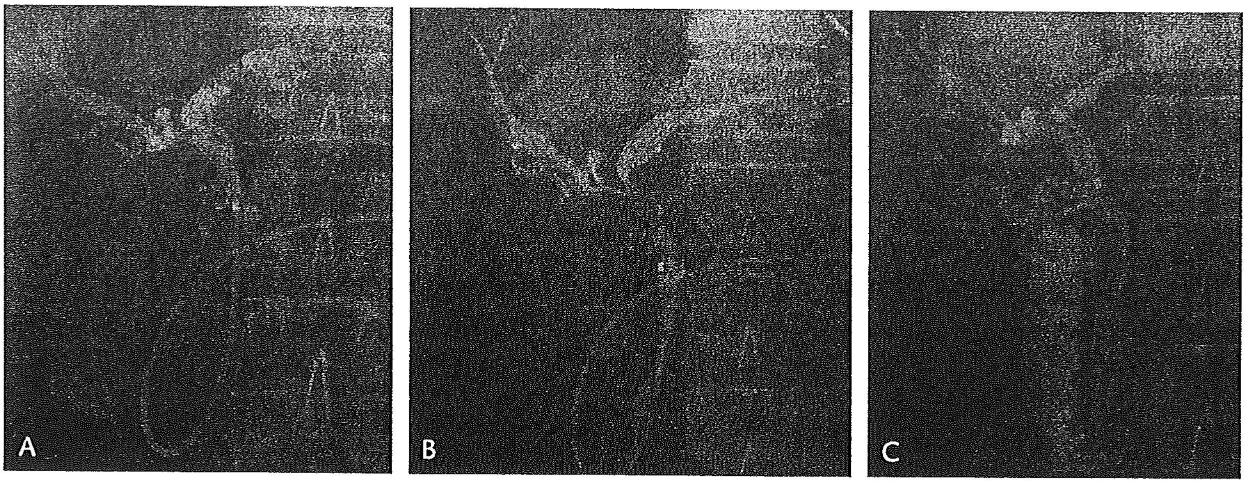

FIGURE 1. Serial cholangiography via an ENBD tube. A, On the initial cholangiography, only lower bile duct was stenotic. B, Two days later, stenosis of the hilar bile duct appeared in addition to that of the lower bile duct. C, On the seventh day after the start of steroid therapy, stenosis of both the hilar and lower bile ducts improved.

AIP, and the major initial symptom of AIP is obstructive jaundice caused by this stenosis.[1–4] The stenotic portion of the bile duct is usually the lower bile duct (40 [80%] of 50 AIP cases in our series), and stenosis of the hilar or intrahepatic bile duct (4 AIP cases in our series) is sometimes detected. When stenosis is found in the hilar or intrahepatic bile duct, the cholangiographic appearance is very similar to that of primary sclerosing cholangitis.[5,6] However, unlike the progressive features of primary sclerosing cholangitis, sclerosing cholangitis associated with AIP responds well to steroid therapy. Histologically, the wall of the stenotic bile duct is found to be thickened by marked fibrosis, with dense infiltration of IgG4-positive plasma cells and lymphocytes, similar to the findings noted in the pancreas. This fibroinflammatory change is detected extensively in the bile duct wall where stenosis is not obvious on cholangiography.[1,7] Rapid progression of sclerosing cholangitis in the hilar bile duct within 2 days is surprising. Although no cases reported in the literature have had such rapid progressive changes in sclerosing cholangitis, subclinical fibrotic inflammation, which extended into the hilar bile duct, seemed to progress rapidly. Furthermore, it improved only within 7 days with steroid therapy.

Rapid progressive changes in sclerosing cholangitis should be kept in mind during the treatment of AIP.

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Enhanced Inhibition of Tumor Growth With Depletion of CD25 Regulatory Cells and Intratumoral Immunization With Tumor RNA–Pulsed Dendritic Cells in a C57BL/6 Pancreatic Tumor Model

To the Editor:

Immunotherapy, with the help of dendritic cells (DCs), results in significant tumor response in different types of tumors using tumor-specific peptides or tumor lysate or RNA for priming. Dendritic cells activate antitumor immunity via generation of a cytotoxic T lymphocyte (CTL) from native T cells. Unfortunately, Dallal et al[1] found that in pancreatic carcinoma, DCs are, if present at all, located outside the margin of the tumor. Furthermore, accumulation of DCs in the local tumor environment correlates with a better patient survival for numerous types of tumors. Therefore, we assumed that paucity of DCs in pancreatic tumor is one reason for its dreadful course. In our model, we overcome this paucity via ultrasound-guided orthotopic inoculation of DCs in an orthotopic pancreatic tumor. We noticed that the number of DCs in the region of the pancreatic tumor enhanced and remained in the tumor after intratumoral DC implantation because there was no significant difference in the number of DCs recovered from an ex vivo or in vivo injection for 7 days, whereas native tumor (just as in humans) showed no DCs (data not shown). Ultrasound-guided tumor inoculation of pancreatic cancer cells led also to successful formation of solid adenocarcinomas in syngeneic recipients in all of the animals (data not shown). Tumors, as expected, grew rapidly. Wang et al[2] also described Panc02 as extremely aggressive after implantation as manifested by progressive growth in the pancreas, peritoneal dissemination, and distant metastasis to multiple organs, including the liver and lungs. We observed that potent CTL can be induced by

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BRIEF ARTICLES

Sclerosing cholangitis associated with autoimmune pancreatitis differs from primary sclerosing cholangitis

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Abstract

AIM: To clarify the characteristic features of biliary lesions in patients with autoimmune pancreatitis (AIP) and compare them with those of primary sclerosing cholangitis (PSC).

METHODS: The clinicopathological characteristics of 34 patients with sclerosing cholangitis (SC) associated with AIP were compared with those of 4 patients with PSC.

RESULTS: SC with AIP occurred predominantly in elderly men. Obstructive jaundice was the most frequent initial symptom in SC with AIP. Only SC patients with AIP had elevated serum IgG4 levels, and sclerosing diseases were more frequent in these patients. SC patients with AIP responded well to steroid therapy. Segmental stenosis of the lower bile duct was observed only in SC patients with AIP, but a beaded and pruned-tree appearance was detected only in PSC patients. Dense infiltration of IgG4-positive plasma cells was detected in the bile duct wall and the periportal area, as well as in the pancreas, of SC patients with AIP.

CONCLUSION: SC with AIP is distinctly different from PSC. The two diseases can be discriminated based on cholangiopancreatographic findings and serum IgG4 levels.

Key words: Autoimmune pancreatitis; IgG4; Primary sclerosing cholangitis; Sclerosing cholangitis

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INTRODUCTION

Autoimmune pancreatitis (AIP) is a unique form of pancreatitis in which autoimmune mechanisms are suspected of being involved in the pathogenesis. AIP has many clinical, radiological, serological and histopathological characteristics: (1) elderly male preponderance; (2) initial symptom is frequently painless obstructive jaundice; (3) occasional association with impaired pancreatic endocrine or exocrine function, and various extrapancreatic lesions; (4) favorable response to steroid therapy; (5) radiological findings of irregular narrowing of the main pancreatic duct and enlargement of the pancreas; (6) serological findings of elevated serum γ globulin, IgG, or IgG4 levels, along with the presence of some autoantibodies; and (7) histopathological findings of dense infiltration of T lymphocytes and IgG4-positive plasma cells with fibrosis and obliterative phlebitis in the pancreas[1-3]. Bile duct stenosis occurs frequently with AIP, and the major initial symptom in AIP patients is obstructive jaundice. The lower portion of the common bile duct is frequently stenotic. However, when AIP patients develop stenosis in the intrahepatic bile duct, the cholangiographic appearance is similar to that of primary sclerosing cholangitis (PSC)[4,5]. PSC is a progressive disease involving the intra- and extra-hepatic bile ducts. Despite therapy, PSC sometimes leads to liver cirrhosis. However, since AIP patients respond well to steroid therapy, it is necessary to discriminate between sclerosing cholangitis (SC) associated with AIP and PSC. This study aimed to clarify the characteristic features of biliary lesions in AIP patients and compare them with those of PSC.

MATERIALS AND METHODS

Study patients

Over a 27-year-period, 43 patients (36 male and 7 female, average age 66.4 years) at Tokyo Metropolitan Komagome Hospital were diagnosed with AIP based on the following clinicopathological criteria: irregular narrowing of the main pancreatic duct on endoscopic retrograde pancreatography (n = 43), pancreatic enlargement on ultrasonography (US) or computed tomography (CT) (n = 42), presence of autoantibodies (n = 22), elevated serum IgG4 level in excess of 135 mg/dL (n = 31), characteristic histological findings in the pancreas (n = 12), and responsiveness to steroid therapy (n = 32). In the 43 AIP patients, 34 had SC (lower bile duct in 34, and intrahepatic bile duct in 4). During the same time, 4 patients were diagnosed with PSC according to appropriate criteria[6].

Methods

The stenotic portion of the bile duct was examined by endoscopic retrograde cholangiopancreatography and/or magnetic resonance cholangiopancreatography, and wall thickening of the bile duct in which stenosis was not obvious on cholangiography was assessed on CT and US. Two experienced gastroenterologists retrospectively reviewed these imaging findings without information on the patients. Extrapancreatic lesions, including sclerosing sialadenitis, sclerosing cholecystitis, and retroperitoneal fibrosis, were evaluated on physical examination, CT, and US. Serum IgG4 levels were measured in 30 AIP patients and 2 PSC patients. Histological examination and immunostaining with anti-IgG4 antibody were performed on specimens of the extrahepatic bile duct (6 AIP patients and 1 PSC patient) and liver (3 AIP and 2 PSC patients).

Statistical analysis

Statistical differences between the two groups were analyzed first by the Kruskal-Wallis H-test, followed by Mann-Whitney's U-test if significant. Other analyses were performed using Fisher's exact test. In all tests, P < 0.05 was considered statistically significant.

RESULTS

Clinical features

Men were significantly more commonly affected by SC with AIP than by PSC. Patients' age at diagnosis was significantly older in those with SC with AIP. Among the initial symptoms, obstructive jaundice was the most frequently observed in SC with AIP. Elevated serum IgG4 levels were frequent in SC patients with AIP, but not in the 2 PSC patients examined. Sclerosing diseases were frequently associated with SC with AIP. Ulcerative colitis was present in only 2 young PSC patients (Table 1). Thirty-two SC patients with AIP were treated with steroid therapy, and all of them showed a good response. All PSC patients were treated with ursodeoxycholic acid, and 1 patient underwent steroid therapy for associated ulcerative colitis. Cholangiographic findings progressed gradually in three PSC patients, and one patient ultimately required liver transplantation. All SC patients with AIP had a favorable outcome without liver failure.

Table 1 Clinical differences between sclerosing cholangitis with autoimmune pancreatitis and primary sclerosing cholangitis

	SC with AIP	PSC	P value
Average age (yr)	63.8	39.2	< 0.01
Male/Female	29/5	1/3	< 0.05
Obstructive jaundice +/-	30/4	0/4	< 0.01
Elevated serum IgG4 +/-	26/30	0/2	
Associated sclerosing disease +/-	20/14	0/4	< 0.05
Associated ulcerative colitis +/-	0/34	2/2	< 0.01

SC with AIP: Sclerosing cholangitis with autoimmune pancreatitis; PSC: Primary sclerosing cholangitis.

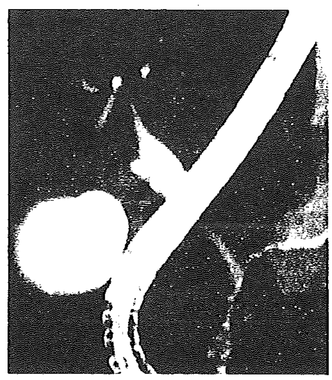

Figure 1 Endoscopic retrograde cholangiography of a patient with autoimmune pancreatitis showing a relatively long stricture of the hepatic hilar bile duct.

Cholangiopancreatographic findings

On pancreatography, narrowing of the main pancreatic duct was detected in all SC patients with AIP, but no abnormal findings were detected in any of the PSC patients. On cholangiography, the intrahepatic bile duct was involved in all PSC patients, but was involved in only four SC patients with AIP (Figure 1). Segmental stenosis of the lower bile duct was observed in all SC patients with AIP, but was not detected in any of the PSC patients. Extensive involvement of the bile duct, showing widespread wall thickening of the middle and upper bile duct where stenosis was not obvious on cholangiography, was detected only in 14 SC patients with AIP, although there was no significant difference between the two groups. A diffusely distributed, beaded and pruned-tree appearance and diverticular formation were detected only in PSC patients (Figure 2 and Table 2). A long stricture was detected in the hepatic hilar region in all 4 SC patients with AIP involving the intrahepatic bile duct.

Histological and immunohistochemical findings

In PSC, the hilar bile duct displayed diffuse fibrosis with moderate lymphoplasmacytic infiltration. The liver of PSC patients showed features of biliary cirrhosis, and fibro-obliterative lesions characterized by onion skin-like periductal fibrosis with predominantly lymphocytic infiltration were observed around the intrahepatic bile

Table 2 Cholangiopancreatographic differences between sclerosing cholangitis with autoimmune pancreatitis and primary sclerosing cholangitis

	SC with AIP	PSC	*P* value
Narrowing of the main pancreatic duct +/-	34/0	0/4	< 0.01
Stenosis of the intrahepatic bile duct +/-	4/30	4/0	< 0.01
Stenosis of the lower bile duct +/-	34/0	0/4	< 0.01
Extensive bile duct wall thickening	14/20	0/4	NS
Beaded appearance	0/34	2/2	< 0.01
Pruned-tree appearance	0/34	3/1	< 0.01
Diverticular formation	0/34	2/2	< 0.01

Figure 2 Endoscopic retrograde cholangiography of a patient with primary sclerosing cholangitis showing beaded and pruned-tree appearance.

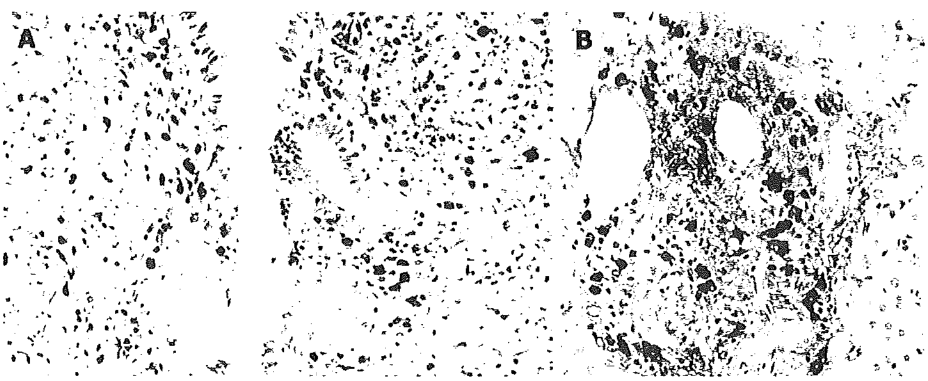

Figure 3 IgG4-immunostaining of the bile duct (A) and liver (B) of a patient with autoimmune pancreatitis. Dense infiltration of IgG4-positive plasma cells was detected in the bile duct wall (A) and the periportal area of the liver (B).

duct. However, infiltration of IgG4-positive plasma cells was not detected in the bile duct or liver.

The histological findings of SC associated with AIP included transmural fibrosis and dense lymphoplasmacytic infiltration of the bile duct wall, along with lymphoplasmacytic infiltration and fibrosis in the periportal area of the liver. Compared with PSC, lymphoplasmacytic infiltration was denser, the degree of fibrosis was less severe, and the onion skin-like appearance was not observed. Dense infiltration of IgG4-positive plasma cells was detected in the bile duct wall (Figure 3A) and the periportal area (Figure 3B), as well as in the pancreas, of patients with AIP.

DISCUSSION

SC is a heterogeneous disease that may be associated with choledocholithiasis, biliary tumor, or infection. SC of unknown origin is called PSC. PSC is progressive despite conservative therapy and involves the intra- and extrahepatic bile ducts, resulting in liver cirrhosis. The effect of steroid therapy is questionable, and liver transplantation currently provides the greatest hope for a possible cure. It occurs among patients in their 30 and 40 s and is frequently associated with inflammatory bowel disease[7,8]. Pancreatograms are not abnormal in most cases[9].

However, an analysis of 192 PSC patients in Japan found that their characteristics differed from those in Western counties, with regard to age distribution and the incidence of complications[10]. In that analysis, the patients were predominantly men, and two peaks in age distribution at diagnosis (20-30 years and 50-70 years) were identified. Compared to younger patients, those aged 40 years or older displayed a lower incidence of associated ulcerative colitis, whereas the incidence of chronic pancreatitis was higher.

SC is frequently associated with AIP, and occurs predominantly in elderly men. The major initial symptom of SC with AIP is obstructive jaundice, which differs from PSC. The most prominent feature on cholangiography for SC with AIP was stenosis of the lower bile duct. When stenosis is found in the intrahepatic or the hilar hepatic bile duct, the cholangiographic appearance is very similar to that of PSC[4,5]. However, a long stricture was detected in the hepatic hilar region in SC patients with AIP, instead of the beaded and pruned-tree appearance that is frequently observed in PSC. Widespread wall thickening of the middle and upper bile ducts was also detected only in SC patients with AIP.

SC patients with AIP responded dramatically well to steroid therapy and showed a favorable outcome[5,9,11]. Histologically, dense infiltration of IgG4-positive plasma cells was detected in the bile duct wall and the periportal area of SC patients with AIP, but it was not detected in PSC patients. SC with AIP is sometimes associated with sclerosing diseases such as sclerosing sialadenitis, sclerosing cholecystitis, or retroperitoneal fibrosis, and these salivary, gallbladder, and retroperitoneal lesions show similar histological findings to those in the bile duct and pancreas. Furthermore, abundant infiltration of IgG4-positive plasma cells is detected in various organs of AIP patients[12,13]. Therefore, we proposed a new clinicopathological entity, an IgG4-related sclerosing disease, which is histopathologically characterized by extensive IgG4-positive plasma cell and T lymphocyte infiltration of various organs. We also suspect that AIP and SC with AIP is a pancreatic and bile duct lesion of this systemic disease[13-15]. Based on the above findings, SC with AIP should be differentiated from

PSC. In particular, since SC with AIP responds well to steroid therapy, discrimination between the two diseases is necessary before making therapeutic decisions. Clinically, serum IgG4 levels and cholangiopancreatographic findings are useful in differentiating between the two diseases.

Considering the predominance of elderly men, the infrequent association with inflammatory bowel disease, and the frequent association with chronic pancreatitis, many older patients diagnosed with PSC in Japan may actually have SC with AIP.

In conclusion, since SC with AIP is induced by different mechanisms to those in PSC, the condition should be differentiated from PSC. The two diseases can be discriminated based on their cholangiopancreatographic findings and serum IgG4 levels.

COMMENTS

Background

When patients with autoimmune pancreatitis (AIP) develop stenosis in the intrahepatic bile duct, the cholangiographic appearance is similar to that of primary sclerosing cholangitis (PSC). PSC is a progressive disease involving the intra- and extrahepatic bile ducts.

Innovations and breakthroughs

Sclerosing cholangitis with AIP is distinctly different from PSC. Only SC patients with AIP had elevated serum IgG4 levels and responded well to steroid therapy. Segmental stenosis of the lower bile duct was observed only in SC patients with AIP, but a beaded and pruned-tree appearance was detected only in PSC patients. Dense infiltration of IgG4-positive plasma cells was detected in the bile duct wall and the periportal area of SC patients with AIP.

Applications

Sclerosing cholangitis with AIP responds well to steroid therapy. The differential diagnosis between sclerosing cholangitis with AIP and PSC is important to ensure optimal patient treatment.

Peer review

The authors described the characteristic features of biliary lesions in autoimmune pancreatitis patients. The paper is well presented and the result are interesting.

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Standard steroid treatment for autoimmune pancreatitis

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See Commentary, p 1438

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ABSTRACT

Objective: To establish an appropriate steroid treatment regimen for autoimmune pancreatitis (AIP).
Methods: A retrospective survey of AIP treatment was conducted in 17 centres in Japan. The main outcome measures were rate of remission and relapse.
Results: Of 563 patients with AIP, 459 (82%) received steroid treatment. The remission rate of steroid-treated AIP was 98%, which was significantly higher than that of patients without steroid treatment (74%, 77/104; p<0.001). Steroid treatment was given for obstructive jaundice (60%), abdominal pain (11%), associated extrapancreatic lesions except the biliary duct (11%), and diffuse enlargement of the pancreas (10%). There was no relationship between the period necessary to achieve remission and the initial dose (30 mg/day vs 40 mg/day) of prednisolone. Maintenance steroid treatment was given in 377 (82%) of 459 steroid-treated patients, and steroid treatment was stopped in 104 patients. The relapse rate of patients with AIP on maintenance treatment was 23% (63/273), which was significantly lower than that of patients who stopped maintenance treatment (34%, 35/104; p = 0.048). From the start of steroid treatment, 56% (55/99) relapsed within 1 year and 92% (91/99) relapsed within 3 years. Of the 89 relapsed patients, 83 (93%) received steroid re-treatment, and steroid re-treatment was effective in 97% of them.
Conclusions: The major indication for steroid treatment in AIP is the presence of symptoms. An initial prednisolone dose of 0.6 mg/kg/day, is recommend, which is then reduced to a maintenance dose over a period of 3–6 months. Maintenance treatment with low-dose steroid reduces but dose not eliminate relapses.

Autoimmune pancreatitis (AIP) is a newly described entity in which autoimmune mechanisms seem to be involved. It is characterised clinically by obstructive jaundice as a frequent initial symptom and an association with diabetes mellitus (DM) and various extrapancreatic lesions. Radiologically, AIP is characterised by enlargement of the pancreas and irregular narrowing of the main pancreatic duct. Serologically, it is characterised by serum immunoglobulin G4 (IgG4) elevation and the presence of autoantibodies. Histopathologically, dense infiltration of lymphocytes and IgG4-positive plasma cells with fibrosis are seen in the pancreas.[1–4] Since the fibroinflammatory process of AIP responds well to steroids, many AIP patients receive steroid treatment.[5–10] Although there are some published reports dealing with steroid treatment for AIP, these previous studies involved only small numbers of patients, and there is little consensus on a steroid treatment regimen. To establish the appropriate steroid treatment regimen, a survey of AIP treatment was conducted in 17 centres in Japan.

METHODS

A retrospective survey of AIP treatment, focusing on steroid treatment, was conducted in the 17 centres that participated in this study. The majority of these centres are major referral centres across Japan with established expertise in the diagnosis and management of AIP. Tokyo Metropolitan Komagome Hospital and Tohoku University served as the coordinating centres.

The diagnosis of AIP was made according to the Asian diagnostic criteria for AIP.[11] To make the diagnosis of AIP, the imaging criterion, consisting of enlargement of the pancreas and irregular narrowing of the main pancreatic duct, must be present, together with the serological criterion (elevated serum IgG or IgG4 levels, or detection of autoantibodies) and/or the histopathological criterion (lymphoplasmacytic sclerosing pancreatitis). AIP can be also diagnosed with fulfilment of both the imaging criterion and a good response to steroid treatment.

Data regarding induction of remission by steroid treatment, maintenance steroid treatment and relapse of AIP were analysed. Remission was defined as the disappearance of clinical symptoms and resolution of the pancreatic and/or extrapancreatic manifestations on imaging studies.[8 12–14] For follow-up after remission, laboratory tests and imaging studies were performed periodically, usually every 3–6 months in the first year. Relapse of AIP was defined as reappearance of symptoms with the development or reappearance of pancreatic and/or extrapancreatic (including bile duct, salivary gland and retroperitoneum) abnormalities on imaging studies and/or marked elevation of serum IgG or IgG4 levels.[8 12–14] Re-elevation of serological levels alone without clinical symptoms or abnormal imaging was not considered to be relapse.

Statistical analysis was performed using Fisher's exact test and Mann–Whitney's U test. Differences with p values of <0.05 were considered significant. The period from the start of steroid treatment to relapse was evaluated using the Kaplan–Meier curve.

After analysis of the data, a consensus meeting was held involving members of the 17 centres to propose a consensus regarding steroid treatment for AIP.

RESULTS

Study subjects

A total of 563 cases of AIP (439 men and 124 women, average age 63.0 years) were confirmed to fulfil the Asian diagnostic criteria for AIP, and they were enrolled in the analyses for steroid treatment of AIP. Of these, 459 (82%) patients with AIP (374 men and 85 women, average age 62.3 years) received steroid treatment. Of the others, 56 patients underwent surgical procedures, and 48 patients were followed-up conservatively.

No patients received any other immunosuppressive treatments such as azathioprine or ulsodeoxycholic acid.

Induction of remission by steroid treatment

The remission rate of patients with AIP was significantly higher in patients who received steroid treatment (98%, 451/459) than in those not given steroid treatment (74%, 77/104; p<0.001) (table 1).

Steroid treatment was administered mainly for obstructive jaundice (247/459 patients, 60%), abdominal pain (51 patients, 11%), associated extrapancreatic lesions such as retroperitoneal fibrosis (50 patients, 11%), diffuse enlargement of the pancreas (45 patients, 10%) and confirmation or differentiation of the diagnosis after a negative investigation of pancreatic cancer (24 patients, 5%).

In patients with DM, before steroid administration, blood glucose levels were controlled using insulin in 104 patients and using oral antidiabetic medicines in 39 patients. Endoscopic or transhepatic biliary drainage was performed in 242 (77%) of 314 patients with obstructive jaundice due to associated sclerosing cholangitis. Endoscopic or transhepatic biliary drainage was performed for patients showing hyperbilirubinaemia of >3 mg/dl in two-thirds of centres.

The initial oral prednisolone dose was 20 mg/day (n = 8, 2%), 30 mg/day (n = 283, 62%), 40 mg/day (n = 160, 35%), 60 mg/day (n = 4, 1%) and others (n = 4, 1%). The initial dose was administered for 2 weeks in three-quarters of cases, and for 3–4 weeks in the remainder. The initial dose was gradually tapered by 5 mg every 1–2 weeks to the maintenance dosage, based on changes in the clinical manifestations, biochemical blood tests (such as serum liver enzymes and IgG or IgG4 levels) and repeated imaging findings. The dose was tapered more gradually, such as 2.5 or 5 mg every 2–8 weeks, after the dose reached 15 mg/day.

The period necessary to achieve remission from the start of initial administration was 6.82 (6.11) months (mean (SD)) in the patients treated with an initial prednisolone dose of 30 mg/day, which was not significantly different from the period (6.34 (8.13) months) in those treated with an initial prednisolone dose of 40 mg/day (p = 0.401) (table 2).

At remission, the enlarged pancreas returned to near-normal size in 239 (80%) of 300 patients. It became atrophic in 58 patients (20%), and showed persistent focal enlargement in 3 patients. Elevated serum IgG4 levels decreased in all patients after the start of steroid treatment, but they failed to normalise (<135 mg/dl) in 115 (63%) of 182 patients. At remission, irregularity of the pancreatic ducts and/or some degree of bile duct stenosis remained in 67 (58%) of 115 patients with persistent elevation of serum IgG4 levels, while it remained in only 18 (27%) of 67 patients with normalised serum IgG4 levels (p<0.001).

Table 1 Remission and relapse rate in patients with autoimmune pancreatitis treated with and without steroid

	With steroid	Without steroid	p Value
Remission rate	451/459 (98%)	77/104 (74%)	<0.0001
Relapse rate	110/451 (24%)	32/77 (42%)	0.003

Maintenance steroid treatment

Maintenance steroid treatment was performed after remission in 377 (82%) of 459 patients treated with steroid. The maintenance oral prednisolone dose was 10 mg/day (n = 27, 7%), 7.5 mg/day (n = 13, 3%), 5 mg/day (n = 238, 63%), 2.5 mg/day (n = 78, 21%) and others. Of the 377 patients who underwent maintenance treatment, the maintenance treatment was stopped in 104 patients (28%) in whom complete radiological and serological improvement was obtained.

Relapse of AIP

The relapse rate of patients with AIP was significantly lower in those who received steroid treatment (24%, 110/451) than in those not given steroid treatment (42%, 32/77; p = 0.003) (table 1). In the patients who received steroid treatment, relapse occurred in the pancreas (n = 57, 52%), bile duct (n = 37, 34%) and extrapancreatic lesions (n = 19; salivary gland swelling (n = 10), interstitial pneumonia (n = 4), interstitial nephritis (n = 2) and others).

There was no correlation between the relapse rate and the initial prednisolone dose (40 mg/day: 19% (31/160) vs 30 mg/day: 23% (65/283), p = 0.402) (table 2). As regards the period from the start of steroid treatment to relapse, 32% (32/99) relapsed within 6 months, 56% (55/99) relapsed within 1 year, 76% (75/99) relapsed within 2 years and 92% (91/99) relapsed within 3 years after starting medication (fig 1). The relapse rate of patients with AIP on maintenance treatment was 23% (63/273), which was significantly lower than that of patients who stopped maintenance treatment (34%, 35/104; p = 0.048). The doses of prednisolone at the time of relapse were 10 mg/day (n = 10, 16%), 7.5 mg/day (n = 7, 11%), 5 mg/day (n = 29, 46%), 2.5 mg/day (n = 8, 13%) and others.

The relapse rate of AIP was significantly higher in patients with persistent elevation of serum IgG4 levels (30%, 34/115) than in those with normalised serum IgG4 levels (10%, 7/69; p = 0.003). Although serum IgG4 levels fluctuated by >30 mg/dl in 94 (55%) of 172 patients during maintenance treatment, re-elevation of serum IgG4 levels was detected in 37 (69%) of 54 patients who relapsed during maintenance treatment.

Of the 89 relapsed patients, 83 (93%) of 89 received steroid re-treatment (prednisolone: 60 mg/day (n = 4, 5%), 40 mg/day (n = 19, 23%), 30 mg/day (n = 39, 47%), 20 mg/day (n = 9, 11%) and others). Steroid re-treatment was effective in 91 (97%) of 94 relapsed patients. Of the 77 patients initially managed without steroid treatment relapses occurred in 32 (42%), and the relapses were treated with steroid with a 100% response rate.

Steroid-related complications

After steroid treatment, mildly or moderately worse glucose tolerance occurred in several patients, but they could be controlled by oral antidiabetic medication or insulin injection. Osteoporosis developed in 10 patients, in whom compression fractures of lumbar vertebrae (n = 5) and avascular necrosis of the femoral head (n = 3) occurred. They were treated with reduction of dosage or cessation of medication. Pneumonia occurred in 3 patients, and they were treated with antibiotics.

Table 2 Period to yield a remission and relapse rate in patients with autoimmune pancreatitis treated with initial prednisolone of 40 and 30 mg/day

	40 mg/day	30 mg/day	p Value
Period to remission (mean (SD), months)	6.34 (8.13)	6.82 (6.11)	0.401
Relapse rate	31/160 (19%)	65/283 (23%)	0.402

There were no deaths attributable to complications of steroid treatment.

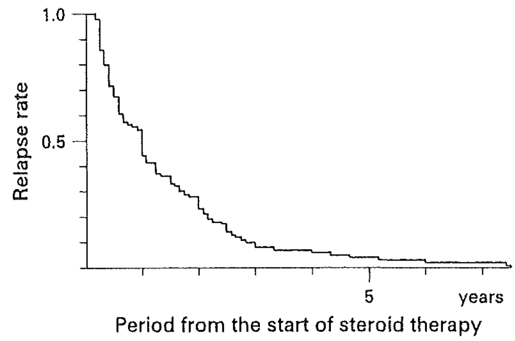

Figure 1 Relapse rate of autoimmune pancreatitis and the period from the start of steroid treatment to relapse.

DISCUSSION

In the analysis of 563 patients with AIP, 82% received oral steroid treatment. The remission rate of patients with AIP was significantly higher in those who received steroid treatment (98%) than in those who did not receive steroid treatment (74%). The relapse rate was significantly lower in patients who received steroid treatment than in those not given steroid treatment. Therefore, the administration of oral steroid appears to be standard treatment for inducing remission in AIP. However, it is most important to distinguish AIP from pancreatic cancer before starting steroid treatment. Facile use of steroids for cases in which the diagnosis of AIP is questionable should be prohibited.[15 16]

The indications for steroid treatment in patients with AIP are thought to be symptoms such as obstructive jaundice due to sclerosing cholangitis, abdominal pain and hydronephrosis due to associated retroperitoneal fibrosis. DM is often (67%[17] to 76%[18] of cases) observed in patients with AIP. Pancreatic exocrine function is also impaired in 88%[19] to 91%[20] of AIP patients. However, as pancreatic exocrine or endocrine dysfunction improves in some patients with AIP after steroid treatment,[17 19 20] steroid treatment may be indicated in patients showing diffuse enlargement of the pancreas, even if they are asymptomatic.

In patients with DM, blood glucose levels should be controlled using insulin before starting steroid treatment. The major presenting complaint of patients with AIP is obstructive jaundice due to associated sclerosing cholangitis (65%[18] to 86%[21] of cases). As steroid treatment may trigger or worsen cholangitis, jaundice is usually managed by endoscopic or transhepatic biliary drainage in patients with obstructive jaundice (usually total bilirubin ⩾3 mg/dl) due to associated sclerosing cholangitis before steroid administration. In the literature, most patients with AIP were treated with 30 or 40 mg/day as the initial prednisolone dose.[5-10] There was no relationship between the period necessary to achieve remission and the initial prednisolone dose. In Japan, 30 mg/day is usually used as an initial dose in patients with standard body weight (~50 kg; 0.6 mg/kg) and 40 mg/day is used in larger patients (body weight >60 kg; 0.67 mg/kg). Therefore, we would recommend that a general initial dose of prednisolone should be 0.6 mg/kg/day.

Since pancreatic enlargement begins to improve from 1 to 2 weeks after medication,[10 12] morphological and serological evaluation for effectiveness of steroid treatment should be performed 2 weeks after starting steroid treatment. A poor response to steroid treatment should raise the possibility of pancreatic cancer and the need for re-evaluation of the diagnosis. When steroid treatment is effective, the dose is tapered by 5 mg every 1–2 weeks until the dose reaches 15 mg/day, while carefully monitoring the patient's symptoms, as well as the biochemical, serological and imaging findings. After that, the dose is tapered more gradually, and the amount of steroid is reduced to a maintenance dose over a period of 3–6 months.

Relapse occurred in 24% of patients with AIP treated with steroid, and it occurred within 6 months after medication in 32%, within 1 year in 56% and within 3 years in 92%. Although it still remains unclear what are useful predictive findings for relapse, it has been reported that markedly elevated serum IgG4 levels and the presence of bile duct stenosis were predictive factors for relapse of AIP.[8] To our knowledge, patients complicated with extrapancreatic lesions such as stenosis of the proximal extrahepatic or intrahepatic bile duct or retroperitoneal fibrosis seem to take longer to achieve remission. In the present study, the patients with elevation of serum IgG4 levels during remission showed persistent abnormalities of the pancreatic and/or bile ducts and more frequent relapses than in those with normalised serum IgG4 levels. Furthermore, in 69% of relapsed patients during maintenance treatment, re-elevation of serum IgG4 levels was detected. Serum IgG4 levels at remission and during follow-up may accordingly be useful to predict or detect relapse earlier.

To prevent relapse, maintenance treatment (5 mg/day) is recommended in almost all patients treated with steroid for at least about 6 months. However, as patients with AIP are typically elderly and are at high risk of developing steroid-related complications, such as osteoporosis, DM and pneumonia, cessation of the medication should be tried. In patients showing complete improvement of cholangiopancreatogram, 1 year after initial administration of steroid, maintenance treatment can be withdrawn. Stopping maintenance treatment should be planned within at least 3 years, because of a lower relapse rate after 3 years. After stopping medication, patients should be followed-up for relapse of AIP. In most recurrent cases, re-administration or dose-up of steroid is effective. In these cases, longer maintenance treatment is necessary to prevent repeated relapse. Therefore, we think that it is now necessary to reduce the relapse rate. Careful, long-term follow-up is also necessary, since pancreatic cancer developed in three patients 3, 3.5 and 5 years after onset of AIP, respectively. On the other hand, 23% of patients with AIP relapsed despite maintenance treatment. We need to identify more useful alternative approaches to maintain remission.

In the Japanese diagnostic criteria for AIP,[15] seronegative AIP cases without histological examination cannot be diagnosed as AIP even if they fulfil the imaging criterion. Kim *et al*[22] reported that a steroid trial was useful for differentiating seronegative AIP from pancreatic cancer, based on marked improvement of pancreatic ductal narrowing, which is evident as early as 2 weeks after the beginning of steroid treatment. When

Box 1 Standard steroid treatment for autoimmune pancreatitis

- Oral steroids is the standard treatment for AIP.
- It is most important to distinguish AIP from pancreatic cancer before starting steroid treatment.
- The indications for steroid treatment in patients with AIP are symptoms such as obstructive jaundice, abdominal pain and hydronephrosis.
- Before steroid treatment, blood glucose level should be controlled using insulin in patients with diabetes mellitus.
- Before steroid treatment, jaundice is usually managed by endoscopic or transhepatic biliary drainage in patients with obstructive jaundice.
- As initial dose of oral prednisolone of 0.6 mg/kg/day is recommended.
- Morphological and serological evaluation for effectiveness of steroid treatment is performed 2 weeks after starting steroid treatment. A poor response to steroid treatment should raise the possibility of pancreatic cancer and the need for re-evaluation of the diagnosis. When steroid treatment is effective, the dose is tapered by 5 mg every 1–2 weeks until the dose reaches 15 mg/day, while carefully monitoring the patient's symptoms, as well as the biochemical, serological and imaging findings. After that, the dose is tapered more gradually to a maintenance dose over a period of 3–6 months.
- To prevent relapse, maintenance treatment (5 mg/day) is recommended in almost all patients treated with steroid for at least about 6 months. In patients showing complete remission 1 year after initial administration of steroid, maintenance treatment can be withdrawn. Stopping maintenance treatment should be planned within at least 3 years.
- In relapsed cases, re-administration or dose-up of steroid is effective.
- A steroid diagnostic trial is not generally recommended. It should only be performed by pancreatologists with extreme caution in limited cases after a negative investigation for pancreatic cancer, including endoscopic ultrasound-guided fine needle aspiration.

response to steroid treatment is added to the diagnostic criteria, the diagnostic sensitivity is increased. However, we are concerned that the facile use of steroid trials will result in delaying pancreatic cancer surgery, which could lead to cancer progression in some cases. Therefore, as described in the Asian diagnostic criteria,[11] a steroid diagnostic trial is not generally recommended and it should only be performed by pancreatologists with extreme caution in limited cases after a negative investigation for pancreatic cancer, including endoscopic ultrasound-guided fine needle aspiration (EUS-FNA; box 1).

In conclusion, oral steroid is a standard treatment for AIP. Indications for steroid treatment in patients with AIP are symptoms such as obstructive jaundice due to sclerosing cholangitis, abdominal pain, and hydronephrosis due to associated retroperitoneal fibrosis. We recommend that the initial dose of prednisolone be 0.6 mg/kg/day, and that it be reduced to a maintenance dose over a period of 3–6 months. To prevent relapses, continued maintenance treatment with low-dose prednisolone for 6 months to 3 years is recommended, but it dose not eliminate relapse entirely.

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ORIGINAL ARTICLE

Amylase α-2A Autoantibodies

Novel Marker of Autoimmune Pancreatitis and Fulminant Type 1 Diabetes

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OBJECTIVE—The pathogenesis of autoimmune pancreatitis (AIP) and fulminant type 1 diabetes remains unclear, although it is known that immune-mediated processes severely compromise the endocrine and exocrine functions in both diseases.

RESEARCH DESIGN AND METHODS—We have screened a λTriplEx2 human pancreas cDNA library with serum from a patient with AIP and obtained positive clones. Sequence analysis revealed that 7 of 10 clones were identical to human amylase α-2A. Using a recombinant COOH-terminal amylase α-2A protein, we developed an enzyme-linked immunosorbent assay system to detect autoantibodies against human amylase α-2A.

RESULTS—All 15 serum samples from patients with AIP recognized the recombinant protein, whereas sera from 25 patients with chronic alcoholic pancreatitis and sera from 25 patients with a pancreas tumor did not. Interestingly, 88% (15/17) of patients with fulminant type 1 diabetes were positive for an autoantibody against amylase α-2A. These antibodies were detected in 21% of patients with acute-onset type 1 diabetes (9 of 42) and 6% of type 2 diabetic patients (4 of 67).

CONCLUSIONS—These results suggest that an autoantibody against amylase α-2A is a novel diagnostic marker for both AIP and fulminant type 1 diabetes and that, clinically and immunologically, AIP and fulminant type 1 diabetes are closely related.
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Recently, autoimmune pancreatitis (AIP), a unique form of chronic pancreatitis, has been reported as a discrete disease entity (1). It is characterized by *1*) irregular narrowing of the main pancreatic duct and swelling of the pancreas, both of which are due to abundant lymphoplasmacytic inflammation to the exocrine pancreas (2); *2*) the increased serum level of IgG and IgG4; *3*) positive autoantibodies such as lactoferrin autoantibody or carbonic anhydrase II (CAII) autoantibody (3,4); and *4*) a high prevalence of diabetes with complications (5).

We recently reported that a high proportion of pancreatic islets and exocrine pancreatic tissues were infiltrated by $CD4^+$ or $CD8^+$ T-cells in the inflammatory process, which might induce diabetes in AIP (6). In addition, treatment with prednisolone improved insulin secretion and glycemic control in AIP patients (5). These data support the concept that autoimmune mechanism(s) plays a pivotal role in the destruction of the endocrine and exocrine pancreas in AIP patients with diabetes.

Clinically, the most common initial symptom of AIP is jaundice, but in some patients, no symptoms or only mild symptoms, frequently without acute attacks of pancreatitis, may be present (7). It is difficult to distinguish AIP from other types of chronic pancreatitis or cancer of the pancreatic head (8). In such cases, detection of autoantibodies is useful for diagnosing AIP, but a proportion of patients with AIP are negative for autoantibodies against lactoferrin and CAII (3,4).

We encountered an AIP patient whose serum IgG and IgG4 levels were 3,498 and 2,430 mg/dl, respectively. It has been reported that median levels (5th and 95th percentiles) of IgG and IgG4 from patients with AIP were 2,389 mg/dl (1,349 and 4,310) and 742 mg/dl (265 and 1,150), respectively (3), so high concentrations of IgG in this case prompted us to search for new autoantigens associated with AIP. We also searched for the presence or absence of new autoantibodies in patients with abrupt onset and severe ketoacidosis-prone type 1 diabetes [called fulminant type 1 diabetes (9,10)], which involve the exocrine pancreas and the endocrine pancreas.

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RESEARCH DESIGN AND METHODS

Serum used for screening the human pancreas cDNA library was obtained from a 67-year-old male patient (A.O.), who was admitted to our hospital complaining of slight abdominal pain and jaundice. Computed tomography revealed an enlarged pancreas, and laboratory findings showed high concentrations of IgG and IgG4. Tests for anti-lactoferrin and anti-CAII antibodies were both positive, but those for anti-nuclear antibody, anti-mitochondrial antibody, and rheumatoid factor were negative.

Additional AIP sera were obtained from 14 newly diagnosed patients at the University of Yamanashi Hospital and Toranomon Hospital, Tokyo. Diagnosis of AIP was based on criteria proposed by the Japan Pancreas Society (11). Our 15 patients filled criterion 1 (narrowing of the main pancreatic duct or enlargement of pancreas by imaging studies), together with criterion 2 (high serum γ-globulin, IgG, or IgG4 or the presence of autoantibodies, such as anti-nuclear antibodies and rheumatoid factor) and/or criterion 3 (marked interlobular fibrosis and prominent infiltration of lymphocytes and plasma cells in the periductal area). Serum samples were taken from 25 patients with chronic alcoholic pancreatitis, who were diagnosed according to a history of alcohol abuse, impaired exocrine pancreatic function, and the presence of calcified precipitates in the pancreas by imaging studies [Japan Pancreas

TABLE 1
Clinical characteristics of subjects

Type of diabetes	n	Age (years)	Sex (male/female)	Duration of diabetes (months)*	Treatment by insulin*
Autoimmune pancreatitis	15	66 (58–75)	14/1	—	8
Before PSL	12				
After PSL	3				
Chronic alcoholic pancreatitis	25	63 (53–70)	18/7	—	10
Pancreatic tumor	25	71 (63–73)	12/13	—	8
Cancer	8				
IPMT	17				
Fulminant type 1 diabetes	17	40 (28–53)	14/3		17
At onset†	13			0.76 ± 0.20	
After onset	4			13.5 ± 2.38	
Acute-onset type 1 diabetes	42	25 (23–33)	14/28	29.0 ± 45.0	42
At onset†	22			0.7 ± 0.9	
After onset	20			51.0 ± 50.0	
Type 2 diabetes	67	62 (58–65)	43/24	130 ± 91.0	37
Hashimoto's thyroiditis	47	60 (55–62)	6/41	—	—
Control subjects	100	47 (40–48)	59/41	—	—

Data are medians (95% CI) or means ± SD. PSL, prednisolone; IPMT, intraductal papillary mucinous tumor. *Duration from the onset of diabetes to the time of sample collection. †At onset; within 3 months after onset.

Society, criteria for chronic pancreatitis 2001 (12)]. Twenty-five serum samples were recruited from patients with pancreas tumor (cancer [n = 8] and intraductal papillary mucinous tumor [IPMT, n = 17]). Fulminant type 1 diabetes (n = 17, 13 cases at the onset and 4 cases after onset) was diagnosed by criteria (fasting C-peptide ≤0.033 nmol/l and A1C ≤8.0% or ∑C-peptide ≤0.540 nmol/l and A1C ≤8.0%) as reported previously (13,14). Fulminant type 1 diabetes associated with pregnancy (15) was excluded from the present study. Acute-onset type 1 diabetes (n = 42) (12) and type 2 diabetes (n = 67) samples were also recruited. The patients' clinical characteristics are summarized in Table 1. Serum from patients with Hashimoto's thyroiditis (n = 47) were also studied. Diagnosis of the disease was made by elastic goiter and autoantibodies against both thyroglobulin and thyroid peroxidase. Control sera were obtained from 100 (59 male and 41 female) healthy volunteers.

Immunoscreening. The λTriplEx2 human pancreas large insert cDNA library (HL5517u) and *Escherichia coli* XL-1 competent cells were obtained from BD Biosciences Clontech (Palo Alto, CA). The plaques on the plate were transferred to nitrocellulose filters presoaked with 10 mmol/l isopropyl-β-D-thiogalactopyranoside (IPTG), washed with Tris-buffered saline (TBS) containing 0.05% Tween 20 (TBST), and blocked with TBST containing 1% BSA. The filters were incubated overnight at 4°C with the sera from the patient with AIP (A.O.) at a dilution of 1:500. After washing four times with TBST, the filters then reacted with goat horseradish peroxidase–conjugated anti-human IgG (American Qualex, San Clemente, CA) at a dilution of 1:2,000 for 30 min at room temperature. The filters were also washed four times with TBST; positive reaction was detected with 3,3′-diaminobenzidine.

Preparation of the recombinant human AMY-2A. A cDNA fragment of the positive clone was amplified by PCR with the sense primer, 5′-ATGGGGATC CTTGGGGTTTCGTACCTTCTGACAGA, and antisense primer, 5′-CTTCGAAT TCCCAATTTAGATTCAGCATGAATTGC. The PCR product was digested with *Bam*HI and *Eco*RI and then ligated into pTrc His B (Invitrogen, Carlsbad, CA). After sequencing, the plasmid was transfected into *E. coli* BL-21 (Novagen, Darmstadt, Germany). The production of the recombinant protein was inducted with 1 mmol/l IPTG and purified by His Bond column chromatography.

Western blot analysis. The 0.1% SDS–15% PAGE and transferring onto the nitrocellulose membrane was carried out as previously described (16) with slight modifications as follows: The membrane was blocked with 5% skim milk and 5% goat serum in TBS and then incubated with sera from the patients with AIP (1:500) overnight at 4°C. After washing five times with TBST, the membrane was reacted with goat horseradish peroxidase–conjugated anti-human IgG (1:2,000) for 30 min at room temperature. Positive reaction was detected by the same way as described in IMMUNOSCREENING.

In vitro translation and immunoprecipitation. A cDNA fragment of AMY-2A was amplified by PCR with the sense primer, 5′-ATGGGGATCCATG TGGGGTTTCGTACCTTCTGACAGA, and antisense primer, 5′-CTTCGAATTC CCAATTTAGATTCAGCATGAATTGC, which added an ATG codon at the NH_2-terminus. The PCR product was digested with *Bam*HI and *Eco*RI and then ligated into pcDNA3.1. ^{35}S-labeled human AMY-2A was prepared with PROTEIN script II (Ambion, Austin, TX) and [^{35}S]methionine (GE Healthcare, Piscataway, NJ). ^{35}S–AMY-2A was incubated with patients' sera (×100) or anti-human amylase antibody (×100; sc-12821; Santa Cruz Biotechnology, Santa Cruz, CA) in 200 μl PBS containing 1% BSA at 4°C overnight, with 10 μl GammaBind G Sepharose (GE Healthcare) added. After further incubation at room temperature for 60 min, the mixtures were centrifuged at 10,000 rpm for 5 min. The pellets were washed three times with PBS containing 0.05% Tween 20 (PBST). Final pellets were directly counted or dissolved with 10 mmol/l Tris-HCl (pH. 6.8) containing 0.1% SDS, boiled for 3 min, and loaded onto a 0.1% SDS–15% polyacrylamide gel.

Enzyme-linked immunosorbent assay for detecting autoantibody against human AMY-2A. Autoantibody against human AMY-2A was measured by enzyme-linked immunosorbent assay (ELISA) using methods previously described (5). In brief, a microtiter plate (Coster 3590; Corning, Horseheads, NY) was coated with 50 μl 0.1 μg recombinant human AMY-2A overnight at 4°C. After washing the plate three times with PBST, the plate was incubated with 200 μl 1% BSA in PBS for 30 min. Next, the patients' sera were tested in triplicate at dilutions of 1:200 in 1% BSA for 1 h. The bound antibody was specially reacted with goat horseradish peroxidase–conjugated anti-human IgG (1:2,000) in 1% BSA for 30 min at room temperature. After washing, the plate was incubated with 100 μl 1-Step Slow TMB-ELISA (Pierce, Rockford, IL) for 30 min. The reaction was terminated by adding 100 μl 1 mol/l H_2SO_4, and absorbance was determined at an optical density of 450 nm. Intra- and interassay coefficient of variation, determined with the same lot of five ELISA plates, were 4.28 and 7.72%, respectively.

Ethics. An ethical committee approved all study protocols, and patients gave informed consent.

Statistical analysis. Statistical analysis was carried out using Fisher's exact test (JMP, Cary, NC), in which we considered statistically significant if P values were <0.05. Receiver operating characteristic (ROC) analysis was carried out with MedCalc (MedCalc Software, Mariakerke, Belgium).

RESULTS

Cloning of cDNAs from human pancreas. We completely screened 2×10^4 plaques with the AIP patient's serum (A.O.) and obtained 10 positive clones. Nucleotide sequencing of the insert cDNAs and a subsequent homology search revealed that 7 of 10 clones were identical to human amylase-2A (AMY-2A). When compared with the nucleotide sequence of the human AMY-2A cloned by Wise et al. (17), four of seven clones contained the full coding sequence, whereas the 5′ ends of the other three clones started from 61, 799, and 897 bp (A in ATG is designated as 1) (Fig. 1). Other nonamylase clones were those of the housekeeping genes, such as the heat shock protein and the nuclear protein.

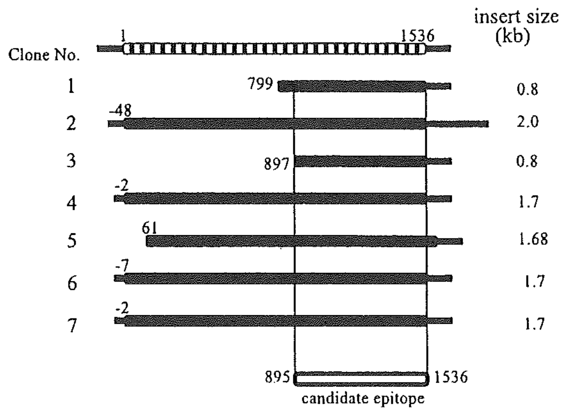

FIG. 1. Cloning of human amylase α-2A cDNAs from λTriplEx2 human pancreas cDNA library. Seven clones of human amylase α-2A cDNAs. Their lengths and 5′-ends are shown (A in ATG is designated as 1). The top bar indicates human amylase α-2A cDNA as reported by Wise et al. (17), and the common regions shared by all seven clones, from codons 299 to 512, are shown in the bottom bar.

Western blot analysis, immunoprecipitation, and ELISA system for detecting anti-human AMY-2A. Because IgG from the AIP patient used for screening recognized four different lengths of human AMY-2A clones, we hypothesized that the regions shared by these four clones, from codons 299 to 512, might contain a common epitope for the patient's IgG (Fig. 1). Therefore, we produced histidine-tagged human AMY-2A from codons 299 to 512 (AMY-2A/299–512) in *E. coli* BL21 and carried out Western blot analysis (Fig. 2*A*). Patient's serum (A.O.) recognized the 30-kDa recombinant protein (*line 1*), but sera from healthy volunteers did not (*lines 3* and *4*). When the patient's serum was preincubated with the recombinant protein, positive staining was abolished (*line 2*), suggesting that the autoantibody reacted with the recombinant protein, which contains the epitope.

Anti-human AMY-2A antibody produced in goat was bound to the in vitro–translated ^{35}S–AMY-2A and was precipitated by protein G–sepharose (Fig. 2*B*). IgG from two patients with AIP also bound to the labeled protein and was precipitated, but the IgG from two healthy volunteers did not (Fig. 2*B*). This recombinant fluid phase autoantibody assay with in vitro transcription and translation of AMY-2A without additional amino acids, such as His-Tag, confirmed the specificity of the autoantibody against the protein.

Next, by coating the protein onto the plate, we developed an ELISA system for detecting anti-amylase antibodies in the serum. When compared with the normal serum, patient sera showed strong signals, which were well correlated with immunoprecipitated ^{35}S–AMY-2A by protein G–sepharose (Fig. 2*C*). This positive reaction in ELISA was displaced in a concentration-dependent fashion by AMY-2A/299–512 (Fig. 2*D*). When the AIP patient's serum (A.O.) was diluted, we could detect positive signals up to ×1,000 dilution (Fig. 2*E*). To obtain a cutoff value for positivity, we carried out ROC analysis of the healthy

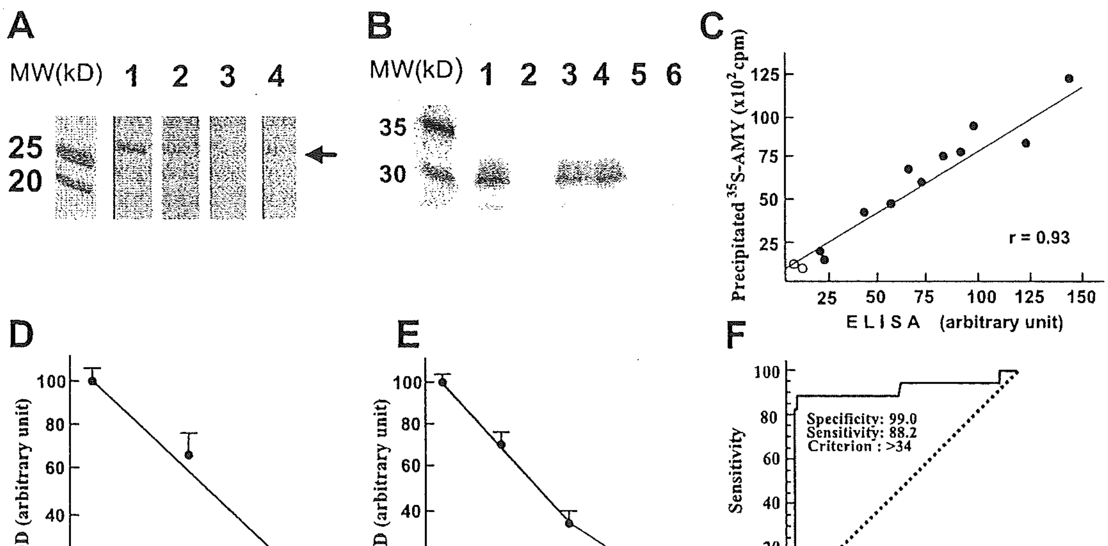

FIG. 2. Western blot analysis and ELISA for detecting anti-human AMY-2A. ***A*****: Western blot analysis. Recombinant human AMY-2A (50 ng) from codons 299 to 512 (AMY-2A/299–512) was electrophoresed in 0.1% SDS–15% polyacrylamide and transferred onto a nitrocellulose filter. The filters reacted with serum (×1,000) from an AIP patient (*line 1*) and normal control sera (*lines 3* and *4*). Line 2, AIP patient's serum preincubated with 1 μg/ml AMY-2A/299–512. MW, molecular weight markers.** ***B*****: Immunoprecipitation of ^{35}S–AMY-2A with antibodies. ^{35}S–AMY-2A was incubated with goat anti-amylase (*line 1*), normal goat IgG (*line 2*), sera from AIP patients (*lines 3* and *4*), and sera from healthy volunteers (*lines 5* and *6*) and then precipitated with protein G–sepharose. The pellets were electrophoresed in 0.1% SDS–15% polyacrylamide and analyzed with Bas 2000 image analyzer (Fujix, Tokyo).** ***C*****: Correlation between the result of ELISA and that of immunoprecipitation. By coating the recombinant human AMY-2A/299–512, we developed an ELISA system for detecting anti-human AMY-2A. Sera from 11 patients with AIP (●) and two normal control subjects (○) were assayed by ELISA and immunoprecipitation for detecting the autoantibody.** ***D*****: Absorption of positive ELISA signal with recombinant AMY-2A. One milliliter of a patient's serum (1:500) was preincubated with the recombinant protein at the indicated dose overnight at 4°C, and then the serum was used as the first antibody. The data are the mean of triplicate values. OD, optical density.** ***E*****: Serum dilution experiment in ELISA assay. Positive serum from patient A.O. was diluted as indicated, and ELISA assay was carried out. The data are the mean of triplicate values.** ***F*****: ROC analysis of the healthy volunteers and fulminant type 1 diabetic patients. We carried out ROC analysis of the healthy volunteers (n = 100) and fulminant type 1 diabetic patients (n = 17) with MedCalc.**

TABLE 2
Criterion values and coordinates of the ROC curve

Criterion	Sensitivity (%)	Specificity (%)	Positive predictive value	Negative predictive value
≥3.2	100.00 (80.3–100.0)	0.00 (0.0–3.7)	14.5	
>11.4	100.00 (80.3–100.0)	9.00 (4.2–16.4)	15.7	100
>11.8	94.12 (71.2–99.0)	9.00 (4.2–16.4)	15.0	90.0
>17.3	94.12 (71.2–99.0)	53.00 (42.8–63.1)	25.4	98.1
>17.5	88.24 (63.5–98.2)	55.00 (44.7–65.0)	25.0	96.5
>34.0*	88.24 (63.5–98.2)	99.00 (94.5–99.8)	93.7	98.0
>34.7	82.35 (56.6–96.0)	99.00 (94.5–99.8)	93.3	97.1
>35.2	82.35 (56.6–96.0)	100.0 (96.3–100.0)	100.0	97.1
>98.4	0.00 (0.0–19.7)	100.0 (96.3–100.0)		85.5

Data in parentheses are 95% CI. *Cutoff value for positivity.

volunteers (n = 100) and fulminant type 1 diabetic patients (n = 17) (Fig. 2*F*). Table 2 shows criterion values and coordinates of the ROC curve. When the value was set as 34 (area under the ROC curve 0.92; significance level P = 0.0001), sensitivity, specificity, and positive predictive value were 88.24, 99.0, and 93.7%, respectively.

Prevalence of autoantibody against human AMY-2A in AIP patients. Using the ELISA system, we determined the prevalence of autoantibody against human AMY-2A in AIP patients and various pancreatic diseases (Fig. 3). All 15 IgGs from patients with AIP were positive for AMY-2A/299–512, whereas 1 of 100 IgGs from control subjects was positive for the antibody (P < 0.001, Fisher's exact test). All the IgGs from the patients with chronic alcoholic pancreatitis (n = 25) or with pancreas tumor (pancreatic cancer, n = 8; IPMT, n = 17) were negative for the antigen. Antibodies were detected in 9% (4/47) of patients with Hashimoto's thyroiditis, a representative organ-specific autoimmune disease (Fig. 3*A*).

Figure 3*B* shows the time course of the autoantibody titer from two AIP patients before and after prednisolone treatment. In patient A.O., IgG4 gradually increased and reached 5,540 mg/dl, but administration of prednisolone initiated a rapid decrease of IgG4 to 571 mg/dl. Before prednisolone treatment, the titer of the autoantibody against AMY remained high, and prednisolone treatment induced a rapid decrease of the titer of AMY-2A autoantibody to a normal level. The fall rate of the antibody titer seemed to be parallel to that of serum IgG4. In patient T.M., administration of prednisolone also rapidly decreased the titer of the autoantibody against AMY. The autoantibodies did not increase even at the drug maintenance dose in both cases.

Prevalence of autoantibody against human AMY-2A in patients with fulminant type 1 diabetes and acute-onset type 1 diabetes. We next studied the prevalence of autoantibody against human AMY-2A in various types of diabetic patients (fulminant type 1 diabetes, n = 17; acute-onset type 1 diabetes, n = 42; and type 2 diabetes, n = 67; Fig. 4). Interestingly, 88% of patients with fulminant type 1 diabetes were positive for the autoantibody, but 1% of control was positive for the antibody (P < 0.001, Fisher's exact test). The autoantibody was detected with low frequency in patients with acute-onset type 1 diabetes

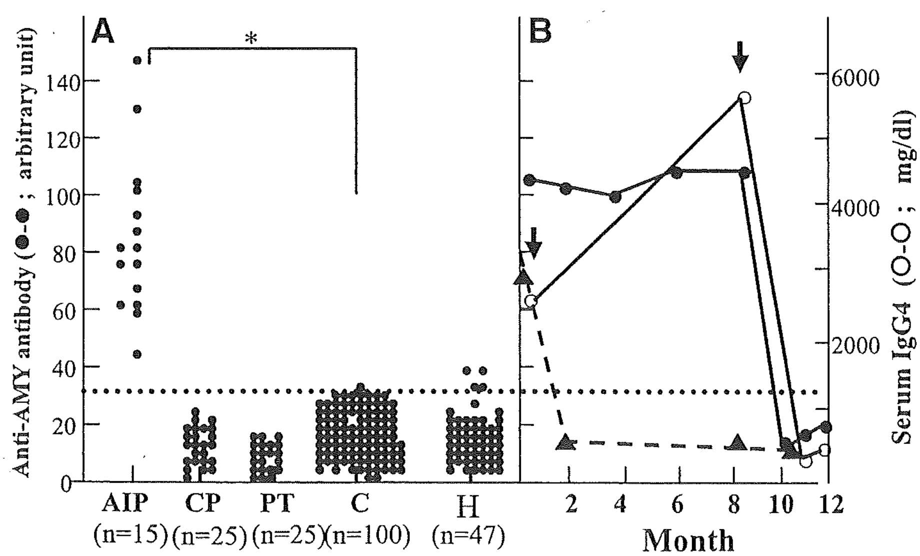

FIG. 3. Prevalence of autoantibody against human AMY-2A in patients with various pancreatic diseases. *A*: Prevalence of autoantibody against human AMY-2A in patients with AIP (n = 15), chronic alcoholic pancreatitis (CP, n = 25), pancreatic tumor (PT, n = 25), control subjects from healthy volunteers (C, n = 100), and Hashimoto's thyroiditis (H, n = 47) was examined by ELISA, as described in RESEARCH DESIGN AND METHODS. The data are the mean of triplicate values. The dotted line shows a cutoff value. Fisher's exact test was carried out between AIP and control groups. *P < 0.001. *B*: Time course of anti-AMY antibody and IgG4 of AIP patients. AIP patient (A.O.), whose IgG was used to screen λTriplEx2 human pancreas cDNA library, was treated with prednisolone (*arrow*). Before and after the treatment, anti-AMY antibody (●-●) and IgG4 (O-O) were measured. In the other AIP patient (T.M.), titer of the anti-AMY antibody (▲-▲) was also measured before and after prednisolone treatment (*arrow*).

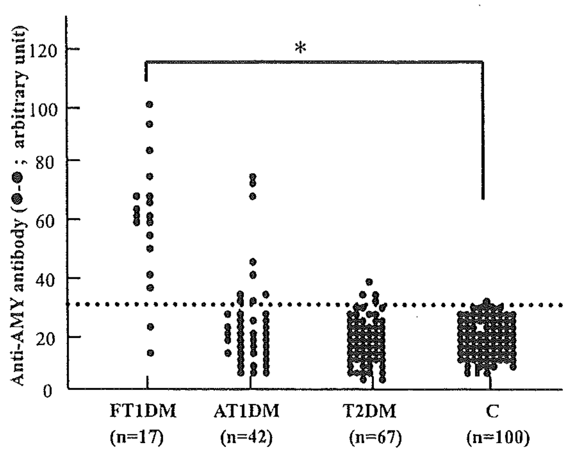

FIG. 4. Prevalence of autoantibody against human AMY-2A in patients with various types of diabetes. Prevalence of autoantibody against human AMY-2A (●) in patients with fulminant type 1 diabetes (FT1DM, n = 17), acute-onset type 1 diabetes (AT1DM, n = 42), type 2 diabetes (T2DM, n = 67), and control subjects from healthy volunteers (C, n = 100) was studied by ELISA, as described in RESEARCH DESIGN AND METHODS. The data are the mean of triplicate values. The dotted line shows a cutoff value. Fisher's exact test was carried out between fulminant type 1 diabetic and control groups. *$P < 0.001$.

(21%, 9 of 42) and patients with type 2 diabetes (6%, 4 of 67).

DISCUSSION

In 2002, Barera et al. (18) reported a case of an 11-year-old girl with celiac disease and hypothyroidism. Because of hyperamylasemia, she was suspected to have chronic pancreatitis, but no pancreatic damage was demonstrated. By using ELISA to detect autoantibodies to amylase, they found that she produced an autoantibody against porcine amylase and that this declined after the institution of a gluten-free diet. In the present study, we also detected an autoantibody against pancreas-specific AMY-2A in all of the AIP patients, but not in patients with chronic alcoholic pancreatitis and with pancreatic tumors.

The presence of autoantibodies against CAII, lactoferrin, and pancreatic secretory trypsin inhibitor (PSTI) has been reported (3,4,19). However, the distribution of these molecules is non–organ specific (20–22), and the prevalence of these autoantibodies against CAII, lactoferrin, and PSTI in AIP is rather low, ranging from 42~73% (3,4,19). Using 13 serum samples from our AIP patients, we carried out ELISA assays for autoantibodies against CAII and lactoferrin. As a result, 66% (10 of 15) were positive for CAII, and 53% (8 of 15) were positive for lactoferrin. Thus, an autoantibody against AMY-2A might be a more sensitive marker for AIP than that of CAII, lactoferrin, or PSTI.

Furthermore, the adoptive transfer of amylase-specific $CD4^+$ T-cells to rats was able to confer pancreatitis, whereas the transfer experiment with lactoferrin-specific or CAII-specific $CD4^+$ T-cells failed to induce experimental pancreatitis (23). Our findings of a high prevalence of autoantibody against AMY-2A in human AIP and the results from the adoptive transfer experiment of amylase-specific $CD4^+$ T-cells to rodents suggest that cellular and/or humoral autoimmunity against AMY-2A plays some role in the pathogenesis of AIP.

Approximately 80% of patients with chronic pancreatitis are alcoholic, the pathogenesis of which still remains unclear. However, it is well known that acute or chronic alcohol exposure suppresses all branches of the immune system (24), and none of our sera from patients with chronic alcoholic pancreatitis were positive for autoantibody against AMY-2A (Fig. 3). Therefore, an assay for autoantibody against AMY-2A is useful for distinguishing AIP from chronic alcoholic pancreatitis.

It is of particular interest that anti–AMY-2A autoantibody is detected in 88% of patients with fulminant type 1 diabetes. Fulminant type 1 diabetes is a recently proposed subtype of type 1B, nonimmune-mediated, or idiopathic type 1 diabetes (9,10). A nationwide survey revealed that fulminant diabetes accounted for ~20% of Japanese type 1 diabetes with ketosis or ketoacidosis and flu-like symptoms frequently observed at onset (25). Clinical characteristics of this subtype of type 1 diabetes are *1*) remarkably abrupt onset of disease; *2*) very short (<1 week) duration of diabetic symptoms; *3*) severe ketoacidosis at diagnosis; *4*) negative status of islet-related autoantibodies, such as GADAb and anti–IA-2 antibody; *5*) virtually no C-peptide secretion (10 μg/day in urine); and *6*) elevated serum pancreatic enzyme levels (26). These features and the absence of insulitis in patients' pancreases have led some to hypothesize that an autoimmune mechanism does not contribute to the development of fulminant type 1 diabetes, but rather that viral infection plays a central role in the pathogenesis of the disease (27). However, we previously demonstrated $CD4^+$ and $CD8^+$ T-cell infiltration to pancreatic exocrine cells and to the islet in an autopsy case deceased immediately after the onset of fulminant type 1 diabetes (28).

Imagawa and Hanafusa (27) also confirmed cellular infiltration of pancreatic islets in patients with fulminant type 1 diabetes. Shimada et al. (29) described a fulminant type 1 diabetic patient with a high serum level of CXCL10, a chemokine that induces migration of activated T-cells to local lesions and GAD-reactive $CD4^+$ cells in the periphery. These results, and the presence of an autoantibody against AMY-2A, suggest that the disease might be autoimmune-related, involving the exocrine and the endocrine pancreas (10,28).

Exocrine dysfunction and impaired glucose tolerance are common features for both AIP and fulminant type 1 diabetes. With regard to the HLA genotype, Kawa et al. (30) demonstrated that the DRB1*0405-DQB1*0401 haplotype is closely associated with AIP in the Japanese population, and Tanaka et al. (31) revealed that the DQA1*0303-DQB1*0401 haplotype is strongly associated with fulminant type 1 diabetes in a homologous manner. When we studied the frequency of this allele in our patients with AIP, 5 of 15 patients were heterozygous for the DRB1*0405-DQB1*0401 haplotype. Although further study with larger sample sizes will be needed, these two reports and our own analysis suggest the importance of the DQB1*0401 allele in both diseases. Furthermore, we are able to detect autoantibody against AMY-2A in both with nearly the same prevalence. Although further investigation is needed, the present results suggest that clinically and immunologically, AIP and fulminant type 1 diabetes are closely related to one another.

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No potential conflicts of interest relevant to this article were reported.

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自己免疫性膵炎は全身疾患か？

神澤 輝実・安食 元・江川 直人

ポイント

- 自己免疫性膵炎は，全身諸臓器にTリンパ球とIgG4陽性形質細胞の密な浸潤を呈する全身性疾患（IgG4関連硬化性疾患）の膵病変の可能性がある．
- 線維化と閉塞性静脈炎を生じる臓器において，以下のような病態による臨床徴候を呈する（膵臓：自己免疫性膵炎，胆管:硬化性胆管炎，胆嚢:硬化性胆嚢炎，唾液腺:硬化性唾液腺炎，後腹膜:後腹膜線維症など）．
- 高率にリンパ節腫大を伴い，診療当初は悪性腫瘍を疑われることが多いが，ステロイド治療が奏効することより，無益な外科手術を行わないためにも，本症を念頭に置くことが肝要である．

自己免疫性膵炎は発症機序に何らかの自己免疫現象の関与が示唆される膵炎として注目されているが，その病因や病態は完全には解明されていない．自己免疫性膵炎には，しばしば胆管狭窄や唾液腺腫大などの膵外病変を合併する．われわれは自己免疫性膵炎患者の全身諸臓器と膵外病変を検索し，自己免疫性膵炎はIgG4が関連する全身疾患（IgG4関連硬化性疾患）である可能性を報告した[1~3]．本稿ではわれわれが提唱したIgG4関連硬化性疾患について述べる．

自己免疫性膵炎患者の全身諸臓器の病理組織像

自己免疫性膵炎の膵の特徴的病理組織像は，リンパ球と形質細胞の密な浸潤と線維化があり（図1），膵内外の静脈に高頻度に閉塞性静脈炎を認める（図2）．この特異的な炎症性変化は，自己免疫性膵炎患者の膵だけでなく，膵周囲後腹膜組織，胆管壁，胆嚢壁，肝内門脈域，唾液腺にも認められる．

免疫組織学的検討では，自己免疫性膵炎の膵に密に浸潤する細胞浸潤は，CD4陽性ないしCD8陽性のTリンパ球とIgG4陽性の形質細胞であった（図3）．これらの細胞浸潤は自己免疫性膵炎患者の後腹膜，胆道系，肝内門脈域，唾液腺，リンパ節，胃粘膜など全身諸臓器にも認められた．

自己免疫性膵炎の膵外病変

文献的に自己免疫性膵炎では，Sjögren症候群，硬化性胆管炎，後腹膜線維症などの他の自己免疫性疾患様病変の合併が報告されてきた．自験例の検索では，自己免疫性膵炎に合併する膵外病変として，下部胆管狭窄（76 %，図4），肝内・肝門部胆管狭窄（15 %，図4），胆嚢壁肥厚（21 %），唾液腺腫大（23 %，図5），後腹膜

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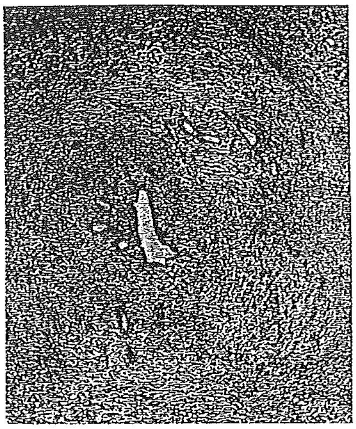

図1　自己免疫性膵炎の膵の病理組織像
リンパ球と形質細胞の密な浸潤と線維化(HE染色)

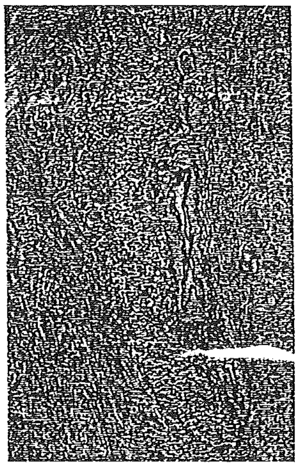

図2　自己免疫性膵炎の膵の病理組織像
膵内外の静脈に高頻度に閉塞性静脈炎を認める(Elastica Van Gienson染色).

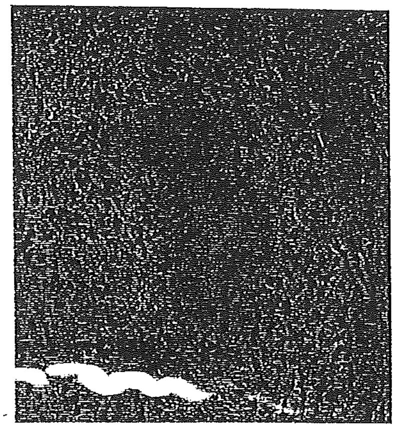

図3　自己免疫性膵炎の膵に認められるIgG4陽性形質細胞の密な浸潤

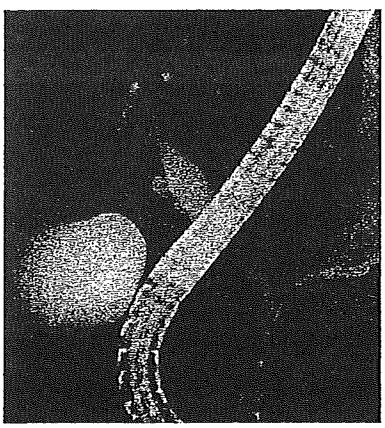

図4　自己免疫性膵炎に認められる肝門部胆管狭窄を示すERCP(内視鏡的逆行性胆管膵管造影)像

図5　自己免疫性膵炎に合併した両側唾液腺腫大（ガリウムシンチ）

線維症(12 %)，リンパ節腫大(36 %)などが認められた．これらの膵外病変は，膵病変と同様にステロイド治療により著しい改善を認めた．膵外病変の組織像は，自己免疫性膵炎の膵と同様で，密なリンパ球と形質細胞の浸潤，線維化と閉塞性静脈炎であり，病理組織学的にsclerosing cholangitis, sclerosing cholecystitis, sclerosing sialadenitis, retroperitoneal fibrosisなどの所見であった．また，腫大したリンパ節を含むこれらのすべての病変で，膵と同様に多数のTリンパ球とIgG4陽性の形質細胞の浸潤を認めた．IgG4陽性の形質細胞の密な浸潤は，アルコール性慢性膵炎，膵臓癌，Sjögren症候群や原発性硬化性胆管炎(primary sclerosing cholangitis：PSC)の患者の諸臓器には認められなかった．

表1　IgG4 関連硬化性疾患

- 全身諸臓器に CD4 ないし CD8 陽性 T リンパ球と IgG4 陽性形質細胞の密な浸潤を呈する全身性疾患である
- 著しい線維化と閉塞性静脈炎を生じる臓器において，以下のごとくの病態の臨床徴候を呈する
  膵臓：自己免疫性膵炎
  胆管：硬化性胆管炎
  胆嚢：硬化性胆嚢炎
  唾液腺：硬化性唾液腺炎
  後腹膜：後腹膜線維症
  大動脈：大動脈炎?
  肝，肺：炎症性偽腫瘍?
- リンパ節腫大を高率に伴う
- 高齢の男性に好発する
- 血中 IgG4 値測定が診断に有用である
- ステロイド治療が奏効する
- 悪性腫瘍を疑診されることが多いので，本症の存在を念頭におくことが肝要である
- IgG4 の役割を含めた発症機序や病態は不明である

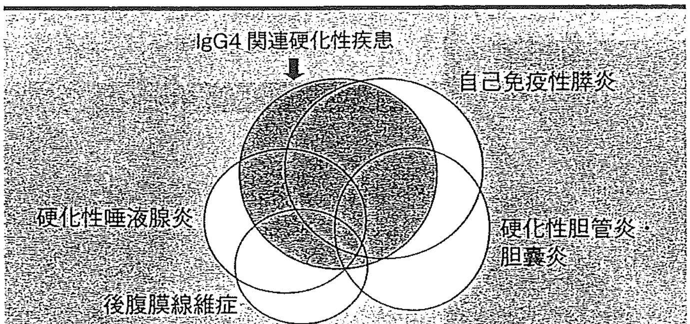

図6　IgG4 関連硬化性疾患の概要

IgG4 関連硬化性疾患の提唱

自己免疫性膵炎には多彩な膵外病変が認められるが，胆道系，唾液腺，後腹膜などの膵外病変の病理組織像は膵と同様であり，さらにほぼ全身諸臓器に IgG4 陽性形質細胞の浸潤が認められた．これらの変化はステロイド治療により改善した．Sjögren 症候群や PSC の臓器に IgG4 陽性形質細胞の浸潤がみられないことより，自己免疫性膵炎の合併する唾液腺や胆管病変は，本来の Sjögren 症候群や PSC とは異なる範疇の病態と考えられた．

そこで，全身諸臓器に CD4 ないし CD8 陽性の T リンパ球と IgG4 陽性の形質細胞の密な浸潤を呈する IgG4 関連硬化性疾患という新しい疾患概念を提唱した．

IgG4 関連硬化性疾患は全身疾患で，線維化と閉塞性静脈炎を生じる膵，胆管，胆嚢，唾液腺，後腹膜などにおいて臨床徴候を呈する．自己免疫性膵炎は本疾患の膵病変であり，その膵外病変は本疾患の諸臓器の病巣である．これらの膵外病変は，自己免疫性膵炎の診断を契機に見つかることが多いが，単独で発症することもある（Mikulicz 病など）．高率にリンパ節腫大を伴う．高齢の男性に好発し，ステロイドが奏

効する．IgG4の役割を含め，詳しい発症機序は不明である．腫瘤の形成とリンパ節腫大により，診療当初は悪性腫瘍が疑われることが多いが，本症はステロイド治療が有効なことより，慎重な鑑別診断を行い，無益な手術を避ける必要がある．血中IgG4値の測定は鑑別診断に有用である(表1，図6)．

おわりに

自己免疫性膵炎は全身諸臓器にTリンパ球とIgG4陽性の形質細胞の密な浸潤を呈し，膵，胆道系，後腹膜，唾液腺などに線維化を起こす全身性疾患(IgG4関連硬化性疾患)の膵病変である可能性を考えたい．

文　献

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2) Kamisawa T, et al：A new clinicopathological entity of IgG4-related autoimmune disease. J Gastroenterol 38：982-984, 2003
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〔Expanded Abstract〕

自己免疫性膵炎と膵臓癌との鑑別方法

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背景と目的

自己免疫性膵炎はステロイド治療に非常によく反応するので，不必要な手術を回避するためにも，適格な診断が必要である．特に膵頭部に腫瘤を形成する自己免疫性膵炎は，局所進行性の膵臓癌との鑑別がしばしば困難である[1~3]．組織学的に，膵頭部腫瘤の多くは膵臓癌であるが，どのような症例に自己免疫性膵炎を疑うべきか？そして，どのように鑑別診断を行っていくか？両疾患の臨床像，血清学的および画像所見を比較検討した．

方　法

膵頭部に腫瘤を形成した自己免疫性膵炎17例を対象とした．これらのうち，膵臓の組織が検索された7例は全て密なリンパ球と形質細胞の浸潤を伴う線維化で，ステロイド治療が施行された11例は全て著効した．同時期に経験した組織学的に確認された局所進行膵頭部癌（ステージIII，IV）70例と比較検討した．

結果と考察

1. 臨床的差異

両群で，診断時年齢，性差，飲酒歴，喫煙歴に差を認めなかった．体重減少は膵臓癌に，動揺性黄疸は自己免疫性膵炎に多くみられた（$p<0.001$）．唾液腺腫大は自己免疫性膵炎の24％にのみ認められた（$p<0.001$）．

2. 血清学的差異

血清IgG4値は，明らかに高頻度に自己免疫性膵炎で高値であった（$p<0.001$）．自己抗体の有無，CA19-9以外の腫瘍マーカー値に差はなかったが，血中CA19-9値は膵臓癌でより高値であった（$p<0.001$）．

3. 画像上差異

CTでは，腫大した膵臓の後期層での造影効果（$p<0.001$），膵周囲のcapsule-like低吸収域（$p<0.001$），膵体尾部の萎縮がない（$p<0.001$），胆管造影にて明らかな狭窄がない部分の胆管壁の肥厚（$p<0.001$），胆嚢壁の肥厚（$p=0.006$），後腹膜腫瘤（$p=0.036$）は，自己免疫性膵炎に高頻度に認められた．血管造影所見には差異を認めなかった．ERPでは，スキップした主膵管狭細像（$p<0.001$），主膵管狭細部からの分枝膵管の派生（$p=0.036$）は自己免疫性膵炎に，主膵管の閉塞（$p<0.001$）は膵臓癌に多く認められた．また，自己免疫性膵炎では膵臓癌に比べて，主膵管狭細部の距離が長く（$p<0.001$），上流主膵管の拡張は軽度（$p<0.001$）であった．唾液腺腫大，後腹膜線維症，肝内胆管狭窄などの膵外病変は自己免疫性膵炎のみに認められた（$p<0.001$）(Table 1)．

4. 膵頭部の腫瘤性病変における自己免疫性膵炎と膵臓癌との鑑別診断のアルゴリズム

客観的に正確な判定が可能で膵臓癌より自己免疫性膵炎をより示唆する6個の画像所見（CTで造影効果を有する膵腫大，CTでcapsule-like低吸収域，ERPで3cm以上の長さの主膵管狭細像，ERPでスキップした主膵管狭細像，ERPで5mm以下の上流主膵管径，唾液腺腫大・後腹膜線維症・肝内胆管狭窄などの膵外病変の存在）を選ぶ