

Immunoglobulin G4-Hepatopathy: Association of Immunoglobulin G4-Bearing Plasma Cells in Liver with Autoimmune Pancreatitis

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Autoimmune pancreatitis (AIP) is characterized by high serum immunoglobulin (Ig) G4 concentrations, lymphoplasmacytic inflammation, and a favorable response to corticosteroid treatment. Since liver dysfunction is frequently seen in AIP patients, we investigated hepatic histopathology and its clinical significance in patients with AIP. We examined the clinical features, histology, and immunoglobulin G (IgG)4-bearing plasma cell infiltration of liver biopsies from 17 patients with AIP and 63 patients with either autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, or chronic viral hepatitis and histological changes in the 7 of 17 livers before and after glucocorticoid therapy. The liver histology of AIP was classified into 5 patterns: evident portal inflammation with or without interface hepatitis (6 cases), large bile-duct obstructive features (8 cases), portal sclerosis (8 cases), lobular hepatitis (5 cases), and canalicular cholestasis (4 cases); some of the histological features coexisted in the same liver. The number of IgG4-bearing plasma cells was significantly higher in AIP patients than controls ($P < 0.01$), and was significantly correlated with serum IgG4 concentration ($P = 0.0014$, $r = 0.709$). Glucocorticoid therapy reduced IgG4-bearing plasma cell infiltration in the liver ($P = 0.031$) and ameliorated other histological findings. In conclusion, virtually all AIP liver biopsies showed evidence of various pathological changes and infiltration of IgG4-bearing plasma cells. These features were ameliorated by steroid therapy, suggesting that the liver is concurrently affected in AIP, and that liver biopsies can provide significant information in the clinical evaluation and diagnosis of AIP. (HEPATOLOGY 2007;46:463-471.)

Autoimmune pancreatitis (AIP) is characterized by irregular narrowing of the main pancreatic duct, swelling of the pancreas, and a favorable response to corticosteroid treatment.¹ This disease is closely asso-

ciated with high serum IgG4 concentrations,² the human leukocyte antigen DRB1*0405-DQB1*0401 haplotype,³ Fc receptor-like gene 3 polymorphisms,⁴ and various other characteristic pathological features such as lymphoplasmacytic infiltration including abundant IgG4-bearing plasma cells, parenchymal fibrosis, and obliterative phlebitis.^{5,6} Similar pathological features have also been reported in several extra-pancreatic organs, including salivary glands (Küttner tumour)⁷ and the lung (inflammatory pseudotumor).⁸

As for the hepatobiliary system, although IgG4-related sclerosing cholangitis and inflammatory pseudotumors have been reported in connection with AIP,⁹ it remains unclear whether other lesions are also present in the liver, as well as the prevalence of liver dysfunction in AIP patients. Additionally, beyond reports of lymphoplasmacytic infiltration with fibrosis in the portal areas¹⁰ and infiltration of IgG4-bearing plasma cells,¹¹ the histopathological features in the liver remain poorly understood in AIP. It is also unknown whether these features are similar to those in the pancreas and are correlated with clinical significance. Recently, Chari et al. have proposed a set of diagnostic criteria for AIP, including histopathology and IgG4 subclass staining, to be "Gold Standard".¹² However, although decreases of serum IgG4 concentra-

Abbreviations: AIH, autoimmune hepatitis; AIP, autoimmune pancreatitis; AST, aspartate aminotransferase; CVH, chronic viral hepatitis; HCV, hepatitis C virus; ERCP, endoscopic retrograde cholangiopancreatography; HBsAg, hepatitis B surface antigen; HPF, high power fields; IDUS, intraductal ultrasonography IQR, interquartile range; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; ROC, receiver operating characteristic.

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Table 1. Demographic and Clinical Characteristics of the 17 Patients with Autoimmune Pancreatitis

Characteristics	Median	IQR		
Median age, years	62	57–66		
Male, n (%)	15 (88)			
Median values (normal values)			Abnormal	(%)
ALT (7–45 IU/l)	76	29–155	10/17	(59)
AST (12–37 IU/l)	39	31–69	9/17	(53)
ALP (124–367 IU/l)	549	339–755	17/17	(100)
Total bilirubin (0.3–1.2 mg/dl)	1.1	0.6–2.0	7/17	(41)
γ GTP (8–50 IU/l)	253	73–434	15/17	(88)
Total protein (6.8–8.3 g/dl)	7.2	6.8–8.4	5/17	(29)
Albumin (4.2–5.1 g/dl)	3.6	3.3–3.9	15/17	(88)
IgG (870–1700 mg/dl)	2443	483–3529	11/17	(65)
IgG4 (<135 mg/dl)	540	243–970	16/17	(94)
Ratio of IgG4:IgG	0.24	0.19–0.40		
IgM (35–220 mg/dl)	81	60–133	1/17	(6)
IgE (<361 IU/ml)	187	84–635	6/17	(35)
			Positive	(%)
ANA (< $\times$ 160)			7/15	(47)
ds-DNA (<12 IU/ml)			1/15	(7)

NOTE: Anti-SS-A, anti-SS-B, anti-ribonucleoprotein, and antimitochondrial antibody-M2 were all negative in 15 cases. Patients with AIP had a significantly higher male ratio compared to patients with AIH (1/17: 6%) and PBC (3/22: 14%) ($P < 0.001$). Median serum IgG4 concentrations and IgG4:IgG ratios were both significantly higher in AIP patients than in those with AIH, PBC, PSC, or CVH ($P < 0.001$).

Abbreviations: AIP: autoimmune pancreatitis; IQR: interquartile range; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; γ GTP, γ -glutamyltransferase; ANA, anti-nuclear antibody; ds-DNA, anti-double stranded DNA.

tions have been closely associated with the improvement of clinical data and imaging findings after administration of corticosteroid therapy,² there are no published data of histological improvements, in particular IgG4 subclass staining, using paired biopsy samples in patients with AIP.

As such, the aims of this study were threefold. First, we sought to determine the relationship, if any, between histopathological features of the liver and the degree of AIP from liver biopsies in AIP patients, autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), and chronic viral hepatitis (CVH). Next, we wanted to determine whether histological features of the liver, including the presence of IgG4-bearing plasma cells, reflected the clinical features of AIP. Lastly, we aimed to prospectively assess the effects of corticosteroid therapy on liver histopathology using paired AIP liver samples before and after steroid treatment.

Patients and Methods

Patients

A total of 80 Japanese patients were enrolled in this study and divided into 5 diagnostic groups on the basis of clinical, epidemiological, and serological features, as indicated below. The protocol was approved by the ethics committee of the Shinshu University School of Medicine and all subjects provided written informed consent. Serum samples were obtained at the time of liver biopsy or surgery and stored at -70°C until testing.

Autoimmune Pancreatitis. Between September 1994

and May 2005, 59 patients (49 men and 10 women; median age 63 years [range 38–76 years]) with AIP were seen at Shinshu University Hospital, Japan. The diagnosis of AIP in all patients was based on criteria released by the Japan Pancreas Society, as reported previously.¹³ Liver histologies were obtained in 18 (31%) of the patients, though one patient was excluded from this study after later being diagnosed with chronic hepatitis C. The patients' characteristics are summarized in Table 1. Initially, endoscopic biliary drainage was performed in 12 patients who appeared jaundiced. High serum IgG4 concentrations (median, 540.0 mg/dl; interquartile range (IQR), 242.8–970.0 mg/dl) were found in 16 of the 17 AIP patients, who were treated with 40 mg of prednisolone daily for 4 weeks that was reduced by 5 mg per week over a period of several weeks, resulting in the improvement of clinical, laboratory and imaging findings. No patients were given azathioprine during follow-up. All 17 patients were negative for hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (HCV), and antimitochondrial antibody-M2.

Autoimmune Hepatitis, Primary Biliary Cirrhosis, and Primary Sclerosing Cholangitis. Seventeen patients with AIH, 22 patients with PBC, and 7 patients with PSC were enrolled as contrast cases. Patients with AIH were diagnosed as definite according to the scoring system from the International Autoimmune Hepatitis Group, and were classified as having type 1 AIH.^{14,15} The diagnosis of PBC was based on internationally accepted criteria, and the antimitochondrial antibody status of each patient was verified.¹⁶ The diagnosis of PSC was

based on typical cholangiographic and liver biopsy criteria.¹⁷ Four of the 7 patients with PSC had ulcerative colitis. All patients with AIH, PBC, and PSC were negative for HBsAg, antibodies to hepatitis B core antigen, and antibodies to HCV.

Chronic Viral Hepatitis. The CVH group included 9 patients with chronic hepatitis C who were positive for antibodies to HCV (EIA-3) and for HCV RNA (AMPLICORE Monitor, Roche Diagnostic, Tokyo, Japan). Eight patients had chronic hepatitis B, and were positive for HBsAg and HBV DNA (AMPLICORE Monitor, Roche Diagnostic). Patients with chronic hepatitis C and B had not been treated with interferon or nucleotide analogues.

Laboratory Testing

Alanine aminotransferase, aspartate aminotransferase (AST), and other relevant biochemical tests were performed using standard methods.¹⁸ Serum IgG4 concentrations were determined by single radial immunodiffusion kits (normal values < 135 mg/dl) as reported previously.² Anti-nuclear antibody titers were determined by immunofluorescence using HEP-2 cells, and a titer of 1:160 or greater was considered positive. Patterns of anti-nuclear antibody reactivity were recorded. Anti-double-stranded DNA, anti-Ro (SS-A), anti-La (SS-B), anti-ribonucleoprotein, and antimitochondrial antibody-M2 were tested by ELISA. Testing for anti-smooth muscle antibody was not performed.

Imaging Modalities

Ultrasonography, computed tomography, magnetic resonance imaging, and endoscopic retrograde cholangiopancreatography (ERCP) were performed in all patients with AIP. Intraductal ultrasonography (IDUS) was performed in 11 patients using a 2.0-mm diameter ultrasonic probe at a frequency of 20 MHz (UM-G20-29R; Olympus Optical Co., Ltd., Tokyo, Japan) to evaluate for thickening of the bile duct wall. Intrahepatic bile duct wall thickness was classified either as severe (obvious stenosis and sclerosis by ERC and IDUS); mild (wall thickening by IDUS and mild stenosis and sclerosis by ERC); or absent (no abnormalities by ERC or IDUS). Ten patients had proximal biliary strictures and 3 had proximal and distal biliary strictures. The remaining 4 patients had no biliary strictures.

Liver Biopsies, Histological Evaluation and Immunostaining

Liver biopsies were performed by percutaneous sampling of the right lobe with a 14-gauge needle in all patients but one, whose liver specimen was obtained during surgery. All biopsies were 1.5 cm or more in length. Liver specimens were taken before administration of corticoste-

roid or ursodeoxycholic acid in patients with AIP, AIH, PBC, and PSC. Additionally, 7 patients with AIP were followed prospectively, and paired liver biopsies were obtained before and after 4 weeks of steroid therapy.

Formalin-fixed and paraffin-embedded specimens were prepared and used for histopathological and immunohistochemical studies. Sections measuring 4 μ m were cut from each paraffin block and stained with hematoxylin and eosin, periodic acid-Schiff after diastase digestion, Azan-Mallory, reticulin, or orcein, with the remaining material used for immunohistochemical analysis. The following 14 histological features were assessed by two experienced pathologists (Y.Z. and Y.N.), who analyzed each feature under code and independently of other data: (1) portal fibrosis (0, absent; 1, periportal fibrosis; 2, bridging fibrosis; 3, bridging fibrosis with lobular distortion; 4, cirrhosis); (2) portal inflammation (–, absent; +, present); (3) interface hepatitis (–, absent; +, present); (4) lobular inflammation (–, absent; +, present); (5) lobular hepatitis (–, 0-2 focal necrosis / high power fields (HPF); +, ≥ 3 focal necrosis / HPF); (6) ductular proliferation (–, absent; +, present); (7) plasma cell infiltration (–, 0-9 cells / HPF in portal area; +, ≥ 10 cells / HPF); (8) eosinophil infiltration (–, 0-4 cells / HPF in portal area; +, ≥ 5 cells / HPF); (9) bile duct damage (–, absent; +, irregularity of cellular and nuclear arrangement of biliary epithelium with or without narrowing of the bile duct lumen); (10) cholangitis simulating chronic nonsuppurative destructive cholangitis (–, absent; +, present); (11) bile duct loss (–, absent; +, present); (12) steatosis (–, absent; +, present in $\geq 30\%$ of hepatocytes); (13) canalicular cholestasis (–, absent; +, present); and (14) orcein-positive granules (0, absent; 1, positive cells in $\leq 1/3$ of periportal areas; 2, positive cells in $1/3$ to $2/3$ of periportal areas; 3, positive cells in $\geq 2/3$ of periportal areas). Indirect immunostaining with goat polyclonal antibody was performed for each IgG subclass (IgG1, IgG2, IgG3, and IgG4), as reported previously.⁵ Counts were tallied for each IgG subclass of positive plasma cells per HPF, and the number of positive cells was expressed as the mean of triplicates. The number of IgG-bearing plasma cells was then calculated by totaling all of the IgG subclass positive plasma cells.

Statistical Analysis

The Mann-Whitney *U* test or Kruskal-Wallis One Way Analysis of Variance on Ranks was used to analyze continuous variables where appropriate. Pearson product moment correlations were used to evaluate the frequencies of IgG4-bearing plasma cells, as well as serum IgG4 concentrations. The Wilcoxon signed rank test was used to compare serum IgG4 concentrations, serum IgG4: IgG ratios, frequencies of

IgG4-bearing plasma cell infiltration in the liver, and the ratios of IgG4:IgG-bearing plasma cells in patients with AIP before and after 4 weeks of glucocorticoid therapy. *P* less than or equal to 0.05 was considered to be significant. Statistical analyses were performed using SigmaStat (version 2.03; SPSS, Chicago, IL).

To differentiate AIP from other diseases, we analyzed receiver-operating characteristic (ROC) curves for IgG4-bearing plasma cells using MedCalc 7.0 software (<http://www.medcalc.de>). The best cutoff values were chosen automatically by MedCalc as the number of IgG4-bearing plasma cells with the highest diagnostic accuracy determined using the area under the curve.

Results

Baseline Clinical Characteristics of Patients

The clinical profile of the experimental patient cohort is shown in Table 1 (and Supplementary Table 1). The median age was 62 years with a male predominance in 15 (88%) of the 17 patients with AIP, both concordant with prior studies. Patients with AIP had a significantly higher male ratio compared to patients with AIH and PBC ($P < 0.001$). Median serum IgG4 concentrations and IgG4:IgG ratios were both significantly higher in AIP patients than in those with AIH, PBC, PSC, or CVH ($P < 0.001$). No patients with PSC had high serum IgG4 concentrations in our cohort.

Histopathology of Liver Biopsies

Portal and periportal fibrosis was present in all but one of the 17 patients, and 7 of them showed bridging fibrosis. No patient had cirrhosis. Portal inflammation was evident in 6 of 17 patients (35%), and 4 (24%) were also associated with interface hepatitis (24%). The majority of liver biopsies (88%) showed features of lobular inflammation including sinusoidal inflammatory cell infiltration, hepatocellular dropout, and pigmented macrophages, to varying degrees. Evident lobular hepatitis, which was defined as ≥ 3 /HPF of focal necrosis was found in 7 patients (41%). Ductular proliferation was found in 10 patients (59%). Plasma cell infiltration and eosinophil infiltration in portal tracts were present in 6 (35%) and 4 patients (24%), respectively. Bile duct damage was found in 10 patients (59%), although chronic nonsuppurative destructive cholangitis and bile duct loss were not found in any of the cases. Steatosis, defined as fat deposition in $\geq 30\%$ of hepatocytes, was present in 2 patients (12%). Canalicular cholestasis mainly involving perivenular areas was found in 53% of patients. Orcein-positive granules, which is a histological feature of chronic cholestasis, was found in the periportal hepatocytes of 4 of 17 patients (24%).

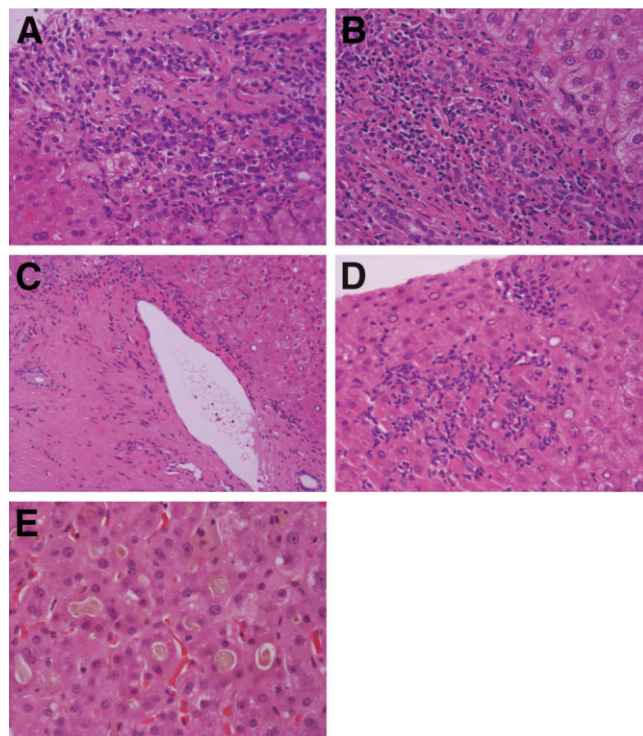


Fig. 1. Five histological patterns observed in liver biopsies of patients with AIP. (A) Portal inflammation pattern: portal tracts are heavily infiltrated with mononuclear cells containing plasma cells, and interface hepatitis is also evident. (B) Large bile duct damage pattern: there is ductular proliferation and portal fibrosis. (C) Portal sclerosis pattern: portal tract is sclerotic and inflammatory cell infiltration is scarce. (D) Lobular hepatitis pattern: there is focal necrosis in the liver parenchyma. (E) Cholestatic pattern: prominent cholestasis is evident in bile canaliculi. (hematoxylin and eosin staining; A, B, D, E, $\times 400$; C, $\times 200$).

Based on the above 14 histological features, we found it possible to comprehensively categorize these AIP liver histologies into 5 histological patterns (Fig. 1 and Table 2) as follows: (1) portal inflammation pattern, with intense portal inflammation with or without interface hepatitis (6 patients, 35%) (Fig. 1A); (2) large duct damage pattern, characterized by bile ductular proliferation, neutrophil infiltration and edematous change in the portal areas (8 patients, 47%) (Fig. 1B); (3) portal sclerosis pattern, exhibiting dense portal sclerosis with scarce portal inflammation (8 patients, 47%) (Fig. 1C); (4) lobular hepatitis pattern, showing lobular inflammation with hepatocellular necrosis resembling viral hepatitis (5 patients, 29%) (Fig. 1D); and (5) cholestatic pattern, with canalicular cholestasis predominantly in the centrilobular area (4 patients, 24%) (Fig. 1E). Multiple histological patterns coexisted in 9 of 17 patients, notably portal inflammation and large bile duct damage patterns (3 patients, cases 1, 6, and 9) and lobular hepatitis and cholestatic patterns (3 patients, cases 6, 10, and 13).

The correlation between clinical characteristics and histological patterns was next examined, revealing signif-

Table 2. Histological Characteristics of the Patients with Autoimmune Pancreatitis

No.	Portal Inflammation	Large Bile Duct Damage	Portal Sclerosis	Lobular Hepatitis	Cholestatic	Total	IgG4-bearing plasma cells (/HPF)
1	+	+	-	-	-	2	29
2	-	-	+	-	-	1	0
3	-	-	-	-	-	0	3
4	+	-	-	-	-	1	7
5	-	-	+	-	-	1	5
6	+	-	-	+	+	3	3
7	-	-	+	-	-	1	0
8	+	-	-	+	-	2	1
9	+	+	-	-	-	2	10
10	-	-	+	+	+	3	19
11	-	-	+	-	-	1	5
12	-	-	+	-	-	1	2
13	-	-	-	+	+	2	8
14	+	+	-	+	-	3	55
15	-	-	-	-	-	0	1
16	-	-	+	-	-	1	6
17	-	-	+	-	+	2	7
	6/17 (35%)	3/17 (18%)	8/17 (47%)	5/17 (29%)	4/17 (24%)		

icant differences in median serum levels of total bilirubin (2.1 versus 1.1 mg/dl; $P = 0.05$) and AST (55.0 versus 35.5 IU/l; $P = 0.05$) in patients with or without the lobular hepatitis pattern, as well as an increase in median total bilirubin level in the cholestatic versus the non-cholestatic group (4.0 versus 1.1 mg/dl; $P = 0.004$).

Immunostaining of IgG4 demonstrated that plasma cells bearing IgG4 were mainly found in the portal tracts, though these numbers varied from case to case (0 to 55 cells/HPF, Table 2). More than 5 positive cells (/HPF) were found in 8 of 17 patients (47%). Interestingly, immunostaining for IgG4 revealed positive staining in the hepatic sinus in 16 of 17 patients with AIP, 2 of 17 patients with AIH, and none in patients with PBC, PSC, or CVH.

Correlation Between IgG4-Bearing Plasma Cells in the Liver and Clinical Features

Fig. 2 shows box plots of the number of IgG4-bearing plasma cells in patients with AIP, AIH, PBC, PSC, and CVH, revealing a significantly higher median number of IgG4-bearing plasma cells in patients with AIP (5.0/HPF [IQR: 1.8-8.5]) compared with other groups (0.0/HPF [IQR: 0.0-0.0]) ($P < 0.01$). Similarly, the median ratio of IgG4: IgG-bearing plasma cells in patients with AIP (0.32 [IQR: 0.20-0.43]) was significantly higher than in control groups (0.00 [IQR: 0.00-0.00]) ($P \leq 0.001$) (Fig. 2).

ROC curve analysis was performed to determine the optimal cutoff values of the number of IgG4-bearing plasma cells to best differentiate patients with AIP from other liver diseases. The selection of the optimal cutoff point value was based on the level at which accuracy was highest. The optimal cutoff value, sensitivity and specific-

ity were 1.0/HPF, 88.2% and 90.5%, respectively, and the area under the curve was 0.918.

A highly significant association was found between serum IgG4 concentrations and IgG4-bearing plasma cells in patients with AIP ($P = 0.0014$, $r = 0.709$). No such correlation was detected in patients with AIH, PBC, PSC, or CVH (data not shown).

We next looked for associations between bile duct thickness and histological findings with the number of IgG4-bearing plasma cells in patients with AIP. The median number of IgG4-bearing plasma cells in patients with severe, mild and absent thickness of bile duct wall were 29.0/HPF, 5.5/HPF and 2.5/HPF, respectively.

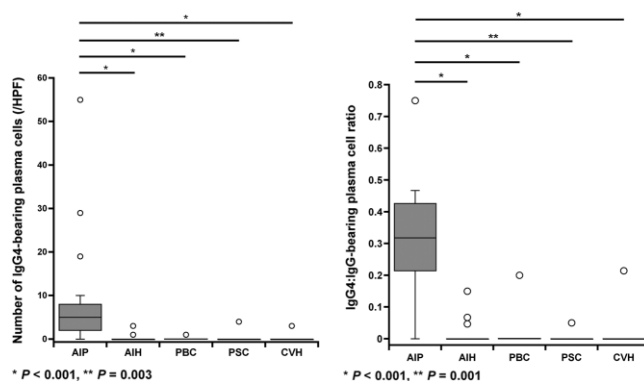


Fig. 2. Number of IgG4-bearing plasma cells in the liver of patients with AIP in comparison with other liver diseases. Boxes represent the IQR of the data. The lines across the boxes indicate median values. Hash marks depict the nearest value within 1.5 times the IQR. Open circles indicate outliers. Presence of IgG4-bearing plasma cells and the IgG4: IgG-bearing plasma cell ratio in the liver of 17 patients with AIP, 17 patients with AIH, 22 patients with PBC, 7 patients with PSC, and 17 patients with CVH.

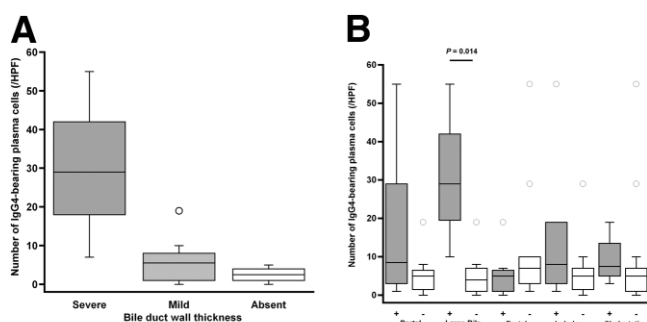


Fig. 3. Correlation between the number of IgG4-bearing plasma cells in the liver with bile duct wall thickness and liver histological patterns. Boxes represent the IQR of the data. The lines across the boxes indicate median values. Hash marks depict the nearest value within 1.5 times the IQR. Open circles indicate outliers. (A) Occurrence of IgG4-bearing plasma cell infiltration in the liver as examined by immunostaining and bile duct wall thickness as evaluated by imaging modalities. (B) Occurrence of IgG4-bearing plasma cell infiltration in the liver and 5 histological liver patterns.

The number of IgG4-bearing plasma cells were found to be positively correlated with the severity of wall thickness ($P = 0.046$) (Fig. 3A). Patients with the large bile duct damage pattern had a significantly higher median number of IgG-bearing plasma cells (68.0 versus 13.5/HPF; $P = 0.014$) and IgG4-bearing plasma cells (29.0 versus 4.0/HPF; $P = 0.014$) (Fig. 3B) compared to those without that pattern, though there were no significant correlations observed between the median number of IgG- or IgG4-bearing plasma cells and the remaining histological patterns. Next, we divided our 17 AIP patients into two groups based on the sum of the 5 histological patterns, 0-1 patterns (9 cases) or 2-3 patterns (8 cases); the median number of both IgG- and IgG4-bearing plasma cells was significantly lower in the former group than in the latter group (13.0 versus 29.0/HPF; $P = 0.049$ and 3.0 versus 9.0/HPF; $P = 0.021$, respectively).

Clinical, Biochemical, Histological Responses to Steroid Therapy in AIP Patients

Paired liver biopsy samples were obtained before and after 4 weeks of glucocorticoid therapy in 7 of the 17 AIP patients. All of these patients experienced remission of symptoms and resolution of abnormalities in imaging studies after therapy as previously reported,¹⁹ and post-therapy levels of AST (55 versus 23 IU/L; $P = 0.031$), alkaline phosphatase (752 versus 389 IU/L; $P = 0.016$), and total protein (7.1 versus 6.3 g/dL; $P = 0.016$) were significantly lower than before treatment (Table 3). Furthermore, both serum IgG4 concentration (249 versus 86 mg/dL; $P = 0.016$) and serum IgG4:IgG ratio (0.198 versus 0.098; $P = 0.016$) were significantly lower at the end of the assessment, consistent with previous reports (Table 3).

Table 3. Clinical Features of 7 Patients with Autoimmune Pancreatitis Before and After 4 Weeks of Glucocorticoid Therapy

Variable	Before Therapy	After Therapy	P Value
ALT (IU /L)	138 (83-176)	89 (62-97)	0.16
AST (IU /L)	55 (50-86)	23 (20-25)	0.031
ALP (IU /L)	752 (509-924)	389 (291-413)	0.016
Total bilirubin (mg /dL)	1.9 (1.1-2.6)	1.1 (0.8-1.4)	0.22
γGTP (g /dL)	253 (90-425)	101 (84-234)	0.078
Total protein (g /dL)	7.1 (6.8-8.4)	6.3 (5.9-6.5)	0.016
Albumin (g /dL)	3.6 (3.2-4.1)	3.8 (3.5-3.9)	0.58
Amylase (IU /L)	92 (71-203)	58 (45-83)	0.047
IgG (mg /dL)	1747 (1432-4166)	1111 (914-1452)	0.016
IgG4 (mg /dL)	248 (169-1683)	86 (70-346)	0.016
Ratio of IgG4:IgG	0.197 (0.111-0.401)	0.098 (0.083-0.261)	0.016
IgE (IU/mL)	106 (45-203)	30 (26-52)	0.031

NOTE. Median (IQR). The Wilcoxon signed rank test.

Abbreviations: IQR: interquartile range; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; γGTP, γ-glutamyltransferase.

Histological changes were assessed using paired liver biopsy samples before and after treatment, revealing prominent improvement of histological features in all patients (Fig. 4A,B). As shown in Fig. 5, the histological features of portal inflammation (57% versus 0%; $P = 0.07$), large bile duct damage (29% versus 0%; $P = 0.46$), lobular hepatitis (71% versus 0%; $P = 0.021$), and cholestatic (43% versus 0%; $P = 0.19$) had clearly improved. Accordingly, the number of infiltrating IgG4-bearing

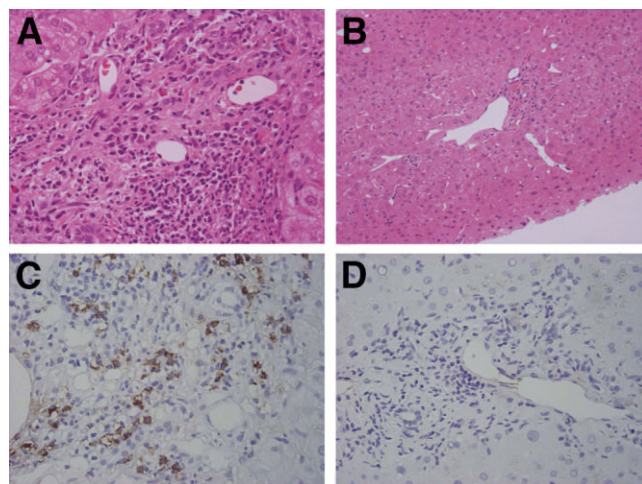


Fig. 4. Histological liver biopsy findings of AIP patients before and after 4 weeks of glucocorticoid therapy. (A) Liver biopsy before glucocorticoid therapy shows periportal fibrosis with inflammatory cell infiltration. (B) Glucocorticoid therapy improves the histopathology. (C) Immunostaining of IgG4 shows that IgG4-bearing plasma cells had infiltrated the portal tract before glucocorticoid therapy. (D) IgG4-bearing plasma cells are visibly decreased after glucocorticoid therapy. (A: hematoxylin and eosin, ×400; B: hematoxylin and eosin, ×200; C, D: immunostaining of IgG4 with hematoxylin, ×400)

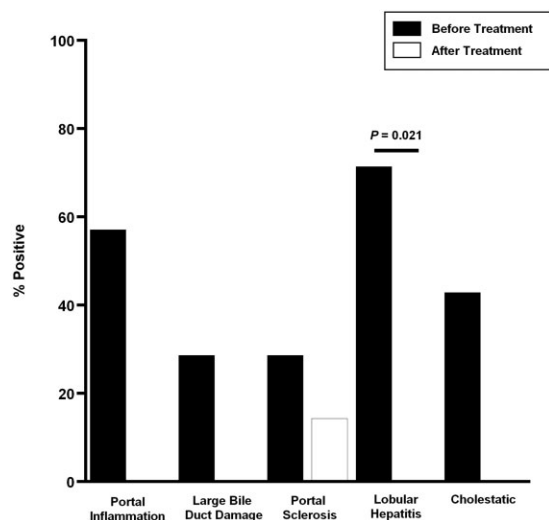


Fig. 5. Comparison of histological changes before and after 4 weeks of glucocorticoid therapy in AIP patients. The prevalence of the 5 histological patterns in the 7 AIP patients before and after 4 weeks of steroid therapy.

plasma cells in the portal areas decreased as well (Fig. 4C,D), as did the median number of IgG4-bearing plasma cells before and after therapy (8.0 [IQR: 1.5-16.8] versus 0.0 [IQR: 0.0-1.0]/HPF; $P = 0.031$).

Discussion

One of our main observations was that serum IgG4 concentration and the degree of IgG4-bearing plasma cell infiltration in the liver was significantly higher in patients with AIP than in other autoimmune liver disease controls. Through analysis using an ROC curve, the cutoff number of IgG4-bearing plasma cells showed high specificity and sensitivity in differentiating patients with AIP from controls, thus achieving a high degree of diagnostic accuracy. Sclerosing cholangitis in AIP, designated as IgG4-related sclerosing cholangitis, is susceptible to steroid therapy and is reversible.⁹ In contrast, PSC is a progressive disease that leads to biliary cirrhosis and no effective medical therapy is currently available.¹⁷ IgG4-related sclerosing cholangitis is similar to PSC with regard to cholangiographic findings, but recent reports have shown that these are in fact different clinical entities.²⁰ PSC patients in our control group had low serum IgG4 concentrations and lacked infiltrates of IgG4-bearing plasma cells.

Surprisingly, there were multiple histological findings in the liver of almost all patients with AIP. After a comprehensive assessment of 14 histological features, AIP liver histopathologies were classifiable into 5 patterns. The portal inflammation pattern suggests that inflammatory processes similar or identical to those involved in the AIP pancreas may develop along the intrahepatic portal

tracts, including the smaller tracts encountered in needle biopsies. The large bile duct damage pattern might be caused by or reflect sclerosing cholangitis in large bile ducts and intra-pancreatic bile ducts because these histological alterations are known to occur secondarily to obstruction or stenosis of large bile ducts. The portal sclerosis pattern might reflect prolonged portal tract damage due to inflammatory processes involving the portal tracts, or secondary portal tract damage relating to sclerosing cholangitis that involves the large or extrahepatic bile ducts. The lobular hepatitis pattern comprised changes similar to those seen in viral and other types of hepatitis, and differed from the biliary or portal damage observed in patients with AIP. In patients exhibiting the cholestatic pattern, canalicular cholestasis found mainly in perivenular areas, was significantly related to high total serum bilirubin levels, perhaps reflecting extensive biliary damage, as seen in the large bile duct damage pattern. Two patients had hepatic steatosis in the absence of diabetes or obesity. Although diabetes mellitus is a complication of 67% of cases of AIP in Japan, steatosis was infrequent in our cohort. Among all of these various pathological patterns, the portal inflammation, large bile duct damage, portal sclerosis, and cholestatic patterns may be explainable by the hepatobiliary involvement of similar or identical pathological processes previously speculated on in the pancreatic lesions of AIP, or may reflect secondary hepatobiliary damage in this pathological process. The lobular hepatitis pattern is most intriguing and seems to have different pathological connotations. Perhaps hepatic parenchyma or hepatocytes are target tissues of a "disease complex" that includes AIP and IgG4-related sclerosing cholangitis, so raising the question of whether some cases of chronic or acute hepatitis of indeterminate nature could be a variant of this process (Umemura et al. unpublished data). The striking improvement of lobular hepatitis after steroid therapy supports this explanation, but further data are needed.

Another important finding from our study was that abundant IgG4-bearing plasma cells had infiltrated into portal areas of the liver of most patients with AIP. The median number of IgG4-bearing plasma cell infiltration was significantly higher in patients with large bile duct damage, and positively correlated with the degree of bile duct wall thickness. Additionally, large bile duct damage was found in two patients (cases 1 and 14) with severe bile duct wall thickness, suggesting a correlation between histology and imaging findings. We found this relationship to be gradually progressive, implying that the degree of IgG4-bearing plasma cell infiltration in the liver could be associated with the severity of sclerosing pancreaticocholangitis type lesions. Furthermore, our results indicate that patients with two or more of the five

histological patterns of liver injury had a higher degree of IgG4-bearing plasma cell infiltration, also suggesting a correlation with disease severity.

Interestingly, this study shows a strong correlation between the number of IgG4-bearing plasma cells in liver specimens and values of serum IgG4 in patients with AIP. It appears that IgG4 in serum and tissue run in parallel. This indicates that serum IgG4 concentrations may reflect the load of infiltrating IgG4-bearing plasma cells in the liver. Additionally, these two indices were found to be closely associated with disease activity, though there are insufficient data on pancreatic histology in our study to assess whether the number of IgG4-bearing plasma cells in the pancreas correlated with serum IgG4 concentration as well.

In terms of response to steroid treatment, we showed that the degree of infiltration of IgG4-bearing plasma cells, and serum IgG4 concentrations, decreased dramatically after just four weeks of therapy. Furthermore, all of the histological pattern improved during steroid therapy. These results confirm that glucocorticoid therapy ameliorates both the clinical and histological findings of patients with AIP. However, it is possible that relief of jaundice influenced histological improvements, particularly the large bile duct or cholestatic pattern. As to progress, 16 patients continue to receive corticosteroid (2.5 - 5mg/day) and none has had elevated liver enzymes up to September 2006.

Diagnostically, Chari et al.¹² reported that pancreatic core biopsies are often insufficient since non-surgically obtained biopsy specimens do not show the complete spectrum of changes of lymphoplasmacytic sclerosing pancreatitis due to small sample size and lack of a duct or vein. In contrast, liver biopsies are safe and provide sufficient tissue. We found that 16 patients had at least one liver histology pattern, and 15 patients demonstrated IgG4-bearing plasma cell infiltration. Thus, histological study and IgG4 immunostaining of liver biopsies can provide firm supportive evidence when a diagnosis of AIP is suspected.

The fundamental nature of this IgG4-associated disease is enigmatic. An IgG4 immune response to an antigen often occurs in allergic disorders. Hence a possible mechanism of damage to pancreatic acinar cells, cholangiocytes and hepatocytes is via an allergic type of reaction to the target tissue cells initially induced by a dietary or bacterial antigen. Comparing cases of pemphigus vulgaris, IgG4-type autoantibodies react to the cell adhesion molecule desmoglein-3.²¹ In some ethnic groups, pemphigus vulgaris is associated with HLA-DRB1*0402, and HLA-DRB1*0402 molecules may form a complex with peptides produced by the processing of desmoglein-3. Such peptides stimulate specific T cells, which secrete cytokines and promote the production of desmoglein-3—

specific IgG4-type antibodies, resulting in the formation of skin lesions. AIP is associated in Japan with the HLA-DRB1*0405-DQB1*0401 haplotype.³ As in pemphigus vulgaris, these HLA molecules may present an as yet unidentified antigenic peptides and promote the production of antigen-specific IgG4-type antibodies, with ensuing tissue damage.

In conclusion, histological findings in the liver in AIP are highly variable although broadly classifiable into specific patterns that clearly relate to disease severity, and one component in common is prominence of IgG4 bearing plasma cells. Treatment with glucocorticoid therapy ameliorates this IgG4-bearing plasma cell infiltration, and other histological abnormalities in AIP. Indeed the diagnosis of AIP may be confirmed though liver biopsy findings that are quite distinct from those in other autoimmune diseases that affect the liver.

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Supplemental Table 1. Demographic and clinical characteristics of the patients with AIH, PBC, PSC, and CVH

Characteristics	AIH (n=17)	PBC (n=22)	PSC (n=7)	CVH (n=17)
Median age, yrs (IQR)	65 (55-70)	60 (48-65)	41 (33-56)	53 (39-65)
Male, n (%)	1 (6)	3 (14)	5 (71)	9 (53)
Median values (IQR)				
ALT (IU/L)	102 (61-175)	40 (30-64)	122 (60-138)	65 (42-172)
AST (IU/L)	83 (61-171)	40 (30-60)	84 (46-131)	53 (39-83)
ALP (IU/L)	391 (285-507)	482 (396-615)	814 (717-1048)	265 (221-324)
Total bilirubin (mg/dL)	1.1 (0.7-1.4)	0.7 (0.6-0.9)	1.7 (1.0-2.4)	0.8 (0.7-0.9)
γGTP (g/dL)	119 (65-179)	176 (121-213)	250 (180)	47 (34-78)
Total protein (g/dL)	8.4 (7.8-9.2)	7.8 (7.7-8.2)	7.7 (6.9-8.2)	7.5 (7.3-8.2)
Albumin (g/dL)	3.9 (3.8-4.1)	4.4 (4.2-4.5)	4.5 (3.0-4.5)	4.5 (4.3-4.6)
IgG (mg/dL)	2980(2268-3228)	1557 (1336-1749)	2013 (1659-2270)	1654 (1375-2261)
IgG4 (mg/dL)	68 (26-105)	26 (17-29)	64 (20-102)	34 (27-51)
Ratio of IgG4:IgG	0.03 (0.01-0.04)	0.02 (0.01-0.02)	0.03 (0.01-0.06)	0.02 (0.01-0.03)
IgM (mg/dL)	189 (122-267)	365 (169-550)	213 (106-290)	106 (81-133)
ANA (%)	17/17 (100)	10/22 (45)	5/7 (71)	(0)
AMA (%)	(0)	22/22 (100)	(0)	(0)

Abbreviations: AIH: autoimmune hepatitis; PBC: primary biliary cirrhosis; PSC: primary sclerosing cholangitis; CVH: chronic viral hepatitis; IQR: interquartile range; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; γGTP, γ-glutamyltransferase; ANA, anti-nuclear antibody.