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Abstract
Primary biliary cirrhosis (PBC) is a chronic progressive cholestatic liver disease characterized by immune-mediated destruction of the small- and medium-sized intrahepatic bile ducts and the presence of antimitochondrial antibodies (AMA) in the serum. AMA are detected in over 90% of patients with PBC, whereas their prevalence in the general population is extremely low, varying from 0.16% to 1%. Previous studies have shown that the unique characteristics of biliary epithelial cells undergoing apoptosis may result in a highly direct and very specific immune response to mitochondrial autoantigens. Moreover, recent studies have demonstrated that serum from AMA-positive PBC patients is reactive with a number of xenobiotic modified E2 subunits of the pyruvate dehydrogenase complex, which is not observed in the serum of normal individuals. These findings indicate that chemicals originating from the environment may be associated with a breakdown in the tolerance to mitochondrial autoantigens. While it is currently generally accepted that AMA are the most specific serological markers of PBC, more than 60 autoantibodies have been investigated in patients with PBC, and some have previously been considered specific to other autoimmune diseases. This review covers the recent progress in research on the pathogenetic and clinical significance of important autoantibodies in PBC. Determining the pathogenic role of those autoantibodies in PBC remains a priority of basic and clinical research.

Key words: Primary biliary cirrhosis; Autoantibodies; Anti-mitochondrial antibodies; Anticentromere antibodies; Anti-gp210 antibodies

Core tip: While the presence of antimitochondrial antibodies is pathognomonic to Primary biliary cirrhosis (PBC), more than 60 autoantibodies have been detected in patients with PBC. Antinuclear antibodies (ANA) become positive in approximately 30% to 50% of patients with PBC. Among ANA, anti-gp210 and anticentromere antibodies have been indicated as significant prognostic markers. Previous studies have shown that unique characteristics of biliary epithelial cells during apoptosis may result in the presence of a direct and specific immune response to mitochondrial autoantigens. Moreover, recent studies have indicated that chemicals originating from the environment are associated with a breakdown in the tolerance against mitochondrial autoantigens.

Yamagiwa S, Kamimura H, Takamura M, Aoyagi Y. Autoantibodies in primary biliary cirrhosis: Recent progress in research on the pathogenetic and clinical significance. World J Gastroenterol 2014; 20(10): 2606-2612 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i10/2606.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i10.2606
INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic progressive cholestatic liver disease characterized by immune-mediated destruction of small- and medium-sized intrahepatic bile ducts and the presence of antimitochondrial antibodies (AMA) in serum of affected patients\(^1,2\). PBC is considered a model autoimmune disease on the basis of several features, including the presence of a highly direct and very specific immune response to mitochondrial autoantigens, female predominance, and homogeneity among patients\(^3\). Despite the fact that the mitochondrial targets are ubiquitous proteins expressed in all nucleated cells, the immunopathology of PBC is characterized by the presence of CD4\(^+\) and CD8\(^+\) T-cell infiltrates in the liver and targeted destruction of biliary epithelial cells (BECs)\(^4,5\). This suggests that BECs may have unique immunological characteristics. While it is currently accepted that AMA are the most specific serological markers of PBC, more than 60 autoantibodies have been investigated in PBC patients, some having previously been considered specific for other autoimmune diseases\(^6,7\).

The immune system has an extraordinary capacity of preventing self-antigens from provoking an inflammatory reaction. Therefore, the presence of autoantibodies is the consequence of a breakdown in or complete failure of B cell tolerance to corresponding autoantigens\(^7\). However, antibodies against self-antigens are also found in cancer, during massive tissue damage, and even in healthy subjects. Natural autoantibodies (NAAs) are immunoglobulins produced at tightly regulated levels in the complete absence of external antigenic stimulation\(^7\). Although NAAs have long been considered a sign of a breakdown in tolerance, these antibodies appear to play an important role in the innate immune system as a first line of defense against pathogens as well as in the prevention of autoimmune diseases\(^8,9\). It was recently demonstrated that NAAs bind to apoptotic cells and thereby facilitate their uptake by dendritic cells, thus preventing activation of the adaptive immune system by molecules released upon apoptosis, which may trigger autoimmune events\(^5,10\). Considering the many nonspecific autoantibodies are detected in patients with PBC, we speculate that these antibodies may be NAAs that are not associated with the development of autoimmunity. Interestingly, the majority of NAAs are of the IgM type, with a smaller proportion of IgG and IgA types\(^11\). The elevated serum IgM level, which is a well-known characteristic of PBC, may reflect such an increase of IgM-type NAAs, although recent studies have stressed that environmental factors also play a major role\(^12,13\). However, the importance of innate immunity, including NAAs, in autoimmune responses has been only recently appreciated, and its role remains to be clarified. The present review focuses mainly on recent progress in studies of the pathogenetic and clinical significance of the more significant autoantibodies detected in PBC patients.

ANTIMITOCHONDRIAL ANTIBODIES

AMA are detected in over 90% of patients with PBC, whereas their prevalence in the general population is extremely low, varying between 0.16% and 1%, and only reaching 8% in hepatitis C virus (HCV)-infected patients\(^13\). AMA seropositivity is a strong predictor for the development of PBC. Mitchison et al\(^14\) found that only two of 29 AMA-positive healthy patients had a normal liver histology pattern. Ten years later, 76% of those patients had developed clinical signs and symptoms of PBC\(^14,15\). Thus, despite their high predictive value, AMA are not useful for prognostication in terms of clinical course of PBC. Moreover, most studies indicate that AMA levels are unaffected by treatment\(^13\).

The autoantigens of AMA have been identified as the E2 subunits of the 2-oxo-acid dehydrogenase complexes, including the E2 subunits of the pyruvate dehydrogenase complex (PDC-E2), branched chain 2-oxo acid dehydrogenase complex, 2-oxo-glutarate dehydrogenase complex, and the E3 binding protein of dihydrolipoamide dehydrogenase\(^16\). The AMA target antigens are all localized within the inner mitochondrial matrix and catalyze the oxidative decarboxylation of 2-oxo-acid acid substrates\(^17\). In approximately 95% of patients, AMA are directed towards the 74 kD mitochondrial polypeptide identified as PDC-E2. During apoptosis of BECs, PDC-E2 remains immunologically intact without being glutathiolated, and becomes the source of the PDC-E2 apotope. The term apotope specifies an epitope created during the processes of apoptosis\(^18\). PDC-E2 contained within apoptotic bodies can be recognized by circulating AMA, and the resulting apotope-AMA complex then stimulates the innate immune systems in genetically susceptible individuals\(^21\). Immune destruction is restricted to BECs due to their unique physiology and is exacerbated by retention of PDC-E2 in apoptotic blebs resulting from apoptosis of BECs\(^21\). Several previous studies of the liver in PBC patients have reported accentuated apical expression of PDC-E2 in BECs, and induction of PDC-E2 cell-surface expression during BEC autophagy\(^22\). The accumulation of autophagic vacuoles appears to be critical for the cell-surface expression of PDC-E2, and such expression may play a role in antigen presentation on BECs, followed by autoimmune-mediated cell injuries of BECs\(^23\).

Association between xenobiotic and antimitochondrial antibodies

Although bacteria and viruses may induce PBC through molecular mimicry, other environmental factors, xenobiotics, or chemical compounds foreign to a living organism may exert similar effects\(^18\). Previous analysis of the specificity of anti-PDC-E2 has revealed a number of anti-PDC-E2 antibody subpopulations that recognize either the PDC peptide, PDC peptide conjugated with lipoic acid, or lipoic acid itself\(^23,24\). The immunodominant...
epitope of PDC-E2 is the lipoylated domain, and the lipoic acid residue attached to AMA epitopes is necessary for autoantibody binding\cite{24}. Moreover, recent studies involving quantitative structure-activity relationship analysis have demonstrated that serum from AMA-positive PBC patients, but not from controls, is reactive with a number of xenobiotic modified PDC-E2 structures\cite{25}. These findings indicate that chemicals of environmental origin may be associated with the breakdown of tolerance to mitochondrial autoantigens.

**ANTINUCLEAR ANTIBODIES**

Antinuclear antibodies (ANA) are serological markers that can be found in a wide variety of liver diseases (drug-induced, viral, alcoholic hepatitis, nonalcoholic steatohepatitis, AIH, PBC, and PSC) and non-hepatic autoimmune diseases (Hashimoto thyroiditis, systemic lupus erythematosus, Sjögren syndrome), as well as in a subgroup of healthy individuals, though usually at a low titer levels\cite{38}. Antinuclear indirect immunofluorescence patterns are characterized by anti-multiple nuclear dots, rim-like/membrane antibodies and less specific anticentromere antibodies (ACA). The molecular target of anti-multiple nuclear dots is the protein sp100 and the promyelocytic leukemia (PML) protein\cite{39-40}. The antirim-like/membranous antibodies are mainly represented by the protein glycoprotein (gp)-210\cite{12,27}. Muratori et al\cite{20} demonstrated that ANA were present in almost 50% of patients with PBC and that their prevalence reached 85% in AMA-negative sera. Specifically, 27% of patients had anti-sp100, 16% had anti-gp210, and 16% had anticientromere antibodies. In other reports, the prevalence of ANA in PBC patients has been approximately 30%-50%\cite{6,36}. Although the significance and predictive value of ANA have been confirmed in other autoimmune diseases such as type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosus, and inflammatory bowel disease, the predictive significance of ANA in PBC remains unclear\cite{20,39}.

**Anticentromere antibodies**

In patients with calcinosis cutis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia (CREST) syndrome or limited cutaneous systemic sclerosis, ACA positivity may be as high as 50%-90%. Although they are not specific to systemic sclerosis, ACA are usually associated with a good prognosis for that condition\cite{12,38}. In patients with PBC, the ACA positivity rate is approximately 30%-60\cite{6,36}. Nakamura et al\cite{34} conducted a retrospective multi-center cohort study of 276 patients with biopsy-confirmed PBC, and found that positivity for ACA was a significant risk factor for the development of portal hypertension. The findings from histological observations indicate that the presence of ACA is most significantly associated with a relatively severe ductular reaction\cite{31}. In PBC, the production of cytokines or growth factors from inflammatory cells, rather than retention of bile constituents, is potentially critical for the induction of ductular reaction\cite{32}. In chronic hepatitis, this type of ductular reaction is also known to promote fibrosis via the production of transforming growth factor-β, monocyte chemoattractant protein-1, and platelet-derived growth factor by proliferating ductular epithelia\cite{33,34}. Therefore, it is reasonable to speculate that more severe ductular reaction may play a crucial role in the progression to portal hypertension in PBC patients who are ACA-positive. However, the mechanism of bile duct damage appears to lead to chronic cholestasis and development of biliary cirrhosis, even in ACA-positive patients\cite{35,36}.

**Antinuclear pore antibodies (gp210)**

A number of nuclear antigens have been recognized as targets of ANA in patients with PBC, including several components of the nuclear pore complex, such as the gp210 and p62 proteins\cite{41}. Gp210 is an integral glycoprotein of the nuclear pore consisting of three main domains: a large glycosylated luminal domain, a single hydrophilic transmembrane segment, and a short cytoplasmic tail. Gp210 is recognized by antibodies in approximately 25% of patient with PBC\cite{6,36}. The association between the presence of anti-gp210 antibodies and the outcome of PBC was first reported by Itoh et al\cite{42}, and subsequently confirmed by several additional studies\cite{38-40}. Ishibashi et al\cite{33} and Nakamura et al\cite{34} reported that PBC patients with consistently high levels of anti-gp210-C terminal peptide antibody have a higher risk of progression to end-stage hepatic failure than do those without such antibodies, or those in whom antibody levels are initially positive but drop to low levels after treatment with ursodeoxycholic acid. These reports demonstrate that antibodies against nuclear pore complexes, especially gp210-C terminal peptides, may serve as important surrogate predictors of PBC progression to end-stage hepatic failure. It has been shown that the presence of anti-gp210 antibodies was most significantly associated with more severe interface hepatitis and lobular inflammation\cite{31}. Furthermore, there was a tendency for relatively more severe ductopenia (ductular reaction) in late stages of disease anti-gp210-positive patients\cite{31}. These findings suggest that two main processes - bile duct destruction and interface hepatitis - are more severe in PBC patients positive for anti-gp210 antibodies compared to those negative for anti-gp210 antibodies; thus, leading to more frequent progression to end-stage hepatic failure\cite{31}. Nakamura et al\cite{34} found that expression of gp210 antigens was increased on the nuclear envelope of epithelial cells in small bile ducts in the liver of PBC patients and that the intensity of gp210 staining by immunofluorescence was positively correlated with the intensity of inflammation around small bile ducts. These observations indicate that gp210 may be a target antigen, reactivity against which plays an important role in both bile duct destruction and interface hepatitis.
Antinuclear dot antibodies (Anti-sp100)

Sp100 was discovered in the context of leukemic transformation and as an autoantigen in PBC[^43]. Sp100 antigen is a 480 amino acid peptide with a calculated molecular weight of 53 kDa that shows aberrant electrophoretic mobility to 100 kDa[^44]. The prevalence of anti-sp100 antibodies in patients with PBC is approximately 25%. Anti-sp100 antibody, which has a specificity of 94%, has an important diagnostic role in PBC, particularly in AMA-negative patients[^39,45,46]. However, anti-sp100 antibodies have been increasingly found in many other autoimmune diseases, including systemic lupus erythematosus and systemic sclerosis. Interestingly, among PBC patients, approximately 74% of those with urinary tract infections are positive for anti-sp100, whereas the positivity rate is only 4.8% in those without such infections[^47]. Given the high specificity of anti-sp100 as an immunoserological hallmark of PBC, these findings support the hypothesis that some bacterial infections might be involved in the induction of PBC-specific autoimmunity.

### OTHER AUTOANTIBODIES IN PBC

Other autoantibodies against nuclear constituents (dsDNA, ssDNA, histone, sel-70, Sm, SSA-SSB, RNP, J0-1, U1RNP) have also been detected in PBC, mostly in conjunction with rheumatic co-morbidities[^41]. Anti-p97/VCP (valosin containing protein) antibodies are detected in approximately 12.7% of PBC cases, and their presence suggests a less progressive disease course and a benign prognosis[^48-50]. Eosinophil peroxidase (Anti-EPO) antibodies have been detected in 52.5% of patients with PBC and 29.0% of patients with AIH. PBC patients who were positive for anti-EPO antibodies had a significantly smaller number of peripheral eosinophils than did patients who were anti-EPO negative[^51]. As mentioned above, more than 60 autoantibodies have been detected in PBC patients, but some are not specific for any disease, while some are thought to be more closely related to other autoimmune diseases[^5]. Among those autoantibodies, the more significant autoantibodies detected in PBC patients are summarized in Table 1[^6,13,46-68]. A comprehensive review of these autoantibodies in PBC was published by Hu et al[^3] in 2010.

### Line immune assay

Previous studies of autoantibodies in PBC have primarily focused on only a single type of autoantibody, whereas the positivity pattern of different autoantibodies in a single serum sample, as well as their clinical significance, have not been elucidated. A line immune assay (LIA) kit that can simultaneously measure different autoantibodies known to be involved in autoimmune disease has recently become available and has been employed in clinical practice. Using a LIA kit that can detect 9 autoantibodies against AMA-M2, M2-3E (a fusion protein of the E2 subunits of alpha-2-oxoacid dehydrogenases of the inner mitochondrial membrane), sp100, PML, gp210, Ro-52, LKM-1 (liver-kidney microsomes-1), LC-1 (cytosolic liver-pancreas antigen), Saito et al[^68] examined the prevalence and positivity pattern of those autoantibodies in 80 patients with PBC, 40 patients with AIH, and 16 patients with PBC-AIH overlap. They found that the prevalence of positivity for anti-sp100, anti-PML, anti-gp210, anti-Ro-52, and ACA were 13.8%, 8.7%, 40%, 27.5%, and 32.5% in PBC, respectively. In the PBC-AIH overlap group, the prevalence of both anti-gp210 (68.7%) and anti-Ro-52 (81.2%) were significantly higher than those in the PBC and AIH groups. The authors concluded that LIA is useful for the diagnosis of PBC and PBC-AIH overlap, although AMA-M2 should be measured by the conventional ELISA-based method, as LIA is less sensitive than ELISA in detecting AMA[^68].

### CONCLUSION

We have provided an overview of the recent developments in the general understanding of the pathogenetic and clinical significance of the autoantibodies that significantly impact PBC. Although there have been substantial advances, determining the pathogenic role of autoantibodies in PBC remains a priority of basic and clinical research.
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