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INTERNATIONAL FORUM

Clinical significance of immunoglobulin G4-associated autoimmune hepatitis

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Abstract
Background Immunoglobulin (Ig) G4-associated autoimmune hepatitis (AIH) is a recently identified and possibly new disease entity. However, the epidemiology and clinical features of IgG4-associated AIH remain uncertain. The aim of this study was to determine the prevalence and the clinical, serological, and histological characteristics of IgG4-associated AIH.

Methods We examined the clinical features, serum IgG4 concentration, liver biopsy histology, and IgG4-bearing plasma cell infiltration of 60 patients with type 1 AIH and 22 patients with autoimmune pancreatitis.
Results High serum IgG4 concentration (≥135 mg/dL) and IgG4-bearing plasma cell infiltration in the liver (≥10/high-power fields [HPFs]) were found in 2 of the 60 (3.3%) patients with type 1 AIH. These patients had high serum levels of IgE, giant cell change, and rosette formation in the liver. Although corticosteroid therapy reduced the serum IgG4 concentration and normalized liver enzymes and histology, one patient developed IgG4-related sclerosing cholangitis after 5 years of follow-up.
Conclusions Because IgG4-associated AIH was found in over 3% of Japanese patients with type 1 AIH in our cohort, further studies are needed on this possible new disease entity and its impact on the diagnostic guidelines of AIH.

Keywords Autoimmune hepatitis · Autoimmune pancreatitis · IgG4 · Histology · Sclerosing cholangitis

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Introduction

Autoimmune hepatitis (AIH) is an organ-specific autoimmune disease characterized by chronic inflammation of the liver, elevated transaminase levels, hypergammaglobulinemia, serum autoantibodies, histological evidence of interface hepatitis, and a favorable response to immunosuppressive treatment [1–3]. Type 1 AIH is the major form of AIH in Japanese and Caucasoid adults, and can be distinguished by the presence of circulating anti-nuclear antibodies (ANA) and/or anti-smooth muscle antibodies (SMA). The diagnosis of type 1 AIH is based on the

revised scoring system developed by the International Autoimmune Hepatitis Group (IAIHG) [4].

We recently reported a case suggesting the existence of a new disease entity termed "immunoglobulin G4 (IgG4)-associated AIH" [5]. Although the revised IAIHG disease score of this patient was 18 and representative of definite AIH, a high serum IgG4 concentration was detected before the administration of corticosteroid therapy. Moreover, immunostaining of liver tissues prior to treatment showed abundant plasma cells with strong immunohistochemical reactivity to IgG4. Abdominal computed tomography, endoscopic retrograde cholangiography, and magnetic resonance cholangiopancreatography showed no abnormalities of the extrahepatic bile ducts or pancreas. Raised serum IgG4 concentration and IgG4-bearing plasma cell infiltration have high sensitivity and specificity for the diagnosis of autoimmune pancreatitis (AIP) [6–8] and IgG4-related sclerosing cholangitis or IgG4-associated cholangitis [9, 10]. Thus, we suggested that IgG4-associated AIH was in fact an IgG4-related disease [5]. Since then, the epidemiology and clinical significance of IgG4-associated AIH have remained largely unknown. Chung et al. [11] recently conducted IgG4 immunostaining of liver biopsies from 26 patients with AIH. Although they reported that 9 out of the 26 patients had infiltration of more than 5 IgG4-positive plasma cells per high power field (HPF) in the liver, no cases in their cohort showed high serum IgG4. We have also found that patients with AIP had histological liver findings that included portal inflammation, large bile duct damage, portal sclerosis, lobular hepatitis, and cholestasis and/or IgG4-bearing plasma cell infiltration in the liver, and have accordingly proposed that histological liver changes in AIP be considered as an IgG4 hepatopathy as well [8]. In the present study, we investigated serum IgG4 concentrations and IgG4 immunostaining of the liver in patients with type 1 AIH, compared the findings with those in AIP, and proposed diagnostic criteria for IgG4-associated AIH.

Methods

Subjects

Between June 1985 and December 2006, 70 consecutively treated type 1 AIH patients (12 men and 58 women; median age 57 years, interquartile range [IQR] 45–63 years)were seen at Shinshu University Hospital, Japan. All patients were diagnosed as having probable or definite AIH according to the scoring system of the IAIHG. Among them, 60 (86%) subjects from whom serum samples and paraffin-embedded liver biopsy samples before administration of corticosteroids were available were registered for this study; their characteristics are summarized in Table 1. No patients had a pancreatic mass or common bile duct strictures detectable by ultrasonography, computed tomography, and/or magnetic resonance imaging. All 60 patients were treated with corticosteroids, and none were given azathioprine during follow-up. All patients were negative for hepatitis B surface antigen, antibody to hepatitis B core antigen, antibody to hepatitis C virus, and antibody to human immunodeficiency virus. No patients were positive for antimitochondrial antibody-M2. No patients had potential drug-induced liver injury. Twenty-two patients with AIP were enrolled as control cases. All were biopsied. The diagnosis of AIP was based on criteria released by the Japan Pancreas Society [12]. Serum samples were obtained at the time of liver biopsy and stored at −70°C until testing. The protocol of this study conformed to the Declaration of Helsinki, and was approved by the ethics committee of Shinshu University School of Medicine.

Laboratory tests

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and other relevant biochemical tests were performed using standard methods [13]. ANA and SMA were determined by indirect immunofluorescence on murine tissue sections as reported previously [8]. A serum titer of 1:40 or greater was considered positive for ANA and SMA. Patterns of ANA reactivity were also recorded. Anti-double-stranded DNA (<12 IU/mL), anti-Ro (SS-A), anti-La (SS-B), and antimitochondrial antibody-M2 were tested by enzyme-linked immunosorbent assay (ELISA) as reported previously [8]. Serum IgG4 concentrations were determined by single radial immunodiffusion (IgG4-SD RID kit, RN109.3; Binding Site, Birmingham, UK) as reported previously [6] or by a nephelometric assay (IgG4 BN^{TM}II kit, NK009.T; Binding Site) (normal value < 135 mg/dL).

Genomic DNA from the patients was isolated by phenolic extraction of sodium dodecyl sulfate-lysed and proteinase K-treated cells as described previously [14]. HLA class I and II alleles were determined using a Micro SSPTM DNA Typing Kit (One Lambda, Canoga Park, CA, USA). DNA typing of DRB1 and DQB1 alleles was performed by polymerase chain reaction-restriction fragment length polymorphism analysis as previously described [14, 15].

Histological evaluation and immunohistochemistry of IgG and IgG4

Liver biopsies were performed by percutaneous sampling of the right lobe with a 14-gauge needle in all but one

Table 1 Demographic and clinical characteristics of AIH and AIP patients

Characteristics	AIH ($n = 60$)	AIP ($n = 22$)	P value	AIH	
				IgG4-AIH ($n = 2$)	Classical AIH ($n = 58$)
Median age, years (IQR)	58 (46–64)	63 (57–66)	0.06	48 (42–54)	58 (46–65)
Female, *n* (%)	49 (82)	3 (14)	<0.001	1 (50)	48 (83)
AIH score, median (IQR)	17 (16–19)	9 (6–10)	<0.001	17 (16–19)	17 (16–19)
Definite, *n* (%)	48 (80)	0 (0)	<0.001	2 (100)	46 (79)
Probable, *n* (%)	12 (20)	11 (50)		0 (0)	12 (21)
Median values (IQR)					
ALT (n.v. 7–45 IU/L)	573 (153–1090)	66 (27–140)	<0.001	497 (253–739)	573 (150–1103)
AST (n.v. 12–37 IU/L)	437 (150–907)	36 (24–59)	<0.001	425 (199–650)	437 (145–907)
ALP (n.v. 124–367 IU/L)	418 (295–548)	481 (322–753)	0.429	539 (472–605)	401 (294–507)
Bilirubin (n.v. 0.3–1.2 mg/dL)	2.2 (1.0–8.5)	1.1 (0.6–1.8)	0.002	5.6 (2.5–8.6)	2.1 (1.0–8.2)
Alb (n.v. 4.2–5.1 g/dL)	3.6 (3.0–3.9)	3.7 (3.4–4.0)	0.314	3.0 (2.8–3.2)	3.6 (3.1–3.9)
IgG (n.v. 870–1700 mg/dL)	2940 (2330–3493)	2494 (1598–3268)	0.096	4015 (2403–5627)	2940 (2328–3464)
IgG4 (n.v. <135 mg/dL)	23 (18–50)	699 (346–1335)	<0.001	560 (557–642)	22 (18–47)
IgG4:IgG	0.009 (0.006–0.015)	0.253 (0.189–0.410)	<0.001	0.173 (0.114–0.232)	0.009 (0.006–0.014)
IgE (n.v. <361 IU/mL)	64 (20–381)	246 (105–700)	0.010	1490 (1330–1650)	58 (18–306)
High IgG4 levels, *n* (%)	4 (7)	21 (95)	<0.001	2 (100)	2 (3)
ANA-positive, *n* (%)	60 (100)	17 (77)	<0.001	2 (100)	58 (100)
SMA-positive, *n* (%)	30/54 (56)	0 (0)	<0.001	1 (50)	29/52 (56)
ds-DNA-positive, *n* (%)	4 (7)	1 (5)	0.593	1 (50)	3 (5)
SS-A-positive, *n* (%)	5 (8)	0 (0)	0.200	0 (0)	5 (9)
SS-B-positive, *n* (%)	1 (2)	0 (0)	0.732	0 (0)	1 (2)
HLA-DR4, *n* (%)	38/56 (68)	9/15 (60)	0.557	1 (50)	37/54 (69)

AIH autoimmune hepatitis, *AIP* autoimmune pancreatitis, *IgG4-AIH* IgG4-associated autoimmune hepatitis, *IQR* interquartile range, *n.v.* normal value, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *Alb* albumin, *ANA* anti-nuclear antibody, *SMA* anti-smooth muscle antibody, *ds-DNA* anti-double stranded DNA

patient, whose liver specimen was obtained during surgery. All biopsy specimens were 1.5 cm or more in length. Liver specimens were taken before the administration of corticosteroid therapy in the 60 patients with AIH and the 22 patients with AIP. Formalin-fixed and paraffin-embedded specimens were prepared and used for histopathological and immunohistochemical studies. Sections measuring 4 μm were cut from each paraffin block and stained with hematoxylin and eosin, periodic acid-Schiff after diastase digestion, Azan–Mallory, silver impregnation reticulin, or silver impregnation orcein, with the remaining material being used for immunohistochemical analysis. The following [11] 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 (0, absent; 1, inflammation in ≤1/3 of periportal areas; 2, inflammation in 1/3 to 2/3 of periportal areas; 3, inflammation in ≥2/3 of periportal areas); [3] interface hepatitis (0, absent; 1, interface hepatitis in ≤1/3 of periportal areas; 2, interface hepatitis in 1/3 to 2/3 of periportal areas; 3, interface hepatitis in ≥2/3 of periportal areas); [4] lobular hepatitis (1, 0–2 focal necrosis/high-power field (HPF); 2, ≥3 focal necrosis/HPF; 3, zonal necrosis); [5] plasma cell infiltration (1, 0–9 cells/HPF in portal area; 2, 10–19 cells/HPF; 3, ≥20 cells/HPF); [6] eosinophil infiltration (0, 0–4 cells/HPF in portal area; 1, ≥5 cells/HPF); [7] syncytial giant cell change (0, absent; 1, present); [8] rosette formation (0, absent; 1, present); [9] bile duct loss (0, absent; 1, present); [10] bile duct damage (−, absent; +, irregularity of cellular and nuclear arrangement of the biliary epithelium with or without narrowing of the bile duct lumen); and [11] cholangitis (0, absent; 1, present).

Immunostaining for IgG and IgG4 was performed using mouse monoclonal antibodies against human IgG (Dako Cytomation, Glostrup, Denmark), and human IgG4 (ZYMED Laboratory, San Francisco, CA, USA) as reported previously [9]. Counts were tallied for IgG- and IgG4-bearing plasma cells per HPF, and the number of positive cells was expressed as the mean of triplicates and compared. The ratio of IgG4-positive to IgG-positive cells was also calculated for each case.

Statistical analysis

The Mann–Whitney *U*-test was used to analyze continuous variables where appropriate. The χ^2 test with Yates's correction was used for the analysis of categorical data. In cases where the number of subjects was less than 5, Fisher's exact test was used. $P \leq 0.05$ was considered to be significant. Statistical analysis was performed using SPSS software (version 15.0J; SPSS, Chicago, IL, USA).

Results

Clinical characteristics of patients

The clinical profile of the experimental patient cohort is shown in Table 1. Forty-nine (82%) were women, and the median age at presentation was 58 years. All patients were graded by the IAIHG scoring system prior to treatment. Of them, 48 (80%) patients satisfied the criteria for a definite diagnosis of AIH and the remaining 12 patients met the requirements for a probable diagnosis. Although no AIP patients fulfilled the definite criteria for AIH, 11 of the 22 (50%) patients met the probable criteria for AIH as well. The median IAIHG score was significantly higher in patients with AIH than in those with AIP. AIH patients also had significantly higher median serum levels of ALT, AST, and total bilirubin. There were significant differences in positivity for ANA and SMA in AIH versus AIP, but the prevalence of HLA DR4 did not differ between the two groups.

The median serum IgG4 concentration, IgG4: IgG ratio, and serum IgE level were significantly higher in AIP patients than in the patients with AIH. However, an elevated serum IgG4 concentration (≥135 mg/dL) was detected in 4 of the 60 (6.7%) patients with AIH, and the serum IgG4 concentrations and IgG4: IgG ratios in each of these patients were 146, 215, 557, and 642 mg/dL and 0.041, 0.072, 0.232, and 0.114, respectively. We previously performed receiver operating characteristic curve analysis to determine the optimal cutoff value of the serum IgG4: IgG ratio to best differentiate patients with AIP from those with other diseases [8]. This value was 0.073, meaning that 2 of the 60 (3.3%) AIH patients had an extraordinarily high serum IgG4 concentration and IgG4: IgG ratio.

Histopathology of liver biopsies in patients with AIH and AIP

As shown in Table 2, portal and periportal fibrosis was present in all 60 patients with AIH, and 33 of them (55%) showed bridging fibrosis. Twenty-two patients (37%) had cirrhosis. Portal inflammation was evident in all but one patient, and 55 (92%) also showed associated interface hepatitis. Lobular hepatitis, which was defined as focal necrosis ≥3/HPF, was found in 39 patients (65%). Marked plasma cell infiltration (≥20/HPF) and eosinophil infiltration (≥5/HPF) in portal tracts were present in 30 (50%) and 18 patients (30%), respectively. Giant cell change and rosette formation were found in 7 (12%) and 9 patients (15%), respectively. Only 3 patients had both giant cell change and rosette formation. Bile duct damage was found in 10 patients (17%), but chronic nonsuppurative destructive cholangitis and bile duct loss were not found in any of the patients.

Immunostaining for IgG and IgG4 demonstrated many IgG- and IgG4-bearing plasma cells in the portal tract. The number of IgG4-bearing plasma cells in patients with AIP (6.0/HPF; IQR, 2.3–12.3) was significantly higher than that in patients with AIH (0.0/HPF; IQR, 0.0–3.8; $P < 0.001$), but the number of IgG-bearing plasma cells did not show a significant difference between the two groups. More than 5 IgG4-positive cells (/HPF) were found in 5 of the 60 (8%) patients with AIH. The numbers of IgG4-positive cells and ratios of IgG4: IgG bearing plasma cells in each of these

Table 2 Histological characteristics of patients with AIH and IgG4-AIH

	AIH ($n = 60$)	IgG4-AIH ($n = 2$)	Classical AIH ($n = 58$)
Bridging fibrosis	33 (55%)	2 (100%)	31 (54%)
Cirrhosis	22 (37%)	0 (0%)	22 (38%)
Portal inflammation (≥2/3 of periportal areas)	43 (72%)	2 (100%)	41 (71%)
Interface hepatitis (≥2/3 of periportal areas)	23 (38%)	2 (100%)	21 (36%)
Lobular hepatitis (zonal necrosis)	15 (25%)	2 (100%)	13 (22%)
Plasma cells (≥20/HPF)	20 (33%)	2 (100%)	18 (31%)
Eosinophils (≥5/HPF)	18 (30%)	1 (50%)	17 (29%)
Giant cell change	7 (12%)	2 (100%)	5 (9%)
Rosette formation	9 (15%)	2 (100%)	7 (12%)
Bile duct damage	10 (17%)	0 (0%)	10 (17%)

AIH autoimmune hepatitis, *IgG4-AIH* IgG4-associated autoimmune hepatitis, *HPF* high-power field

patients were 6, 6, 7, 24, and 29/HPF and 0.082, 0.102, 0.071, 0.282, and 0.528, respectively. The latter 2 patients were also the patients with elevated serum IgG4 and elevated IgG4: IgG ratios.

All patients with AIP in this study had relevant histological liver findings and/or IgG4-bearing plasma cell infiltration in the liver, and were thus considered to have an IgG4 hepatopathy.

Clinical significance of IgG4-associated autoimmune hepatitis

In our cohort, a high serum IgG4 concentration and more than 10 IgG4-positive cells (/HPF) were found in 2 patients. As shown in Table 1, the 2 patients with IgG4-associated AIH had higher median serum levels of IgG4, higher IgG4: IgG ratios, and higher IgE levels compared with the 58 patients with classical AIH. The HLA DRB1*0405 allele was found in one patient with IgG4-associated AIH. Liver biopsies of the 2 patients showed similar chronic active hepatitis with bridging fibrosis. Hepatic activity was high, and both patients showed interface hepatitis and zonal necrosis. Interestingly, giant cell change and rosette formation were simultaneously observed in both patients with IgG4-associated AIH (Table 2). The median numbers of IgG- and IgG4-bearing plasma cells in the patients with IgG4-associated AIH (70.3/HPF and 26.5/HPF) tended to be higher than those for the patients with classical AIH (24.5/HPF and 0.0/HPF) and AIP (14.5/HPF and 6.0/HPF).

Clinical course of IgG4-associated autoimmune hepatitis

Case 1 in this study was previously reported as the first proposed case of IgG4-associated AIH [5]. The patient's serum IgG4 concentrations and liver enzymes eventually normalized with continuous low-dose corticosteroid therapy (5.0–10.0 mg/day). However, after 5 years of follow-up, her serum alkaline phosphatase and γ-glutamyl transpeptidase rose to 622 and 936 IU/L, respectively. Although no abnormalities of the bile duct were found before administration of corticosteroid therapy, distal and proximal biliary strictures became evident by endoscopic retrograde cholangiography (Fig. 1) and magnetic resonance cholangiopancreatography. In addition, abundant IgG4-bearing plasma cell infiltration was found in a biopsy of the common bile duct, despite serum IgG4 concentrations remaining normal at 75 mg/dL. Hence, she was the first patient diagnosed with IgG4-associated AIH who later developed IgG4-related sclerosing cholangitis.

Case 2 was a 42-year old man who was admitted to our hospital because of elevated liver enzymes [16]. Serum IgG and IgE concentrations prior to corticosteroid therapy were extremely high. His ANA antibody, anti-double-strand DNA, and SMA were all positive. A first liver biopsy showed changes associated with typical AIH: interface hepatitis, lobular hepatitis, rosette formation, syncytial multinucleated giant cell change, and marked plasma cell infiltration. No biliary epithelial changes were found. Imaging modalities showed no abnormalities of the extrahepatic bile ducts or pancreas. A serum IgG4 concentration of 642 mg/dL was detected in stored serum, and immunostaining of liver tissue showed abundant plasma cells with strong immunohistochemical reactivity to IgG4. He fulfilled the criteria for definite AIH and was administered corticosteroids at 60 mg/day. Four weeks after the initiation of the corticosteroid treatment, serum IgG4 and IgE concentrations were decreased to 452 mg/dL and 909 IU/L, respectively. Although a second liver biopsy performed 7 months after the first showed remaining portal sclerosis, almost all the other histological findings were improved except for mild portal inflammation. Serum IgG4 and IgE concentrations were normalized. Furthermore, IgG4-bearing plasma cell infiltration in the liver was absent (0/HPF). After 13 years of follow-up, his transaminases are still elevated but below 100 IU/L with continuous low-dose corticosteroid therapy (5.0 mg/day), and image modalities show no abnormalities. His ANA antibody titer (1: 1280) and anti-double-strand DNA (>400 IU/mL) are still abnormal.

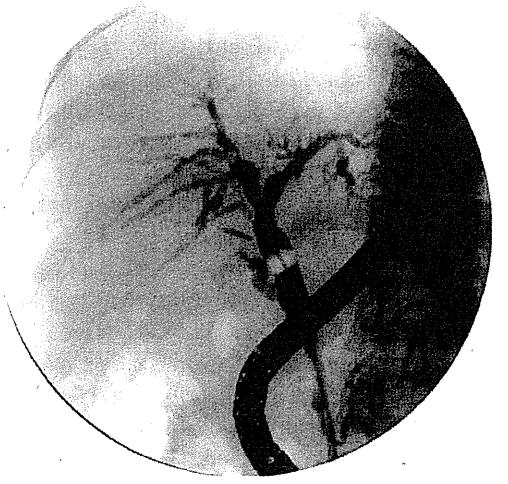

Fig. 1 Endoscopic retrograde cholangiography after 5 years of follow-up, showing smooth narrowing of the common bile duct and intrahepatic biliary strictures

Discussion

In an earlier report, a strong and unexpected association was seen between serum IgG4 concentration and IgG4-

bearing plasma cell infiltration in the liver in one patient with type 1 AIH, raising the possibility of a new disease entity, termed 'IgG4-associated AIH' [5]. In the present study, we investigated serum IgG4 concentration and IgG4 immunostaining of the liver in 60 Japanese patients with type 1 AIH and looked for correlations with liver histology and clinical features in comparison with AIP.

Based on our findings, we have provisionally set the diagnostic criteria for IgG4-associated AIH as follows: (1) having definite AIH according to the IAIHG scoring system, (2) serum IgG4 concentration ≥135 mg/dL, and (3) immunostaining of IgG4 showing infiltration of ≥10/HPF IgG4-bearing plasma cells in the portal tract. We ultimately identified 2 patients (3.3%) among our AIH cases in the present cohort who fulfilled these criteria. This rate was quite low, as we had expected. Conversely, Chung et al. [11] recently reported that 9 of 26 patients with AIH showed more than 5 IgG4-positive plasma cells (/HPF) in the liver. Although serum IgG4 concentration in all patients was normal at less than 80 mg/dL, these cases were classified as having IgG4-associated AIH, thus being distinct from IgG4-related disease. In general, the concept of an IgG4-related disease is high serum IgG4 concentration and abundant infiltration of IgG4-bearing plasma cells in the affected organs. Although it is possible that several of their patients had IgG4-positive cell infiltration without high serum IgG4 concentration, it is difficult to draw the conclusion that all of their cases can be classified as having an IgG4-associated disease. Koyabu et al. [17] have recently reported that an IgG4/IgG1-bearing plasma cell ratio of >1 in the liver is specific for IgG4-related diseases. In our 2 cases, the ratio of IgG4: IgG1-bearing plasma cells in the liver was >1. Hence, this ratio might be a useful marker for the diagnosis of IgG4-related diseases.

We recently reported IgG4 hepatopathy in AIP patients because almost all the patients had liver dysfunction and histological changes such as interface and lobular hepatitis [8]. Our patients with IgG4-associated AIH in the present study had high serum IgG4 and abundant infiltration of IgG4-bearing plasma cells in the liver, and therefore we believe this disease entity should be considered as an IgG4-associated disease (IgG4 hepatopathy) rather than AIH. Using the IAIHG scoring system, 50% of our 22 AIP patients had probable AIH. Hence, we excluded all AIH cases listed as probable for a more precise evaluation of IgG4-associated AIH. We also selected 135 mg/dL as the cutoff value for serum IgG4 because of its high accuracy for AIP diagnosis [6]. Because IgG4-bearing plasma cell infiltration is a characteristic finding in AIP [6, 18], IgG4-related sclerosing cholangitis, and IgG4-associated cholangitis [9, 10] we included the criteria of the infiltration of more than 10 IgG4-bearing plasma cells (/HPF) in the liver as well. Moreover, we believe that AIH patients with pancreatic abnormalities should be excluded from the diagnosis of IgG4-associated AIH, because a prior study showed that interface hepatitis was found in 24% of patients with AIP [8]. Although all the AIP patients in the present study had relevant histological liver changes and/or IgG4-bearing plasma cell infiltration in the liver, no patients fulfilled the criteria for definite AIH. Hence, they were considered to have AIP with histological damage in the liver, not IgG4-associated AIH. A relatively small number of patients with AIH were studied in this report, because the number of patients having both stored sera and paraffin-embedded liver biopsy samples is small. As such, a larger sample group will be needed to compare IgG4-associated AIH with classical AIH.

IgG4-related diseases are primarily found in the pancreatobiliary system and hepatic parenchyma [6–9]. Our patients were mainly affected in the liver and their disease resembled AIH both clinically and pathologically [5, 16]. The differences between IgG4-associated AIH and comorbid hepatic injury in AIP are considered to be: (1) patients with IgG4-associated AIH have a much higher degree of IgG4-bearing plasma cell infiltration in the liver compared not only with classical AIH, but also with AIP; (2) giant cell change and rosette formation are obvious; and (3) bile duct damage or loss is not found. Taken together, it appears that the histological liver findings of IgG4-associated AIH seem to be more severe compared with the findings in classical AIH and AIP.

Interestingly, one patient with IgG4-associated AIH who was administered low-dose corticosteroid therapy developed IgG4-related sclerosing cholangitis after 5 years of follow-up. Because we did not perform a bile duct biopsy prior to treatment, we cannot exclude the possibility that she had sclerosing cholangitis at that time. However, no bile duct abnormalities were seen by imaging modalities or in liver biopsy samples before therapy. AIH/primary sclerosing cholangitis overlap syndrome has been reported with an increasing incidence in a number of studies [19–24]. Although these studies did not examine serum IgG4 concentration or IgG4-immunostaining of liver biopsies, it is possible that a case of IgG4-associated AIH with sclerosing cholangitis might have been included in their populations.

The fundamental nature of IgG4-related diseases is enigmatic. Because IgG4 and IgE immune responses to antigens often occur in allergic disorders [25, 26], damage to pancreatic acinar cells, cholangiocytes, and hepatocytes via an allergic type of reaction to target tissue cells initially induced by a dietary or bacterial antigen is plausible. In addition, Zen and colleagues [27], who are coauthors of the present study, recently reported that IgG4-related diseases were characterized by the overproduction of T-helper 2 and regulatory cytokines, both of which are closely involved in the pathogenesis of allergic disorders. Our present patients

with IgG4-associated AIH had high serum IgG4 and IgE concentrations and lobular hepatitis with marked IgG4-bearing plasma cell infiltration, suggesting that an allergic reaction to hepatocytes or molecules in the liver parenchyma might be a possible pathogenesis of this disease. However, such a notion is highly speculative and would require additional in-depth studies to show mast cell involvement in the liver tissues of AIP and IgG4-associated AIH patients. At present, we can only describe the observation of high serum IgE concentrations and cannot provide a sound scientific basis for its occurrence.

Susceptibility to AIH and AIP is influenced by genetic factors, specific HLA alleles, amino acid sequences at the presentation site of the HLA molecule [28, 29], and Fc receptor-like gene 3 and cytotoxic T-lymphocyte antigen 4 single nucleotide polymorphisms [28, 30–33]. These genetic markers should be investigated in our 2 patients.

In conclusion, IgG4-associated AIH was found in over 3% of classical AIH cases in a Japanese cohort and is characterized by high serum IgG4 and IgE concentrations and IgG4-bearing plasma cell infiltration in the liver. Because IgG4-associated AIH is clearly an IgG4 hepatopathy, this disease should be differentiated from classical AIH. Further studies are needed to clarify the epidemiology and pathogenesis of this possible new disease entity.

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Review Article

Hepatobiliary membrane transporters in primary biliary cirrhosis

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The secretion of bile normally depends on the function of a number of membrane transport systems in hepatocytes and cholangiocytes. The transport of solutes from the blood to the bile is driven by transport systems in the plasma membrane of the basolateral and canalicular surfaces of the hepatocytes. In cholestatic animal models, the expression of hepatobiliary transporters changes in response to functional impairment of the efflux of bile salts and various organic anions. In recent years, several studies have led to an improved understanding of the function and regulation of hepatobiliary transport systems in patients with primary biliary cirrhosis (PBC). This review focuses on the adaptations in hepatobiliary transporters in PBC patients.

Key words: ABC transporters, hepatobiliary transporters, nuclear hormone receptor, primary biliary cirrhosis

INTRODUCTION

THE LIVER PLAYS an essential role in removing toxic compounds from the body. Normal hepatobiliary secretion and enterohepatic circulation are required for the elimination of endobiotic toxic compounds such as cholesterol, bilirubin, and their metabolites from the body, as well as for the maintenance of lipid and bile acid homeostasis. The liver also provides the body's primary means of eliminating xenobiotic compounds such as drugs and carcinogens. These xenobiotic and endobiotic compounds are taken up by the liver from portal blood and secreted into the bile by distinct transport proteins that are localized in the sinusoidal and canalicular membranes. Hepatic transport proteins have unique substrate specificities and capacities. At physiological pH, taurine- and glycine-conjugated bile acids exist in anionic form and are unable to cross membranes by diffusion; they are thus completely dependent on membrane transport proteins for hepatocyte entry and exit.[1]

Primary biliary cirrhosis (PBC) is a disease of unknown cause in which intrahepatic bile ducts are progressively destroyed by biliary epithelial cells (BEC) apoptosis.[2,3] In PBC patients, our previous studies showed the diminished tight junction of BEC,[4] the enhanced expression of heat shock protein 70 (HSP 70),[5] and the altered localization of alkaline phosphatase (ALP) in the liver.[6] PBC is characterized by chronic inflammation that targets mainly the small bile ducts, resulting in duct destruction (ductopenia) and proliferation. PBC causes functional impairment of bile salt and organic anion efflux. In response, hepatobiliary transporters are alternatively expressed to prevent bile acid accumulation in hepatocytes.

This review focuses on the adaptations of hepatobiliary transporters in cholestatic disorders, particularly PBC.

HEPATOBILIARY TRANSPORTERS

THE MAIN ORGANIC solutes of bile are bile salts, phospholipids, and cholesterol, which form mixed micelles in bile. Bile acid homeostasis is maintained by the coordinated regulation of genes involved in synthesis, detoxification, and transport. The overall process of bile formation by hepatocytes can be divided into four phases based on function, using a modification of Phase I and II drug metabolizing enzymatic reactions. Phase 0 involves hepatic uptake mechanisms. Phase I is distinguished by enzymatic hydroxylation reactions and Phase II by enzymatic conjugation reactions such as

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sulfation and glucuronidation. Phase III involves hepatic efflux mechanisms. Each of these phases is regulated by a series of transporters (Phases 0 and III) or enzymatic reactions (Phases I and II).[7]

Phase 0. Bile acid uptake: Sodium taurocholate cotransporter and sodium-independent organic-anion transporter

Bile salts are the most abundant solutes in bile. There are two sinusoidal systems for bile-salt uptake in hepatocytes: a sodium-taurocholate cotransporter (NTCP: SLC10A1) and a sodium-independent organic-anion-transporting polypeptide (OATP) (Fig. 1). Sodium-dependent uptake of bile salts through the NTCP is driven by an inwardly-directed sodium gradient generated by Na^+/K^+-ATPase and a membrane potential generated in part by a potassium channel. In contrast to conjugated bile salts, the unconjugated bile salt cholate, the organic anion sulfobromophthalein, and numerous other lipophilic albumin-bound compounds are transported from plasma to the hepatocytes by sodium-independent transport systems, including OATP.[8] OATP is a member of a large super-family of transporters that includes OATP1B1 (SLCO1B1, formerly OATP-C), OATP1B3 (SLCO1B3, formerly OATP8), OATP1A2 (SLCO1A2, formerly OATP-A), and OATP2B1 (SLCO2B1, formerly OATP-B). OATP is localized on the basolateral membrane of hepatocytes, along with a sodium-hydrogen exchanger and a sodium-bicarbonate symporter.

In the advanced stages of PBC, the expression of hepatic NTCP protein is diminished.[9] Impairment of NTCP in humans results in reduced Na^+-dependent uptake of conjugated bile salts. However, sodium-independent mechanisms for hepatic bile salt uptake persist due to continued expression of several other basolateral membrane organic anion transporters, possibly OATP1B3 in humans.[10,11] In the advanced stages of PBC[9] and primary sclerosing cholangitis (PSC),[12] the expression of OATP1B1 is reduced. Reduced expression of these uptake transporters disrupts the detoxification function of hepatocytes (Figs 2,3).

Nuclear hormone receptors play an important role in bile acid and cholesterol metabolism and in many other metabolic and transport functions in the liver. These receptors are typically ligand-dependent transcription factors whose normal ligands are generally lipophilic and include steroid hormones, bile acids, fatty acids, vitamins, and prostaglandins. The farnesoid X receptor (FXR) induces the synthesis of the suppressor protein "short heterodimer partner" (SHP). SHP inhibits fetoprotein transcription factor (FTF) and hepatocyte nuclear factor 4 (HNF4) transactivation of cholesterol 7α-hydroxylase (CYP7A1) (Fig. 4), the rate-limiting enzyme in bile acid biosynthesis. In human cells, the regulatory mechanism is complex; bile acids induce SHP expression via FXR, which reduces HNF4α-binding to bile acid response elements in the NTCP promoter. In turn, this inhibits the *trans*-activating effects on hepatocyte nuclear factor 1α (HNF1α). HNF1α expression is highly dependent on activation by HNF4α, which is the main regulator of NTCP expression.[13,14] Bile acids also have SHP-independent effects on HNF4α binding. However, SHP has no direct effect on NTCP promoter activity, and bile-acid-induced signaling pathways via

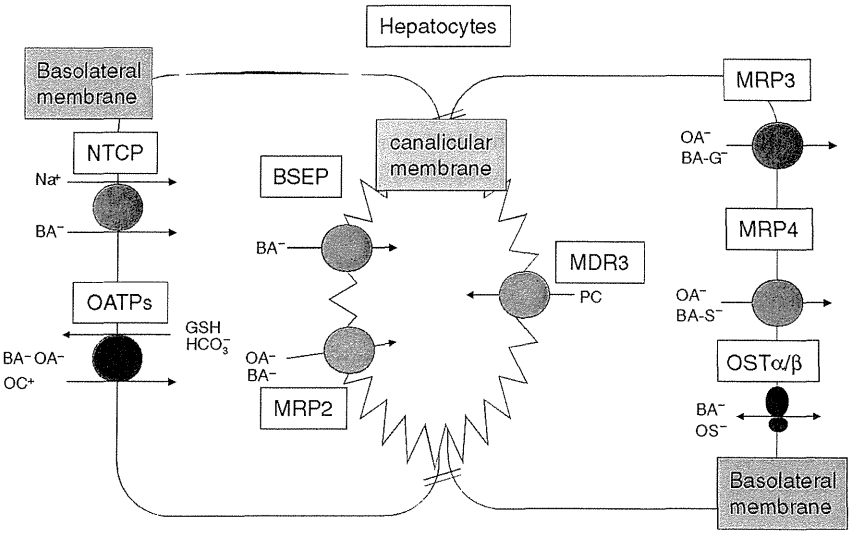

Figure 1 Hepatobiliary transporters in hepatocytes. BA, bile acids; BA-S, sulfated bile acid; BA-G, bile acid glucuronide; BSEP, Bile salt export pump; MRP2, Multidrug resistance-associated protein 2; MRP3, Multidrug resistance-associated protein 3; MRP4, Multidrug resistance-associated protein 4; MDR3, Multidrug resistance P-glycoprotein 3; NTCP, Sodium taurocholate cotransporting polypeptide; OA, organic anion; OATPs, Organic anion transporting polypeptides; OC, organic cation; OS, organic solute; OSTα-β, Organic solute transporter α-β; PC, phospholipids.

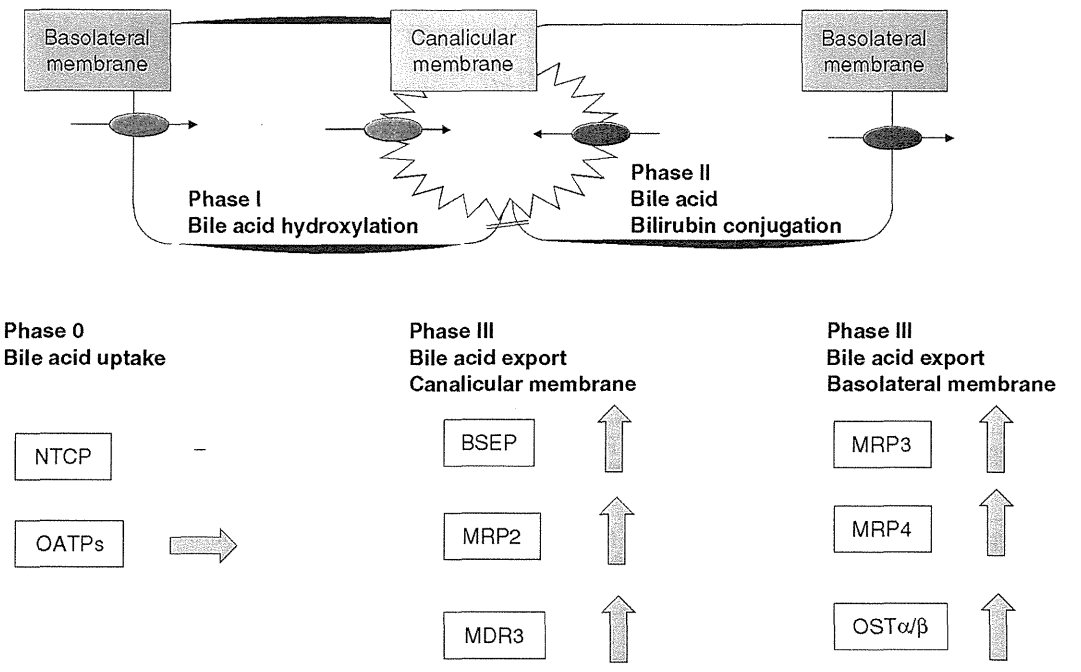

Figure 2 Hepatobiliary transporters expression in the early stage primary biliary cirrhosis (PBC). BSEP, Bile salt export pump; MRP2, Multidrug resistance associated protein 2; MRP3, Multidrug resistance associated protein 3; MRP4, Multidrug resistance associated protein 4; MDR3, Multidrug resistance 3 p-glycoprotein; NTCP, Sodium taurocholate cotransporting polypeptide; OATPs, Organic anion transporting polypeptides; OSTα-β, Organic solute transporter α-β; ↑, upregulation; →, unchanged; ↓, downregulation.

c-Jun N-terminal kinase may be involved. Recent studies suggest that the human NTCP gene is also activated by the glucocorticoid receptor (GR) and peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC-1α) and can be suppressed by bile acids via an SHP-dependent mechanism.[15] Transcription mechanisms are not known for OATPs in cholestatic patients. *In vitro* studies indicate that OATP1B1, like NTCP, is regulated by both FXR/SHP-dependent and -independent mechanisms, where HNF1α is also a primary transcription factor.

Phase I. Bile acid hydroxylation and bile acid synthesis: Cholesterol 7α-hydroxylase

Bile acids are formed from cholesterol in the liver via enzymatic pathways that are mediated by CYP7A1. This enzyme catalyzes the first and rate-limiting step in the classical bile acid synthetic pathway[16] and is thus a key enzyme in cholesterol homeostasis. Transcription of the gene encoding *CYP7A1* is repressed by bile acids via multiple mechanisms. The gene is downregulated by sterol regulatory element binding proteins when plasma cholesterol levels are low, and it is upregulated by the nuclear liver X receptor (LXR) when cholesterol levels, particularly oxysterol, are high.[17] The effect of this upregulation is to increase the production of bile acids and reduce the level of cholesterol in hepatocytes. Deficiency of CYP7A1 increases the possibility of cholesterol gallstones.[17]

The expression level of hepatic CYP7A1 mRNA is elevated in PBC patients with early-stage disease. However, this level is lower in patients with end-stage PBC.[18] During cholestasis, elevated levels of bile acids inhibit bile acid synthesis by activating FXR, which induces SHP and inhibits CYP7A1 gene transcription.[19] FXR and LXR inhibit and stimulate CYP7A1 transcription, respectively. In addition, α-fetoprotein transcription factor is an essential transcriptional factor for CYP7A1 expression. Activation of FXR enhances the expression of its target gene, SHP, and suppresses CYP7A1 transcription. This suppression occurs via the FXR-SHP-FTF cascade through inactivation of FTF, which abolishes its positive effect on transcription.

Cytochrome P450 3A4 (CYP3A4) is a major enzymatic determinant of drug and xenobiotic metabolism.

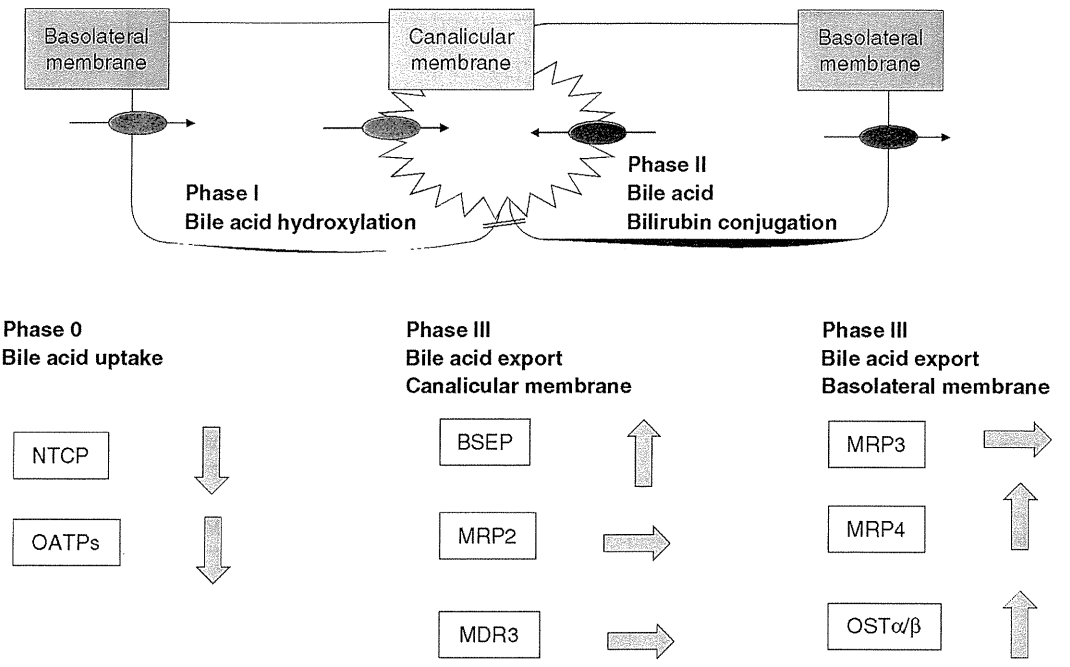

Figure 3 Hepatobiliary transporters expression in the late stage primary biliary cirrhosis (PBC). BSEP, Bile salt export pump; MRP2, Multidrug resistance associated protein 2; MRP3, Multidrug resistance associated protein 3; MRP4, Multidrug resistance associated protein 4; MDR3, Multidrug resistance 3 p-glycoprotein; NTCP, Sodium taurocholate cotransporting polypeptide; OATPs, Organic anion transporting polypeptides; OSTα-β, Organic solute transporter α-β; ↑, upregulation; →, unchanged; ↓, downregulation.

CYP3A4 plays a role in xenobiotic responses through the hydroxylation and detoxification of cholestatic bile acids such as lithocholic acid (LCA). CYP3A4 can be regulated by pregnane X receptor (PXR),[20] FXR,[21] vitamin D receptor (VDR),[22,23] and constitutive androstane receptor (CAR).[24,25] PXR is activated by the drug rifampicin. In PBC patients, CYP3A4 mRNA is only mildly altered.[26]

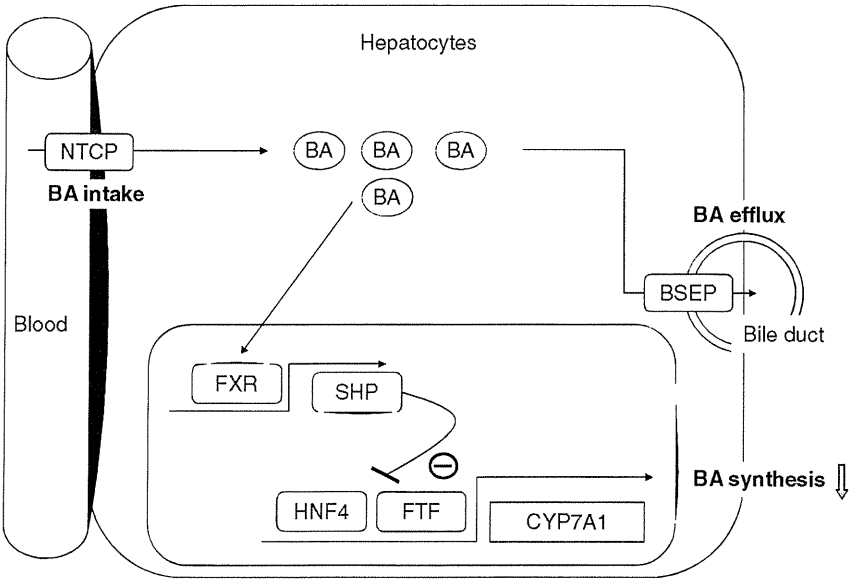

Figure 4 Farnesoid X receptor/short heterodimer partner (FXR/SHP) pathway. Bile acids activate FXR, which induces SHP expression. SHP then inhibits fetoprotein transcription factor (FTF) and hepatocyte nuclear factor 4 (HNF4) transactivation of CYP7A1 (FXR/SHP pathway). BA, bile acid; BSEP, Bile salt export pump; CYP7A1, cholesterol 7α-hydroxylase; NTCP, Sodium taurocholate cotransporting polypeptide.

Phase II. Bile acid and bilirubin conjugation

Glucuronidation, sulfation, and amidation reactions are the major Phase II pathways for diminishing the toxicity of bile acids during cholestasis. Bile acids are conjugated with glucuronide and sulfate in the cholestatic liver. These modifications facilitate bile acid excretion into the blood via basolateral export pumps and subsequent elimination by kidney.[27] Bile acid sulfation is suggested to be a primary target for regulation under cholestatic conditions because of its capability to detoxify and enhance bile acid elimination.[28] Bile acid sulfation is catalyzed by sulfotransferase 2A1 (SULT2A1). In PBC patients, the levels in urinary sulfated bile acids (USBA) are elevated. Moreover, USBA levels are higher in PBC patients with cirrhosis than in those without cirrhosis.[29]

Phase III. Canalicular export pumps

Bile salt export pump

Bile acid secretion is ATP-dependent, and the majority is mediated by the bile salt export pump (BSEP: ABCB11) at the canalicular membrane of hepatocytes (Fig. 1). Mutations in the BSEP gene lead to the inherited cholestatic disorder *progressive familial intrahepatic cholestasis type 2* (PFIC2).[30] Polymorphisms in BSEP have also been associated with intrahepatic cholestasis of pregnancy,[31,32] benign recurrent cholestasis,[33] and drug-induced cholestasis.[34] BSEP protein transport activity is inhibited by glybenclamide and troglitazone by direct binding of these compounds to BSEP,[35–37] causing drug-induced cholestasis. BSEP protein and mRNA levels in the liver are increased in PBC patients.[18,38,39] BSEP expression is maintained in patients with PBC (Figs 2,3).

The expression of the BSEP gene is highly regulated by the heterodimer FXR/RXR in both humans and rodents. Bile acids have been identified as ligands for FXR,[40] resulting in feed-forward regulation of BSEP by the substrates it transports. By increasing BSEP mRNA expression in response to elevated levels of intracellular bile acids, increased efflux of bile acids from the hepatocytes results in the return of intracellular bile acids to normal levels. Physiological dependence on FXR for regulation of BSEP gene expression was confirmed in Fxr –/– mice which showed markedly reduced basal expression levels of BSEP,[41] along with a complete lack of BSEP induction by bile acids.[42]

Multidrug resistance P-glycoprotein 3

Multidrug resistance P-glycoprotein 3 (MDR3: ABCB4) is localized at the canalicular membrane of hepatocytes and is responsible for the ATP-dependent translocation of phosphatidylcholine from the inner to outer leaflets of the membrane bilayer (the phospholipid export pump) (Fig. 1). This function is carried out in rodents by multidrug resistance protein 2 (Mdr2: Abcb4). Mutations in MDR3 cause progressive cholestatic liver injury (progressive familial intrahepatic cholestasis). Sclerosing cholangitis-like changes in Mdr2–/– mice suggest that MDR3 defects could be involved in adults with PSC. The expression of MDR3 is not altered in acquired forms of cholestasis in humans. MDR3 and BSEP genetic variations are not responsible for the pathogenesis of PBC or PSC.[43] A number of studies of MDR3 in PBC patients demonstrate elevated MDR3 mRNA levels,[9,44,45] while others report normal MDR3 mRNA levels (Figs 2,3).[46,47] MDR3 is induced by bezafibrate in the human liver;[48] MDR3 then increases biliary phospholipid secretions in bile. This process decreases the cytotoxic effect of hydrophobic bile acids on biliary epithelial cells. Clinical studies have shown that combination therapy with bezafibrate and ursodeoxycholic acid (UDCA) improves the biochemical profile of patients with PBC who are non-responsive to UDCA monotherapy.[49] The positive effects of bezafibrate in PBC patients might be due to MDR3 upregulation.

Multidrug resistance-associated protein 2

Multidrug resistance-associated protein 2 (MRP2: ABCC2) mediates ATP-dependent, multispecific organic-anion transport (e.g. bilirubin diglucuronide, sulfates, and glutathione conjugates) into bile; a major determinant of bile salt-independent bile flow by glutathione (GSH) transport. In humans, mutations in MRP2 cause Dubin-Johnson syndrome,[50] a type of hereditary conjugated hyperbilirubinemia. Mutations in MRP2 result in defective excretion of bromsulfophthalein (BSP), indocyanine green (ICG), oral cholecystographic agents, antibiotics such as ampicillin and ceftraxone, heavy metals, and a variety of amphipathic organic anions, including divalent bile acids, conjugated bilirubin, coproporphyrin isomer series 1, and leukotrienes. Studies of MRP2 expression in patients with cholestatic alcoholic hepatitis suggest that MRP2 mRNA is not significantly reduced in this inflammatory disorder.[46] The expression levels of hepatic MRP2 protein and mRNA are also unchanged in PBC patients.[9] In early-stage PBC, MRP2 mRNA levels are elevated; however, the expression of MRP2 is unchanged in late-stage PBC (Figs 2,3).[39]