

—Review Article—

## Autoimmune Cholangitis: Is It an Antimitochondrial Antibody Negative Primary Biliary Cirrhosis?

Katsuhisa OMAGARI

Second Department of Internal Medicine, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan

Autoimmune cholangitis/cholangiopathy (AIC) is a recently described disease characterized by chronic cholangitis resembling primary biliary cirrhosis (PBC) with a high frequency of antinuclear antibodies (ANA) and with sero-negativity for antimitochondrial antibodies (AMA). Whether AIC is a disease entity distinct from PBC and autoimmune hepatitis (AIH) or whether it is an AMA-negative variant of PBC or a cholangiopathic variant of AIH have so far been controversial. We recently examined the specificities of AMA and ANA in Japanese patients with AIC, PBC and AIH by immunofluorescence, immunoblotting and enzyme inhibition assays using various mitochondrial and nuclear autoantigens including 2-oxo-acid dehydrogenase complex, Sp100, gp210, and p62, and found that AIC and PBC had similar patterns of immunoreactivity. However, this duo is of interest because, usually, among sets of autoimmune syndromes, differences in serological targetting are matched by differences in clinical presentation: AIC and PBC seem to be an exception to this rule. While it is true that a single etiological agent can produce a wide range of disease expression, it is possible that seemingly similar clinicopathological features can be induced by pathogenetic mechanisms caused by a diversity of etiological agents.

---

**Key Words:** autoimmune cholangitis, primary biliary cirrhosis, autoimmune hepatitis, antimitochondrial antibody, antinuclear antibody

### Introduction

Autoimmune hepatitis (AIH), primary biliary cirrho-

sis (PBC), and primary sclerosing cholangitis (PSC) are regarded as autoimmune liver diseases. AIH is a chronic necroinflammatory liver disease of unknown etiology and characterized by immunological features, including hypergamma-globulinemia and circulating autoantibodies such as antinuclear antibodies(ANA).<sup>1)</sup> On the other hand, PBC is a chronic, progressive cholestatic liver disease of unknown cause that usually affects middle-aged woman.<sup>2)</sup> Antimitochondrial antibodies (AMA) are found in 95% of patients with PBC, and they have a specificity of more than 98% for this disease.<sup>3)</sup> The distinction between AIH and PBC is in general based on above characteristic clinical, histologic, and immunologic features. However, there have been several reports on liver diseases marked by coexisting features of PBC and AIH.<sup>4)5)</sup>

In this context, considerable interest has been generated in a novel chronic liver disease recognized as autoimmune cholangitis/cholangiopathy (AIC) since the first report of this disease in 1987.<sup>6)</sup> In that report and subsequent studies,<sup>7-12)</sup> the clinical and histopathological features of AIC were characterized as an inflammatory intrahepatic cholangitis with sero-negativity for AMA and a high frequency of ANA. The disease resembles PBC in many respects including survival experience,<sup>12)</sup> although early reports cited a more favorable response to treatment with corticosteroids.<sup>6-8)</sup> Thus, it is unclear at present whether AIC is distinct from PBC<sup>7)9)</sup> or whether AIC is considered as an AMA sero-negative variant of PBC,<sup>10-15)</sup> or a cholangiopathic variant of AIH.<sup>8)16)</sup> Detailed serological specificities of AMA and ANA, tested using recently developed assays, have not been described hitherto in AIC although the study of a single case of AIC led us to surmise that the disease resembled PBC since the serum reacted by immunoblotting with an E2 subunit (50-52 kDa) of one of the lower molecular weight 2-oxo-acid dehydrogenase complexes (2-OADC), antigenic components specific for PBC serum, and

---

**Address Correspondence:** Katsuhisa Omagari, M.D.

Second Department of Internal Medicine,  
Nagasaki University School of Medicine,  
1-7-1 Sakamoto, Nagasaki 852-8501, Japan  
TEL: +81-95-849-7281, FAX: +81-95-849-7285

exhibited by immunofluorescence the multiple nuclear dots and nuclear membrane patterns indicating the presence of anti-Sp100 and anti-gp210.<sup>13)</sup>

### Definition of "autoimmune cholangitis/ cholangiopathy"

The term "immunocholangitis" was introduced first by Brünner and Klinge<sup>6)</sup> in 1987. The authors described a condition seen in three women who had clinical, histological and biochemical liver abnormalities fulfilling the criteria of PBC but were sero-positive for ANA and sero-negative for AMA. The three patients responded well to immunosuppressive treatment with azathioprine and prednisolone. Carrougher et al.<sup>7)</sup> also reported a relatively young woman with clinical, pathologic, and serologic features of both AIH and PBC. The positive ANA, negative AMA, and favorable response to corticosteroid therapy were similar to the cases reported by Brünner and Klinge.<sup>6)</sup> Ben-Ari et al.<sup>8)</sup> described four such patients who were also sero-positive for smooth muscle antibody (SMA).

Subsequent to the above three reports, attention has focused on a subgroup of patients with clinically and histologically diagnosed PBC who are sero-negative for the classical immunofluorescence pattern of AMA but sero-positive for ANA. The subgroup is termed "immunocholangitis",<sup>6)</sup> "autoimmune cholangitis",<sup>9)12)14)17)</sup> "autoimmune cholangiopathy",<sup>8)16)18)</sup> "primary autoimmune cholangitis",<sup>10)</sup> or "immune cholangiopathy".<sup>19)</sup> Ben-Ari et al.<sup>8)</sup> suggested that AIC probably represented a subgroup of type 1 AIH with predominant bile duct damage because the patients responded well to prednisolone therapy, in contrast to those with classical PBC. However, some other reports indicated that AIC can be considered to be the same as AMA-negative PBC since an only consistently distinguishing feature between the two disease entities was the autoantibody (AMA and ANA) profile as both had otherwise virtually identical clinical and histopathologic features.<sup>12)20)</sup> Moreover, Sherlock indicated only a partial response to prednisolone therapy in their subsequent study;<sup>14)</sup> inflammation was reduced but serum gamma-glutamyl transpeptidase level remained at the high levels and bile duct lesions persisted. Furthermore, Colombato et al.<sup>16)</sup> reported a patient with AIC in whom this entity was the result of consecutive typical PBC followed by typical steroid-dependent AIH. Thus, "sero-conversion" from AMA positivity to negativity by immunofluorescence or immunoblotting, or *vice versa*,<sup>21)</sup> is a rare but recognised clinical situation in PBC,<sup>22)</sup> and the clinical

entity of AIC is so far unestablished (Table 1). AIC is not very rare since the frequency of this disease was reported in one study to be 11% among 225 patients with various autoimmune liver diseases including PBC, AIH and primary sclerosing cholangitis,<sup>23)</sup> and 20% among another group of 200 patients who had morphologically consistent features of PBC.<sup>12)</sup>

### Immunologic or serological markers in AIC

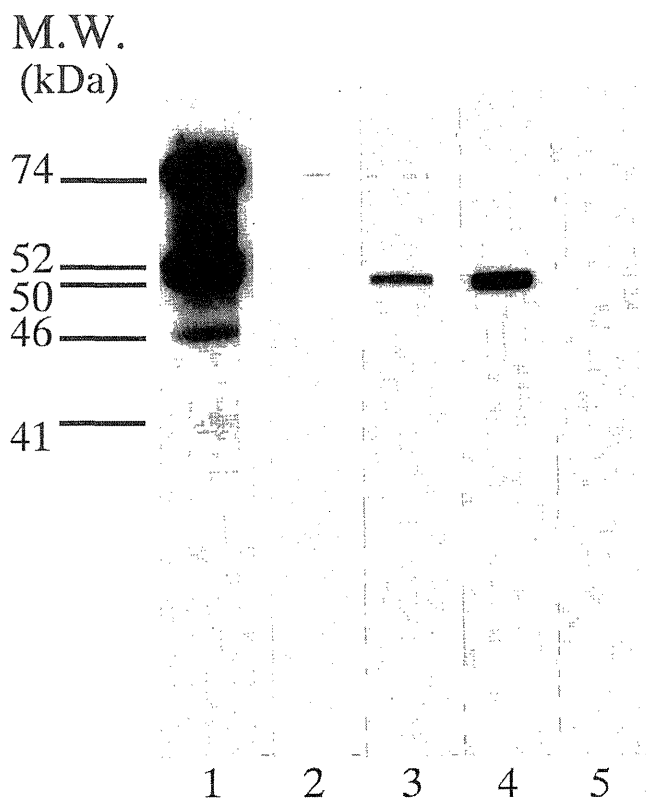
**Table 1.** Clinical features of various autoimmune liver diseases

Criterion	AIH	AIC	PBC	PSC
Target	Hepatocyte	Septal and intra-hepatic bile duct	Septal and intra-hepatic bile duct	Intra- and extra-hepatic bile duct
Sex	Female predominance		Female predominance	Male predominance
Biochemistry	AST, ALT	ALP, $\gamma$ -GTP	ALP, $\gamma$ -GTP	ALP, $\gamma$ -GTP
Autoantibody	ANA	ANA	AMA	p-ANCA ?
Immunoglobulin	IgG	IgG, IgM	IgM	
Histopathology	CAH	CNSDC	CNSDC	Pericholangitis
Treatment	CS	CS ?	UDCA	UDCA ?

AIH: autoimmune hepatitis, AIC: autoimmune cholangitis, PBC: primary biliary cirrhosis, PSC: primary sclerosing cholangitis, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase,  $\gamma$ -GTP: gamma-glutamyl transpeptidase, ANA: antinuclear antibody, AMA: antimitochondrial antibody, p-ANCA: perinuclear-anti-neutrophil cytoplasmic antibody, CAH: chronic active hepatitis, CNSDC: chronic non-suppurative destructive cholangitis, CS: corticosteroid, UDCA: ursodeoxycholic acid

Hitherto, no specific immunologic or serological patterns of reactivity in AIC have been described, except the presence of an antibody to human carbonic anhydrase II (CA-II), one of a family of zinc metal enzymes, reported by Gordon et al.<sup>19)</sup> However, Muratori et al.<sup>18)</sup> reported that the antibody to CA-II seemed to be commonly found in a variety of pathological and normal conditions and was therefore unlikely to be a serological marker specific to AIC. On the other hand, Michieletti et al.<sup>9)</sup> reported that 3 of 20 patients diagnosed as PBC who were AMA-negative and ANA-positive by immunofluorescence showed the positive results of antibodies to 2-OADC enzymes by immunoblotting and ELISA. Of these 3 sera, 2 reacted with pyruvate dehydrogenase complex (PDC), which has been characterized as the immunodominant autoantigen in PBC, and 1 with branched-chain oxo-acid dehydrogenase complex (BCOADC) by immunoblotting. Thus, if AIC is, in fact, a part of the spectrum of PBC, detailed studies on the profile of immunoreactivities with 2-OADC enzymes can be informative. We examined the specificities of AMA and ANA in 21 Japanese patients with AIC, 37 with classical PBC and 16 with AIH by immunofluorescence, immunoblotting and enzyme inhibition assays using various mitochondrial and nuclear

autoantigens including 2-OADC, Sp100, gp210, and p62.<sup>24)</sup> By immunoblotting, in which the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy in our assay condition were 99%, 86%, 89%, 99%, and 93%, respectively,<sup>25)</sup> and notwithstanding the negative result for AMA by immunofluorescence, 5 (24%) of the 21 sera from AIC reacted with the 74kDa component of bovine heart mitochondria (PDC-E2), and 9 (43%) with the lower molecular weight E2 subunits of 2-OADC enzymes (Figure 1). The antinuclear autoantibodies in AIC reacted with centromere, Sp100 and nuclear pore complex proteins as in PBC, but preferentially with the nuclear pore complex. Our results have demonstrated that AIC and PBC are virtually similar diseases.



**Fig. 1** Representative immunoblots from preparation of bovine heart mitochondria probed with PBC serum (lane 1) and AIC sera (lanes 2-5). Sera were used at a dilution of 1:1,000 in lane 2, and 1:5,000 in lanes 1, 3-5. Positive control PBC serum with known reactivity with 2-OADC enzymes at molecular weights (M.W.) of 74 kDa (PDC-E2), 52 kDa (protein X), 50 kDa (BCOADC-E2), 46 kDa (OGDC-E2) and 41 kDa (PDC-E1 alpha) is shown in lane 1. IgG class reactivities of AIC sera are shown with a single protein at M.W. of 74 kDa (PDC-E2) in lane 2, proteins at M.W. of 74 kDa (PDC-E2) and 50 kDa (BCOADC-E2) in lane 3, a single protein at M.W. of 50 kDa (BCOADC-E2) in lane 4, and a non-reactive AIC serum is shown in lane 5.

### Proposed revised categories of "autoimmune cholangitis"

The term "primary biliary cirrhosis" is clearly a misnomer for a disease that has a long precirrhotic stage and "cirrhosis" is a relatively late feature of the disease.<sup>26)</sup> "Chronic nonsuppurative destructive cholangitis" or "(primary) autoimmune cholangitis" would be a more appropriate description, however, "primary biliary cirrhosis" is too entrenched a term to be supplanted. Goodman et al.<sup>12)</sup> and Dhillon<sup>27)</sup> have proposed revised categories of a group of conditions in which chronic nonsuppurative destructive cholangitis is seen histologically. They tentatively divided so called "autoimmune cholangitis" into four groups (Table 2).

### Future work

It remains to be determined whether the identification of the mitochondrial and nuclear antigens reacting with PBC- or AIC-specific autoantibodies will help **Table 2.** Revised categories of "autoimmune cholangitis" proposed by Dhillon<sup>27)</sup>

Serology	ANA (-)	ANA (+)	Total
AMA(+)	Type Ia (classical PBC) 31%*	Type Ib (PBC+AIH overlap syndrome) 37%*	68%*
AMA(-)	Type IIa (seronegative PBC) 12%*	Type IIb (autoimmune cholangiopathy) 20%*	32%*
Total	43%*	57%*	

\*Data from Goodman et al.<sup>12)</sup>

ANA: antinuclear antibody, AMA: antimitochondrial antibody,  
PBC: primary biliary cirrhosis,  
AIH: autoimmune hepatitis

to unravel the pathogenesis of the disease. First, we could direct attention to genetic factors, particularly to human leukocyte antigens (HLA), which may determine the autoantibody response to an etiologic agent, although HLA only weakly predisposes to PBC *vis-a-vis* other autoimmune diseases.<sup>28)</sup> Second, we noted in our previous study<sup>29)</sup> that in Japanese patients with PBC there was a relatively high frequency of reactivity with the lower molecular weight components of 2-OADC such as BCOADC-E2, and this same bias was also evident in Japanese patients with AIC.<sup>24)</sup> It would be interesting to elucidate whether this same trend is also evident in Caucasian patients with AIC. Third, a longitudinal follow-up studies on serological reactivities of AMA and ANA in patients with PBC,

AIC and AIH will be useful since it is well known that in PBC the AMA titres often fluctuate considerably from a negative result to a positive result, and *vice versa*, during the course of the disease.<sup>12)</sup> If so, the diagnosis of PBC or AIC might depend on the "phase" of the same disease. Such studies are in progress in our laboratory. Again, this duo is of interest because, usually, among sets of autoimmune syndromes, differences in serological targetting are relevant to differences in clinical manifestations: AIC and PBC are an exception to this rule.<sup>24)</sup>

### Acknowledgements

The author thanks Drs. Hideki Kinoshita, Isao Matsuo, Kanae Yamaguchi, Kazuo Ohba, Hiroaki Hazama, Jun-ichi Masuda, and Professor Shigeru Kohno, from the Second Department of Internal Medicine, Nagasaki University School of Medicine, for their excellent technical assistance, support and review of this manuscript.

### References

- 1) Krawitt EL. Autoimmune hepatitis. *N Engl J Med* 334: 897-903, 1996
- 2) Kaplan MM. Primary biliary cirrhosis. *N Engl J Med* 335: 1570-1580, 1996
- 3) Omagari K, Rowley MJ, Whittingham S, et al. Autoantibodies to M2 mitochondrial autoantigens in normal human sera by immunofluorescence and novel assays. *J Gastroenterol Hepatol* 11: 610-616, 1996
- 4) Klöppel G, Seifert G, Lindner H, et al. Histopathological features in mixed types of chronic aggressive hepatitis and primary biliary cirrhosis. *Virchows Arch A Pathol Anat Histopathol* 373: 143-160, 1977
- 5) Lindor K, Wiesner RH, LaRusso NF, et al. Chronic active hepatitis: overlap with primary biliary cirrhosis and primary sclerosing cholangitis. In *Chronic active hepatitis. The Mayo Clinic experience* (Czaja AJ, Dickson ER eds.; Marcel Dekker, New York) pp. 171-187, 1986
- 6) Brünner G, Klinge O. A cholangitis with antinuclear antibodies (immunocholangitis) resembling chronic destructive non-suppurative cholangitis. *Dtsch med Wschr* 112: 1454-1458, 1987
- 7) Carrougher JG, Shaffer RT, Canales LI, et al. A 33-year-old woman with an autoimmune syndrome. *Semin Liver Dis* 11: 256-262, 1991
- 8) Ben-Ari Z, Dhillon AP, Sherlock S. Autoimmune cholangiopathy: Part of the spectrum of autoimmune chronic active hepatitis. *Hepatology* 18: 10-15, 1993
- 9) Michieletti P, Wanless IR, Katz A, et al. Antimitochondrial antibody negative primary biliary cirrhosis: a distinct syndrome of autoimmune cholangitis. *Gut* 35: 260-265, 1994
- 10) Taylor SL, Dean PJ, Riely CA. Primary autoimmune cholangitis. An alternative to antimitochondrial antibody-negative primary biliary cirrhosis. *Am J Surg Pathol* 18: 91-99, 1994
- 11) Lacerda MA, Ludwig J, Dickson ER, et al. Antimitochondrial antibody-negative primary biliary cirrhosis. *Am J Gastroenterol* 90: 247-249, 1995
- 12) Goodman ZD, McNally PR, Davis DR, et al. Autoimmune cholangitis: A variant of primary biliary cirrhosis. Clinicopathologic and serologic correlations in 200 cases. *Dig Dis Sci* 40: 1232-1242, 1995
- 13) Omagari K, Ikuno N, Matsuo I, et al. Autoimmune cholangitis syndrome with a bias towards primary biliary cirrhosis. *Pathology* 28: 255-258, 1996
- 14) Sherlock S. Autoimmune cholangitis: A unique entity? *Mayo Clin Proc* 73: 184-190, 1998
- 15) Mohr L, Heintges T, Hensel F, et al. Treatment of autoimmune cholangitis. *Dig Dis Sci* 43: 2160-2163, 1998
- 16) Colombato LA, Alvarez F, Cote J, et al. Autoimmune cholangiopathy: The result of consecutive primary biliary cirrhosis and autoimmune hepatitis? *Gastroenterology* 107: 1839-1843, 1994
- 17) Heathcote J. Autoimmune cholangitis. *Gut* 40: 440-442, 1997
- 18) Muratori P, Muratori L, Lenzi M, et al. Antibodies to carbonic anhydrase in autoimmune cholangiopathy. *Gastroenterology* 112: 1053-1059, 1997
- 19) Gordon SC, Quattrocchi-Longe TM, Khan BA, et al. Antibodies to carbonic anhydrase in patients with immune cholangiopathies. *Gastroenterology* 108: 1802-1809, 1995
- 20) Nakanuma Y, Harada K, Kaji K, et al. Clinicopathological study of primary biliary cirrhosis negative for antimitochondrial antibodies. *Liver* 17: 281-287, 1997
- 21) Horsmans Y, Piret A, Brenard R, et al. Autoimmune chronic active hepatitis responsive to immunosuppressive therapy evolving into a typical primary biliary cirrhosis syndrome: a case report. *J Hepatol* 21: 194-198, 1994
- 22) O'Donohue J, Williams R. Antimitochondrial antibody and primary biliary cirrhosis: can there be one without the other? *J Hepatol* 25: 574-577, 1996
- 23) Czaja AJ. Frequency and nature of the variant syndromes of autoimmune liver disease. *Hepatology* 28: 360-365, 1998
- 24) Kinoshita H, Omagari K, Whittingham S, et al. Autoimmune cholangitis and primary biliary cirrhosis - an autoimmune enigma. *Liver* 19: 122-128, 1999
- 25) Kinoshita H, Omagari K, Matsuo I, et al. Frequency of IgG, IgM, and IgA class autoantibodies against 2-oxo-acid dehydrogenase complex in 102 Japanese patients with primary biliary cirrhosis. *Hepatol Res* (in press)
- 26) Gershwin ME, Mackay I, Coppel R, et al. Clinical, immunologic and molecular features of primary biliary cirrhosis. *Semin Clin Immunol* 7: 5-16, 1994
- 27) Dhillon AP. Autoimmune cholangiopathy and PBC-CAH overlapping syndrome. The classification of primary biliary cirrhosis and its variants. In: Nakanuma Y, ed. *Proceeding of international symposium on liver pathology*. Kanazawa: 40-53, 1997
- 28) Manns MP, Bremm A, Schneider PM, et al. HLA DRw8 and complement C4 deficiency as risk factors in primary biliary cirrhosis. *Gastroenterology* 101: 1367-1373, 1991
- 29) Omagari K, Rowley MJ, Jois JA, et al. Immunoreactivity of antimitochondrial autoantibodies in Japanese patients with primary biliary cirrhosis. *J Gastroenterol* 31: 61-68, 1996