responsiveness [3–6]. Since the fibroinflammatory process of AIP responds well to steroids, autoimmune mechanisms are thought to be involved in the development of AIP. A recent large Japanese study of AIP and guidelines for treatment recommend standard oral steroid therapy with an initial dose of 0.5–0.6 mg/kg/day [7, 8].

While steroid responsiveness as a diagnostic component is not included in the revised Japanese criteria, it is included in the Korean criteria, Mayo Clinic HISORt (Histology, Imaging, Serology, Other organ involvement, and Response to steroids) criteria, and recently proposed Asian criteria [8–11]. The most important issue in AIP management is making the diagnosis of AIP, especially the

mass-forming type, differentiating it from pancreatic or biliary cancers [12–15], although some cases of pancreatic cancer are accompanied by AIP [16–19]. In some tumor-forming AIP cases, the efficacy of a steroid trial has been reported as useful in diagnosing AIP by criteria other than the Japanese diagnostic criteria [20]. Moreover, Korean investigators have reported the usefulness of a 2 weeks' trial of oral steroids in differentiating AIP from malignancy, with continuing administration if AIP is diagnosed [20]. However, it has not yet been established whether or not withdrawal of steroids in reconsidering malignancy presents a risk of postoperative adrenal insufficiency [21–23]. Therefore, it is desirable to have an alternative to the discontinuation of steroid administration immediately after surgery.

Steroid pulse therapy is widely used to initiate treatment in patients with rapidly progressive and immunologically mediated disorders such as acute graft rejection, Graves ophthalmopathy, pemphigus, and severe systemic lupus erythematosus [24–27]. Moreover, high doses of systemic steroid can be given with comparative safety within a period of 1 week [28]. We therefore evaluated the efficacy of short-term steroid pulse therapy, in comparison with oral steroid therapy, in patients with AIP.

Methods

Patients and treatment

For this study, we retrospectively examined the records of all 21 AIP patients treated in our hospitals from November 2004 to May 2009. All patients were diagnosed with AIP according to the clinical diagnostic criteria for AIP proposed by the Research Committee of Intractable Diseases of the Pancreas supported by the Japanese Ministry of Health, Labor, and Welfare, and the Japan Pancreas Society. Following diagnosis, 20 patients with AIP were randomly distributed to two treatment groups by their attending physicians. One patient (case 10) was referred to our hospital after the withdrawal of oral steroid for AIP because his bile duct lesion had not responded to the treatment. Eleven patients (cases 1–11; 5 male and 6 female; aged 47–80 years, with a mean age of 66 years, named the "pulse group") (Table 1) received steroid pulse therapy, and ten patients (cases 12–21; 8 male and 2 female, aged 49–72 years, with a mean age of 69 years, named the "oral group") (Table 2) received oral steroid therapy. For the pulse group, the initial dose of methylprednisolone was 500 mg/day for 3 days each week as 1 course, and we treated them with 2 weekly courses. Then oral prednisolone at 20 mg/day was prescribed as maintenance therapy and the dose was tapered off. For the oral group, ten patients commenced oral prednisolone at 30–40 mg/day. Two weeks after the start of the treatment, oral prednisolone at 20 mg/day was prescribed, and the dose was tapered off. This study was approved by the Kansai Medical University ethics committee.

Serological study

We analyzed immunological findings for the following: IgG, IgG4, antinuclear antibodies (ANA), rheumatoid factor (RF), antimitochondrial antibodies (AMA), myeloperoxidase-antineutrophil cytoplasmic antibodies (MPO-ANCA), anti-Sjögren's syndrome A antibodies (SS-A), anti-Sjögren's syndrome B antibodies (SS-B), anti-thyroid peroxidase antibodies (TPOAb), and anti-thyroglobulin antibodies (TgAb). To compare liver and endocrine function in both groups, we evaluated the serum levels of γ-guanosine triphosphate (GTP) and alanine aminotransferase (ALT) on day 0 (data just before the treatment), and at weeks 2 and 8 after therapy, and checked glycosylated hemoglobin values (HbA1c) at months 1, 3, and 7 after therapy, which closely reflected glucose tolerance at months 0, 2, and 6, respectively. In each evaluation, patients who did not show abnormal values during the clinical course were excluded in order to evaluate the therapeutic effect strictly.

Radiological study

All the patients were examined by contrast-enhanced helical computed tomography (CT), magnetic resonance imaging (MRI), and endoscopic retrograde cholangiopancreatography (ERCP) and underwent liver function tests, combined with bile duct drainage and pathological tests as necessary.

For morphological changes, CT, MRI, and ERCP were studied. The width of the pancreas along its longest axis was measured on CT or MRI images and compared with the transverse diameter of the vertebral body according to the method of Heuck et al. [29]. The pancreas size on the first image was defined as 100%.

Cases showing lower bile duct stricture were classified as follows: 0 = absent, 1 = <0–25%, 2 = <25–50%, 3 = <50–75%, and 4 = <75–100%, according to the method of Craig et al. [30]. Using the method described above, pancreas size was evaluated after 2 weeks on steroids, and stricture of the distal third of the common bile duct was measured after 2 weeks and after 8 weeks.

Statistical analysis

Statistical analysis was performed using the Mann–Whitney *U*-test, Wilcoxon signed-ranks test, paired *t*-test, and

Table 1 Background of AIP patients who received steroid pulse therapy

Patient ID	Age (years)/ sex	Symptoms	IgG <1,700 (mg/dl)	IgG 4 <135 (mg/dl)	Amy <130 (IU/L)	T-Bil <0.9 (mg/dl)	ALT <30 (IU/L)	γ-GTP <35 (IU/L)	ANA	PFD <73.4 (%)	DM	Extrapancreatic lesion	Stenosis on ERCP	Morphological change of the pancreas
1	80/F	Jaundice	2,604	1,230	52	0.9	43	260	−	52.1	−	Sialoadenitis	Head, CBD	FS in head
2	63/M	Epigastralgia	1,714	354	66	0.9	56	222	−	29.7	−	Warthin tumor	Head, CBD	DS
3	54/F	Epigastralgia	1,828	324	561	1.0	100	403	−	NT	−	Hypothyroidism	Body, CBD	DS
4	71/F	None	1,916	295	78	0.6	60	101	−	97.9	−	Sialoadenitis, mediastinum LNS	Head	FS in head
5	66/F	Nausea	1,535	235	77	2.2	118	1,311	−	NT	−	Hypothyroidism, retroperitoneal fibrosis	Head to tail, CBD	FS in head
6	66/M	Epigastralgia, jaundice	2,695	1,790	164	12.5	98	137	−	58.1	+	None	Body, CBD	DS
7	47/F	Jaundice	2,453	629	15	1.0	190	65	−	NT	−	None	Head, body to tail, CBD	DS
8	72/M	Epigastralgia	1,692	452	66	1.3	721	1,352	+	30.9	+	None	Head to tail CBD	FS in head
9	72/M	Epigastralgia, jaundice	1,513	411	32	14.1	114	352	−	NT	+	None	Head, CBD	DS
10	63/M	Malaise	1,514	394	76	0.5	20	82	−	NT	−	None	Head to tail, CBD	FS in head
11	73/F	Epigastralgia	1,598	373	55	0.6	33	588	−	59.7	+	None	Head to tail, CBD	FS in head

AIP autoimmune pancreatitis, *T Bil* total bilirubin, *ALT* alanine aminotransferase, *ID* identification, *γ-GTP* γ-guanosine triphosphate, *Amy* amylase, *ANA* antinuclear antibody, *PFD* pancreatic functional diagnostic test, *DM* diabetes mellitus, *ERCP* endoscopic retrograde cholangiopancreatography, *CBD* common bile duct, *FS* focal swelling, *DS* diffuse swelling, *NT* not tested, *LNS* lymph node swelling

Table 2 Background of AIP patients treated with oral prednisolone

Patient ID	Age (years)/sex	Symptoms	IgG <1,700 (mg/dl)	IgG4 <135 (mg/dl)	Amy <130 (IU/L)	T-Bil <0.9 (mg/dl)	ALT <30 (IU/L)	γ-GTP <35 (IU/L)	ANA	PFD <73.4 (%)	DM	Extrapancreatic lesion	Stenosis on ERCP	Morphological change of the pancreas
12	71/M	Jaundice	3,274	1,870	58	1.0	24	76	−	32.7	−	Mediastinum LNS	Head to tail, CBD	DS
13	66/M	Thirst	4,060	1,170	64	0.4	18	116	−	65.8	−	Sialoadenitis, mediastinum LNS	Tail, CBD	DS
14	58/F	Epigastralgia	2,754	1,110	95	0.9	13	10	−	NT	−	Thyroiditis, mediastinum LNS	Body to tail	FS in head
15	52/F	None	2,190	661	435	0.6	15	15	−	69.7	−	Interstitial pneumonia, Mikulicz tumor	Tail	FS in tail
16	68/M	Fever	1,622	407	34	0.6	30	30	−	73.4	−	Thyroiditis	Head, body, CBD	DS
17	72/M	Vomiting	2,010	773	51	0.3	34	67	+	NT	−	None	Head	FS in head
18	55/M	Epigastralgia	1,461	659	53	0.6	178	240	−	17.9	+	None	Head to tail, CBD	DS
19	63/M	Jaundice	2,073	487	41	1.1	52	149	−	NT	+	None	Head, CBD	FS in head
20	49/M	Jaundice	2,065	479	62	0.9	52	503	−	35.7	−	None	Body, tail, CBD	DS
21	66/M	Malaise	1,607	200	47	1.0	41	189	−	77.9	+	None	Head, CBD	FS in head

Amy amylase, *ANA* antinuclear antibody, *PFD* pancreatic functional diagnostic test, *DM* diabetes mellitus, *ERCP* endoscopic retrograde cholangiopancreatography, *LNS* lymph node swelling, *CBD* common bile duct, *DS* diffuse swelling, *NT* not tested, *FS* focal swelling

Student's *t*-test. In all tests, corrected P values of < 0.05 were considered statistically significant.

Results

Clinical manifestations

After 6 months' treatment, oral prednisolone had been administered to 19 patients in both groups [pulse group: 9 of 11 vs. oral group: 10 of 10; difference not significant (N.S.)], and the median dosage of prednisolone in each group was 10 mg/day (pulse group: 2.5–12.5 vs. oral group: 5–12.5; N.S.). Two patients in the pulse group dropped out of the maintenance therapy. Neither group showed severe or lethal complications.

In both groups, extrapancreatic lesions other than the bile duct lesions were observed in 10 patients (pulse group: 5 of 11 vs. oral group: 5 of 10; N.S.) (Tables 1, 2). No exacerbation of the extrapancreatic lesions was found following either of the treatments (data not shown). Laboratory findings including immunoglobulin, autoantibody, and exocrine function at the treatment start are listed in Tables 1 and 2.

Immunoglobulin

At the beginning of treatment, in both groups, abnormal serum immunoglobulin-G4 (IgG4) values were observed in all patients (Tables 1, 2), and abnormal serum immunoglobulin-G (IgG) values were observed in 13 patients (pulse group: 6 of 11 vs. oral group: 7 of 10; N.S.) (Tables 1, 2). Normalization of the IgG value was shown in all these patients within 6 months (data not shown).

Liver function

At the beginning of treatment, abnormal γ-GTP values were revealed in both groups, in a total of 18 patients (pulse group: 11 of 11 vs. oral group: 7 of 10; N.S.). In the pulse group, the median γ-GTP levels fell, from 222 IU/L (range 65–1,352) at the beginning of treatment to 92 IU/L (22–679) ($P < 0.01$) after 2 weeks of pulse therapy (Fig. 1a), and to 36 IU/L (19–556) ($P < 0.01$) after 8 weeks of pulse therapy (Fig. 1a). In the oral group, however, the median γ-GTP fell insignificantly after 2 weeks of prednisolone treatment, from 149 IU/L (range 67–380) at the beginning of treatment to 125 IU/L (63–274) ($P = 0.083$) (Fig. 1b), although the level fell significantly to 72 IU/L (29–114) ($P = 0.027$) after 8 weeks of prednisolone treatment (Fig. 1b). When limiting results to the patients who showed diffuse pancreatic swelling, γ-GTP was significantly improved in the pulse group after 2 weeks ($P = 0.043$) and 8 weeks ($P = 0.043$) of pulse therapy (Fig. 1c), whereas the improvement in the γ-GTP level was insignificant in the oral group after 2 weeks ($P = 0.27$) and 8 weeks ($P = 0.27$) of prednisolone treatment (Fig. 1d).

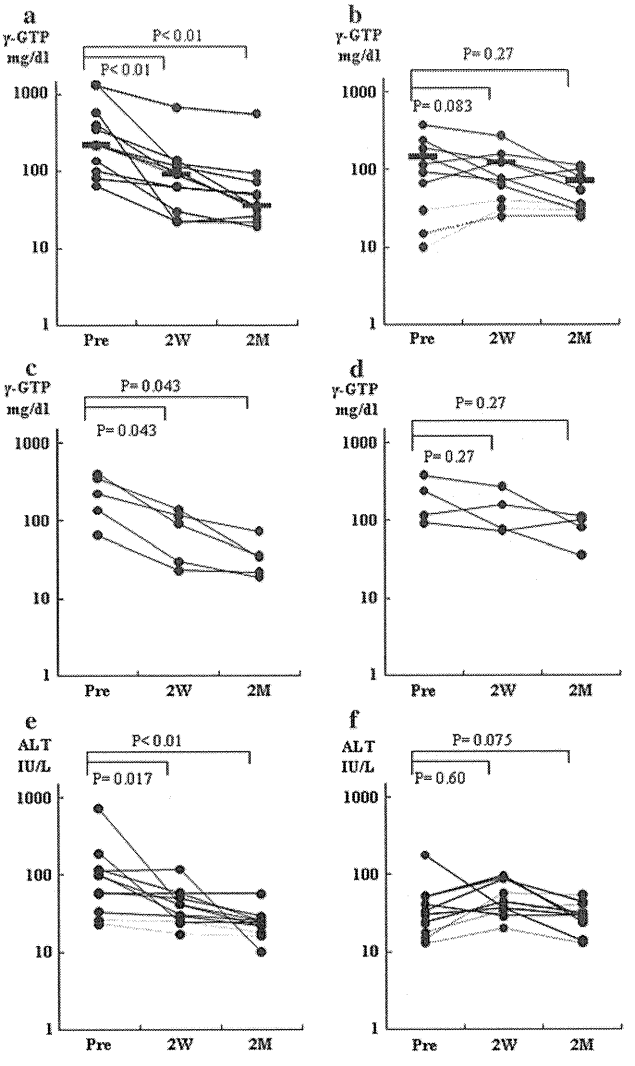

Fig. 1 γ-Guanosine triphosphate (*γ-GTP*) and alanine aminotransferase (*ALT*) changes after steroids. The serum levels of γ-GTP after steroid pulse therapy (**a**), after oral steroid therapy (**b**), those of ALT after steroid pulse therapy (**e**) and those after oral steroid therapy (**f**) were monitored on day 0 (*Pre*), and 2 weeks (*W*) and 8 weeks after therapy. To evaluate the therapeutic effect strictly, the patients (*dotted lines*) who did not show an abnormal value during the clinical course were excluded from this analysis. The serum levels of γ-GTP after 2 weeks on steroids and those of ALT after 2 and 8 weeks on steroids were significantly improved in the pulse group, compared with the oral group. When limiting the patients to those who showed diffuse pancreatic swelling, the serum level of γ-GTP was significantly improved in the pulse group after 2 and 8 weeks of pulse therapy (**c**), compared with the oral group (**d**). *M* Months

At the beginning of treatment, abnormal ALT values were revealed in both groups, in a total of 16 patients

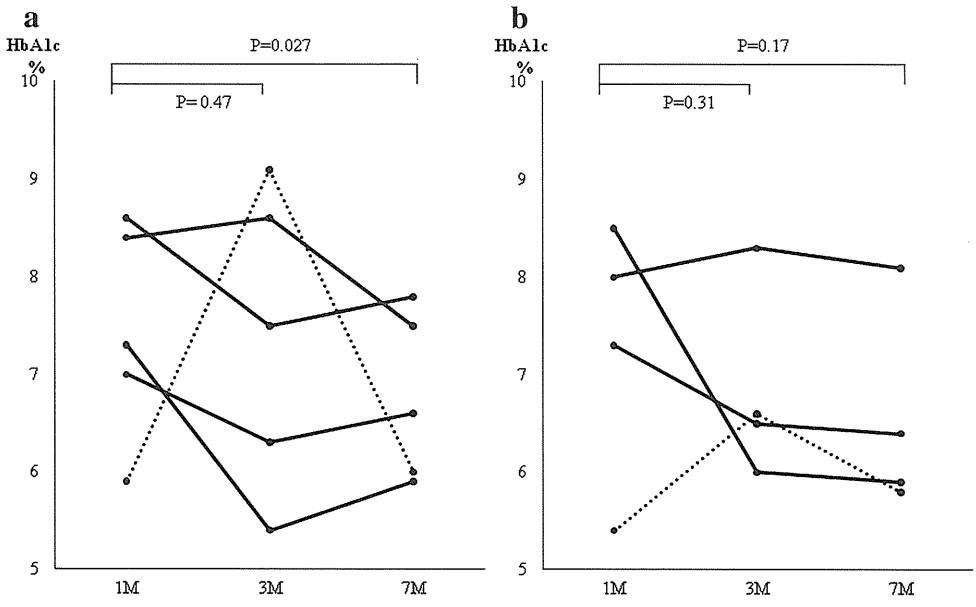

Fig. 2 Glycosylated hemoglobin (*HbA1C*) changes after steroid therapy. The levels of HbA1c after steroid pulse therapy (**a**) and oral steroid therapy (**b**) were monitored at months 1, 3, and 7 after therapy, which closely reflect glucose tolerance at months 0, 2, and 6, respectively. To evaluate the therapeutic effect strictly, patients who did not show abnormal values during the clinical course were excluded from this analysis. *Dotted lines* represent the patients who developed glucose intolerance after 2 months of steroid therapy. The level of HbA1c at month 7 on steroids tended to be improved in the pulse group, compared with the oral group

(pulse group: 10 of 11 vs. oral group: 6 of 10; N.S.). In the pulse group, the median ALT level fell from 100 IU/L (range 33–721) at the beginning of treatment to 41 IU/L (24–117) ($P = 0.017$) after 2 weeks of pulse therapy (Fig. 1e), and to 25 IU/L (10–57) ($P < 0.01$) after 8 weeks of pulse therapy (Fig. 1e). In the oral group, however, the median ALT rose transiently from 46.5 IU/L (range 30–178) at the beginning of treatment to 62 IU/L (29–96) ($P = 0.60$) after 2 weeks of prednisolone (Fig. 1f), but improved to 28 IU/L (14–44) ($P = 0.075$) after 8 weeks of prednisolone treatment (Fig. 1f).

Endocrine function

Before steroid treatment, diabetes mellitus was seen in 7 patients (pulse group: 4 of 11 vs. oral group: 3 of 10; N.S.) (Tables 1, 2). One patient in each group developed glucose intolerance after 2 months of steroid therapy. Including these patients, all the patients with impaired glucose tolerance were treated with dietary measures and received medical therapy while the steroid therapy was maintained. Neither group showed significant improvement in glucose tolerance after 2 months, but at 6 months, the pulse group had improved significantly ($P = 0.027$), whereas the oral group had not ($P = 0.17$) (Fig. 2).

Pancreas size

Both groups evidenced pancreatic swelling: diffuse pancreatic swelling was observed in 10 patients (pulse group: 5 of 11 vs. oral group: 5 of 10; N.S.) and focal swelling was observed in 11 patients (pulse group: 6 of 11 vs. oral group: 5 of 10; N.S.) (Tables 1, 2). The change in pancreas size

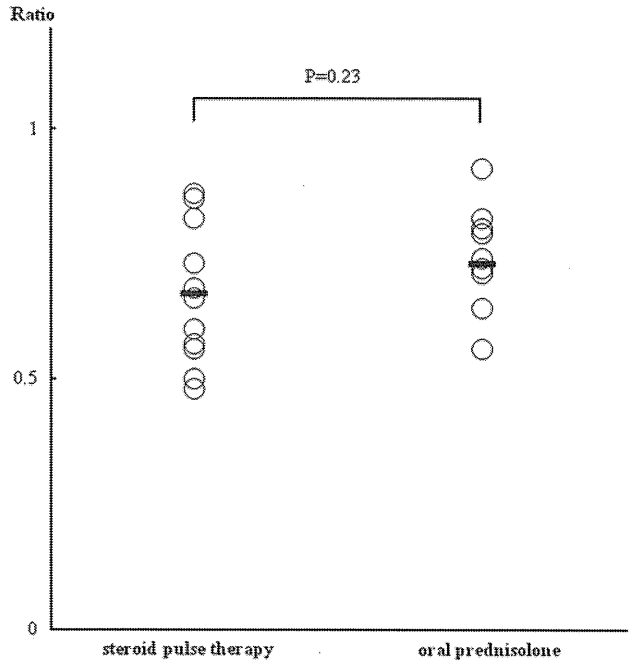

Fig. 3 Pancreas morphology changes after 2 weeks on steroids. Morphological changes of the pancreas after 2 weeks of steroid pulse therapy (*left*) and oral steroid therapy (*right*) were scored. The width of the pancreas along its longest axis was measured on computed tomography (CT) or magnetic resonance imaging (MRI) and compared with the transverse diameter of the vertebral body, referred to in the method of Heuck et al. [29]. The pancreas size on the first image was defined as 100%, and the ratio after 2 weeks with each treatment was measured in the same manner. The *black bars* represent the mean in each group. The two groups showed no significant difference in morphological change of the pancreas after 2 weeks

after 2 weeks showed no significant difference between the groups after treatment (pulse group: 67% vs. oral group: 73.4%, $P = 0.23$) (Fig. 3).

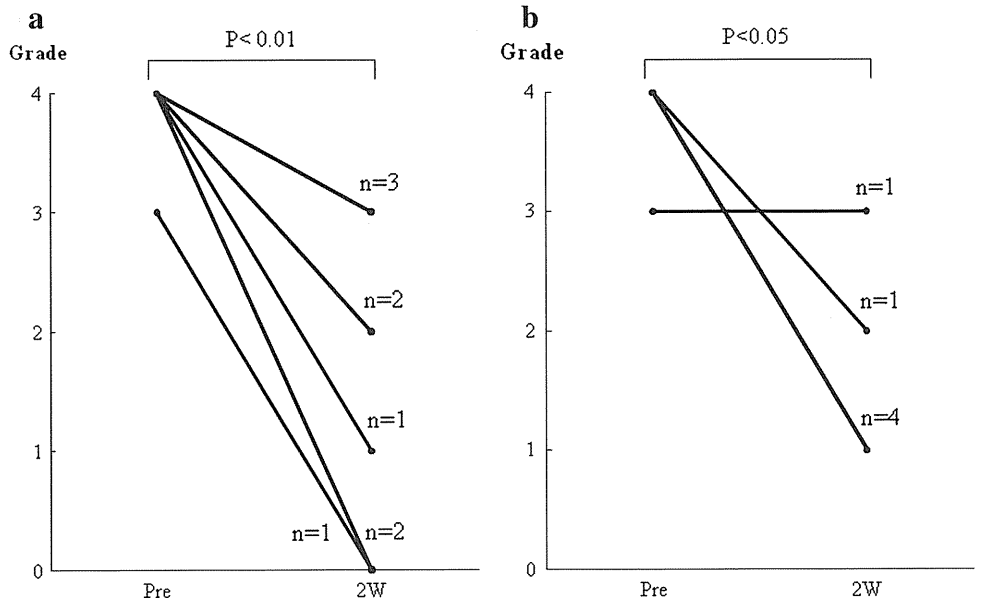

Fig. 4 Lower bile duct stricture changes after steroids. The changes in lower bile duct stricture in the pulse group (**a**) and the oral group (**b**) on day 0 and 2 weeks after therapy were scored as follows: 0 = absent, 1 = <0–25%, 2 = <25–50%, 3 = <50–75%, 4 = <75–100%, referred to in the method of Craig et al. [30]. Bile duct stenosis in the distal third part was significantly improved after 2 weeks in each group

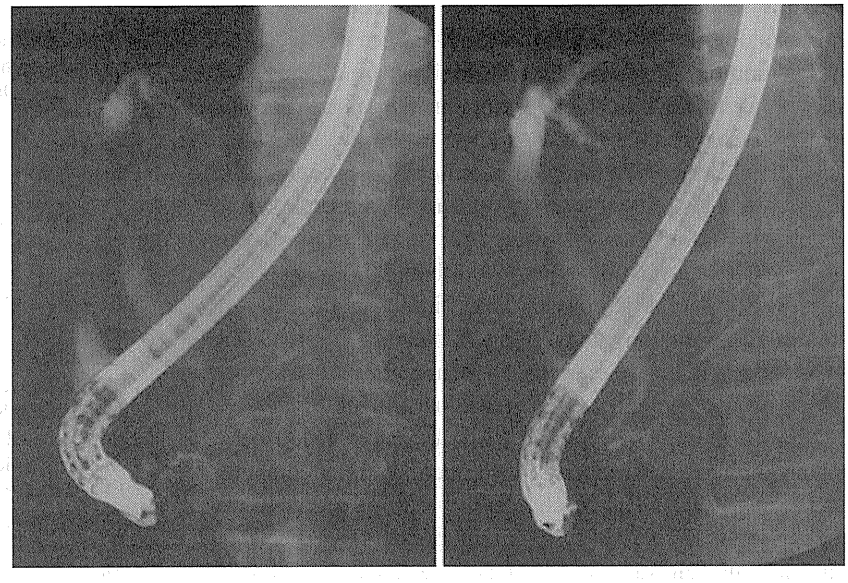

Fig. 5 Endoscopic retrograde cholangiopancreatography (ERCP) images of the impact of steroid pulse therapy on refractory autoimmune pancreatitis (AIP). Although oral prednisolone was commenced, it had had no effect on the biliary stenosis (*left*). Two courses of steroid pulse therapy ameliorated the stenosis dramatically (*right*) (Case 10; reference [31])

Bile duct lesion

After 2 weeks on steroids, significant improvement of lower bile duct stricture was shown in both groups (pulse group: $P < 0.01$, and oral group: $P < 0.05$) (Fig. 4). However, there was one patient (case 10) whose lower bile duct stricture did not improve following oral prednisone treatment, but showed definite improvement with steroid pulse therapy [31] (Fig. 5).

Discussion

Several cases have been reported of pancreatic cancer or bile duct cancer concurrent with AIP [16–19]; because the image findings of AIP often mimic pancreatobiliary malignancies, it is extremely crucial to distinguish AIP from cancers, although this can be difficult [12–15].

In the Asian diagnostic criteria proposed by the Japan-Korea symposium on autoimmune pancreatitis [11], the use of steroids as diagnostic treatment was allowed only when the imaging findings were compatible with AIP and only after there was a negative result for malignancy work-up. Cases of diagnostic treatment using steroids will doubtless increase in future. The usefulness of a 2-week conventional oral steroid diagnostic treatment was also proposed by Moon et al. [20]. However, oral steroid therapy requires a long period for drug tapering, because any patient who has received a glucocorticoid in doses equivalent to at least 20 mg a day of prednisone for >5 days is at risk of

secondary adrenal insufficiency due to hypothalamic–pituitary–adrenal suppression [21–23]. Diagnostic treatment with an oral steroid may cause an undesirable effect when surgical resection is required. Although one article reported that azathioprine was used for refractory AIP [32], it is causative of acute pancreatitis [33]. Therefore, a safe and simple alternative to oral steroid treatment is needed for refractory AIP [34].

Steroid pulse therapy is a well known alternative to oral steroid treatment for autoimmune disorders; it requires no drug tapering [28], and we have already reported cases where steroid pulse therapy was effective for AIP [35], although comparative studies of conventional oral steroid therapy and steroid pulse therapy have not been reported. Here we report that steroid pulse therapy is an effective alternative to oral steroid for the initial treatment of AIP.

The efficacy of oral steroid therapy for AIP is well known, and the improvement of AIP in patients treated with steroids for 2 weeks can be shown in radiographic findings [5, 20, 36]. Our data on pancreas size after 2 weeks did not show a significant difference between oral steroid and pulse therapy. Both therapies were effective for alleviating lower bile duct stricture in the short term and for resolving abnormal IgG values. However, the short-term change in γ-GTP showed significant improvement in the steroid pulse therapy group, but not in the oral steroid group. In one patient, lower bile duct stricture was improved by steroid pulse therapy, although it had not been improved by oral steroids. These findings suggest that steroid pulse therapy may prevent patients from having unnecessary major operations for benign bile duct lesions which do not respond to oral steroid treatment.

Steroid therapy is reported to be effective in approximately half of AIP patients with accompanying diabetes mellitus [37, 38]. The mechanism of steroid action in the recovery of endocrine function in patients with AIP is unclear, but Tanaka et al. [39] have suggested that steroids can suppress the release of cytokines produced by inflammatory cells, and enable islet regeneration and eventual restoration of insulin secretion. However, we have reported that conventional oral steroids did not improve diabetes mellitus in the long term [40]. Although the accumulation of a larger numbers of cases is needed, our study of glycosylated hemoglobin values showed that steroid pulse therapy tended to be more effective than oral steroids for improving glucose tolerance after 6 months of treatment. In our protocol, there was a decisive difference between the two therapies just for the initial 2 weeks, because 20 mg/day of oral prednisolone was prescribed as maintenance therapy after the 2 weeks of steroid pulse therapy. We presume that the steroid pulse therapy was better than the oral steroid therapy for improving glucose tolerance because of its stronger cytokine suppression.

In summary, steroid pulse therapy is an effective alternative initial treatment for AIP, and surpasses conventional oral steroid therapy in the improvement of bile duct lesions. When a differential diagnosis between AIP and pancreatic cancer is difficult clinically or when bile duct lesions do not respond to oral steroid treatment, we recommend steroid pulse therapy as an alternative to oral steroid therapy. In future, the accumulation of a larger number of patients receiving steroid pulse therapy is needed, and prospective studies will be required.

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Idiopathic Duct-Centric Pancreatitis (IDCP) with Immunological Studies

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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

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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

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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

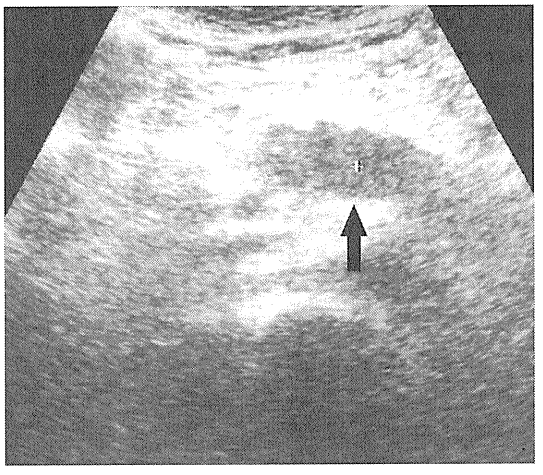

Figure 1. Abdominal ultrasonography (US) of the pancreas. US showed the partially enlarged pancreas body and tail with homogenous hypoechoic pattern (arrow).

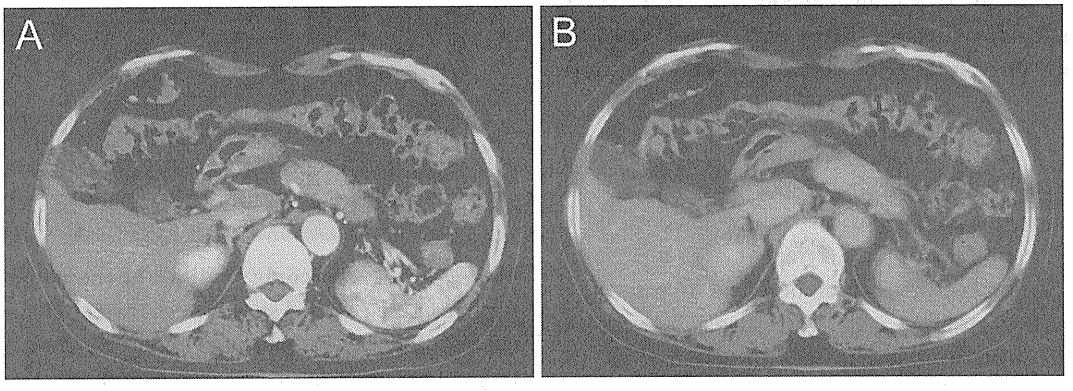

Figure 2. Contrast-enhanced computed tomography (CT) of the pancreas. Contrast-enhanced CT demonstrated moderate swelling in the body and tail of the pancreas with homogenous enhancement, but not capsular-like low density rim or swelling of peripancreatic nodes. (A) early phase (B) delayed phase.