REVIEW ARTICLE

Autoantibodies in liver disease: important clues for the diagnosis, disease activity and prognosis

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Abstract It has been well established that numerous kinds of autoantibodies have been detected in liver disease. Some kinds of autoantibodies may be helpful in the diagnosis of autoimmune liver diseases including autoimmune hepatitis, primary biliary cirrhosis or primary sclerosing cholangitis. However, these autoantibodies are present even in sera of patients with viral hepatitis, drug-induced hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease and hepatocelluar carcinoma as well as in sera of patients with autoimmune liver diseases. Other kinds of autoantibodies are recognized as predictive hallmarks for disease activity or prognosis in liver diseases. On the other hand, treatment with interferon initiates the production of several types of autoantibodies in patients with chronic hepatitis C virus infection. Some of autoantibodies induced by interferon may postulate the treatment outcome in those patients. Recent studies also revealed the close correlation between oxidative stress and the production of autoantibodies in liver diseases. This article primarily reviews the recent advances of autoantibodies in the liver diseases and discusses the clinical significance of these autoantibodies.

Keywords Autoantibodies · Autoimmune liver diseases · Diagnosis · Disease activity · Prognosis

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Introduction

Autoantibodies are produced by humoral immune responses against self-cellular proteins and nucleic acids [1], and have been well established as serological hallmarks of autoimmune disease. Numerous autoantibodies have been isolated from sera of patients with liver diseases. These autoantibodies have often clinical values for the diagnosis, disease activity and/or prognosis. Such autoantigens are primarily engaged in essential cellular functions including DNA replication, DNA transcription, and RNA processing [2].

Autoantibodies in the field of liver disease are mainly classified into two entities: (1) non-organ specific autoantibodies including antinuclear antibodies (ANA), smooth muscle antibodies (SMA), antimitochondrial antibodies (AMA), and antibodies to liver kidney microsome type-1 (LKM-1), (2) liver-specific autoantibodies such as antibodies to soluble liver antigen (SLA) and antibodies to asialoglycoprotein receptor (ASGPR) [3]. The presence of these autoantibodies are essential in the process of diagnosing autoimmune liver diseases, including autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC). However, some of these autoantibodies are occasionally detected even in the sera of patients with liver disease other than autoimmune liver diseases [3–6]. Recent advances in proteomic or microarray analyses have led to the identification of novel autoantigens from patients with AIH [7, 8] or hepatocellular carcinoma (HCC) [9], although the clinical relevance of antoantibodies to the novel autoantigens remains obscure.

In addition, the relationship between insulin resistance in obese human and several types of autoantibodies has been shown. For example, thyroid autoantibodies are frequently found in obese females [10]. Another study

revealed that insulin resistance was closely related to distinct profiles of IgG in an experimental animal model of obesity [11].

Novel concepts have also been proposed in autoimmune liver diseases. IgG4-associated AIH, a new entity which is characterized by a higher IgG4 concentration and IgG4-bearing plasma cells, was proposed by Umemura et al. [12]. However, no peculiar autoantibodies associated with the elevation of IgG4 levels have, thus, far been found among the patients with IgG4-associated AIH. Th17-cells, which arise in the presence of transforming growth factor-beta (TGF-β) and interleukin-6 (IL-6), play important roles in the pathogenesis of autoimmune diseases. However, the putative mechanism by which Th17 cells contribute to the production of autoantibodies remains unclear in autoimmune liver disease [13]. Moreover, Czaja and colleagues [14] proposed a new concept, "autoantibody-negative autoimmune hepatitis". However, most of patients with autoantibody-negative AIH may have unknown autoantibodies that are still undiscovered or have delayed appearance of the conventional autoantibodies.

This review mainly focuses on the current interpretation and potential application of autoantibodies in liver diseases.

Autoantibodies in liver diseases

Table 1 summarizes the numerous types of autoantibodies in the field of liver disease. These autoantibodies are detected in the sera of patients with viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), drug-induced hepatitis, and HCC as well as autoimmune liver diseases. These autoantibodies are rarely present even in the normal healthy control. Some autoantibodies are essential to the diagnosis [3–6], while others have clinical significance for judging the prognosis [15, 16].

Mechanisms of autoantibody production

Impaired regulatory T cells and NKT cells

Regulatory T cells (Treg) are considered to serve as the maintenance of immunological self-tolerance and prevention of autoimmunity. On the other hand, natural killer T (NKT) cells, a T cell subset that co-expresses the NK cell marker, regulate immune system by inducing apoptosis of altered hepatocytes. A decrease in the number and function of Treg [17, 18] and NKT cells [19] eventually leads to the facilitation of type 2 cytokine (IL-4, IL-10) responses

which expand the clone of plasma cells, resulting in the promotion of autoantibody production [20]. These phenomena have already been confirmed in patients with AIH and PBC.

Elevated BAFF levels

B-lymphocyte activating factor (BAFF), a member of the tumor necrosis factor superfamily, is mainly secreted from myeloid cells (macrophages, monocytes, and dendritic cells) and plays crucial roles in the maturation and survival of B cells. Therefore, the elevation of serum BAFF levels contributes to the polyclonal activation of B cells. Serum BAFF levels were closely related to the titers of AMA in patients with PBC [21] and antibodies to cardiolipin in patients with hepatitis C virus (HCV) infection [22].

Molecular mimicry

Molecular mimicry between autoantigens and infectious agents including several kinds of bacteria and viruses may occasionally trigger the autoantibody production. The HCV polyprotein has a molecular mimicry with cytochrome p450 2D6 (CYP2D6), a target antigen of antibodies to LKM-1, and the cross-reactivity initiates the emergence of anti-LKM-1 in patients with chronic HCV infection [23].

Molecular mimicry and immunological cross-reactivity between Escherichia coli (E.coli) and human PDC-E2, a major target antigen of AMA, result in the production of AMA in patients with PBC [24]. Exposure to chemical xenobiotics including 2-nonynoic acid can also trigger the production of AMA in PBC patients [25].

Innate immunity

Recent studies reveal that the hyper-responsiveness of innate immunity is frequently involved in the pathogenesis of PBC. Exposure to CpG, a ligand to TLR9, resulted in B cell activation and the subsequent facilitation of AMA production [26].

Genetic factors

Genetic predisposition of the host may account for the production of autoantibodies in liver diseases. For example, SMA and high titers of ANA were associated with human leukocyte antigen (HLA)-DR4 in patients with type 1 AIH [27]. We also revealed that antibodies to double-stranded DNA (ds-DNA) were also related to HLA-DR4 in ANA-positive patients with AIH [28].

Table 1 Clinical significance of autoantibodies in liver disease

Autoantibodies	Molecular target	Associated liver diseases	Clinical significance
ANA	Histone	Type 1 AIH, PBC	H3-predominant in type 1 AIH
	Chromatin	Type 1 AIH	Higher IgG levels
	dsDNA	Type 1 AIH	Diseases activity, unfavorable prognosis
	Centromere	PBC, type 1 AIH, HCV (rare)	Complementry marker for PBC in AMA-negative case
	hnRNP	Type 1 AIH	Specific to type 1 AIH
SMA	Actin	Type 1 AIH	Diagnostic marker for type 1 AIH, unfavorable prognosis
	Myosin	HCV	
	Vimentin		
	Tublin	Alcoholic liver diseases	
	Desmin		
Anti-LKM1	CYP2D6	Type 2 AIH, HCV	Diagnostic marker for type 2 AIH
	CYP2A6	HCV (rare)	
Anti-LKM2	CYP2C9	Tienilic acid-included hepatitis	
Anti-LKM3	UGT1A2	HDV, HCV (rare)	
Anti-LC1	Formiminotransferase cyclodeaminase	Type 2 AIH, HCV	Diagnostic marker for type 2 AIH
Anti-SLA/LP	UGA supressor tRNA-associated protien	Type 1 AIH, HCV (rare)	Disease activity, relapse after drug withdrawal
pANCA	Actin	Type 1 AIH	
	HMG	Type 1 AIH	
	Lactoferrin	Type 1 AIH, PBC, PSC, HCV	
	50 kD myeloid-specific nuclear envelope protien	PSC, type 1 AIH	
Anti-ASGPR	Asialoglycoprotein receptor	Type 1 AIH, PBC, HBV, HCV, HDV drug-induced hepatitis	Diseases activity, relapse after drug withdrawal
AMA	PDC-E2	PBC, AIH (rare), HCV (rare)	Diagnostic marker for PBC
	BCOAD-E2, OGDC-E2	PBC	Specific to PBC
tTGA	Tissue transglutaminase	Celiac disease	Disease activity
ASCA	*Saccharomyces cerevisiae*	Type 1 AIH, PBC, PSC, celiac disease	Correlation with IgA levels
Anti-GST	Glutathione S-transferase	Type 1 AIH	Disease activity, unfavorable prognosis
Anti-GW/PB	GW/PB	PBC	
Anti-CRP	CRP	HCV	Predictive marker for concurrent mixed cryoglobulinemia

Diagnostic and prognostic values of autoantibodies in liver disease

Antinuclear antibodies (ANA)

Diagnostic value

The presence of ANA and/or smooth muscle antibodies (SMA) requires the diagnosis of type 1 AIH, the classical type of AIH [29]. However, ANA is also present in the sera of patients with other autoimmune liver diseases including PBC and PSC, and even in the sera of patients with viral hepatitis, drug-induced hepatitis, NAFLD, alcoholic liver disease, and HCC [3–6].

ANA is usually assessed by the indirect immunofluorescent (IIF) method using HEp-2 cells. The target antigens of ANA in type 1 AIH contain a heterogeneous group of structures, such as nuclear DNA, nuclear structural and functional proteins or centromeres [30]: different immunofluorescent staining types including homogeneous, speckled, nucleolar and discrete speckled patterns are shown on HEp-2 cells [31]. We previously revealed that the most common immunofluorescent staining type in patients with AIH type 1 was a homogeneous pattern [31]. Notably, heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1, which belongs to RNA-binding protein, was recently identified by Ballot's group as one of the liver-specific nuclear antigens in type 1 AIH [32]. hnRNP A2/B1 is

largely engaged in the maturation of mRNA precursor and in the transport of mRNA to the cytosolic compartment.

On the other hand, several kinds of ANA specific for PBC have been well studied. These PBC-specific ANA can be mainly divided into two groups by the immunofluorescent pattern on HEp-2 cells: the rim-like membranous pattern and the multiple nuclear dots pattern [33]. ANA showing the rim-like pattern on HEp-2 cells are largely directed against to nuclear pore complexes (gp210 [34] and nucleoporin p62 [35]), while ANA exhibiting multiple nuclear dot pattern are directed against to nuclear body proteins including sp100 [36], promyelocytic leukemia (PML) protein [37], small ubiquitin-related modifiers (SUMO) [38], and more recently sp140 [39] (Table 2). The specificity of anti-gp210 was estimated for more than 96 %, although the prevalence of the antibody ranged from 9.4 to 41.2 % of patients with PBC [40].

ANA were present in approximately 10–40 % of patients with HCV-related chronic liver disease (CLD) [41–44]. Molecular mimicry between viral and self antigens may trigger the immunological cross-reaction. Gregirio and colleagues documented molecular mimicry between HCV polyprotein and matrin, histone H2A or replication protein A [45].

Clinical significance in predicting disease activity and/or concurrent autoimmune diseases

Antibodies to chromatin are directed against the complex of histones and DNA. These antibodies were found in 20–50 % of patients with type 1 AIH [46, 47]. AIH patients with anti-chromatin had a biochemical characteristic of higher IgG levels [46, 47].

Antibodies to histone were present in 35 % of ANA-positive patients with AIH [48]. IgG type antibodies to histone in sera of patients with AIH showed dominant reactivity against H3 among individual histones (H1, H2A, H2B, H3 and H4), while the reactivity in patients with PBC was predominant against H1 [49]. Unfortunately, anti-histone were not associated with disease activity in patients with AIH. However, the titer of anti-H3 was decreased by the treatment with corticosteroid in proportion to the serum ALT level in each individual [49].

In contrast, antibodies to dsDNA were detected in less than half of patients with type 1 AIH [28, 50]. The emergence of anti-dsDNA may reflect higher IgG levels and more frequent failures to the treatment with corticosteroid in patients with AIH. On the other hand, Muratori and colleagues [51] demonstrated that concomitant anti-dsDNA and AMA were the serological profile of AIH/PBC overlap syndrome.

Recently, we revealed that anticentromere antibodies were present in eight (17 %) of 47 patients with type 1 AIH [52]. Those patients with anticentromere antibodies had clinical characteristics of lower serum IgG levels than those with other immunofluorescent staining patterns of ANA, although no significant difference in histological activities between the two groups was found.

ANAs specific to PBC can conveniently predict the disease activity or a concurrent infection. Antibodies to gp210 were associated with severe interface hepatitis lobular inflammation [53]. The frequency of anti-sp-100 in patients with PBC and recurrent urinary tract infection was higher than that in patients with PBC without urinary tract infection [54]. It is of interest that Rigopoulou and colleagues [55] revealed that the IgG3 isotype of ANA specific to PBC was associated with more severe biochemical and histological activities.

The detection of antibodies to SS-A/Ro implied concurrent Sicca syndrome in patients with PBC [56, 57], while anticentromere antibody was associated with CREST syndrome in those patients [57, 58]. Moreover, antibodies to SS-A/Ro52 were predictive markers for a more advanced histological stage at the diagnosis of PBC [57].

ANA status also affected the disease severity in patients with HCV-related CLD. Those patients with ANA frequently had more advanced fibrosis and necroinflammation in the liver than those without ANA [43, 59]. Those patients with ANA had biochemical and immunological characteristics of higher serum alkaline phosphatase and γ-GTP levels, indicating bile duct lesions, as well as ALT and IgG levels [42, 60], and the most commonly speckled pattern on HEp-2 cells by the IIF method [60]. These clinical characteristics were independent of HCV genotypes and loads of HCV-RNA [59, 61].

Table 2 Prognostic values of PBC-specific ANA

Immunofluorescent pattern	Nuclear antigens	Clinical significance
Rim-like/membranous	gp210 nucleoporin/p62 lamin B receptor	Progression to liver failure advanced liver stage
Multiple nuclear dots	Sp100	Recurrent urinary tract infection
	Sp140	Coexistence with anti-Sp100
	PML	Coexistence with anti-Sp100
Discrete speckled	CENP-B	Progression to portal hypertension

Recent studies have revealed that around approximately 20–30 % of patients with non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH) also had ANA [62–64]. One of the studies showed that high titers of ANA, not SMA, in NAFLD were tightly associated with insulin resistance [62]. In general, patients with NASH, who were seropositive for ANA also seemed to have more severe fibrosis and necroinflammation in the liver than those with NASH, who were seronegative for ANA [63].

Prognostic value

The subtype of ANA detected in the sera of patients with PBC exhibits a variety of prognoses in PBC. Antibodies to gp210 might be a predictive factor for the progression to liver failure [65].

The emergence of antibodies to Ro52 alone or in combination with antibodies to SLA in patients with type 1 AIH represented an unfavorable prognosis [66].

Antibodies to chromatin are also recognized as a candidate forecasting relapse after withdrawal of the treatment in patients with AIH [47].

On the other hand, PBC patients with anticentromere antibodies were previously considered to have mild liver injury and show a favorable prognosis [67]. However, recent articles revealed that anticentromere antibodies have been the risk factors for the development of portal hypertension in those patients [65]. Moreover, we previously implied that patients with HCV-related CLD seropositive for anticentromere antibodies more frequently progressed to HCC [68].

Smooth muscle antibodies (SMA)

Diagnostic value

SMA is directed against structures of the cytoskeleton such as microfilaments (actin and myosin), intermediate filaments (vimentin and desmin), and microtubules (tubulin) [69]. In type 1 AIH, SMA are predominantly directed against filamentous (polymerized) actin (F-actin) [70]. Therefore, antibodies to F-actin (anti-F-actin) are serological hallmarks specific to type 1 AIH [71]. The analysis of anti-F-actin is usually performed by an indirect IIF method using fibroblasts, which have peculiar stress fibers [72]. Phalloidin-treated rat hepatocytes have been also used as substrates for IIF, which were stained with a polygonal pattern on the hepatocytes [73]. However, the assay for anti-F-actin gives controversial results because of a conformational change of actin epitopes [72]. Moreover, the IIF procedure for detecting anti-F-actin is somewhat complicated, and interpretation of the results depends on the operator's experience. Recently, a commercially available kit using the VSM 47 cells line, which is obtained from the thoracic aorta of rat embryo, as a novel substrate enabled us to analyze anti-F-actin more easily [74].

Other methods for the detection of anti-F-actin include immunoprecipitation, ELISA, protein binding assay, and passive hemoagglutination and immunoblot techniques [71, 73]. The trouble is that a gold standard method for anti-F-actin have not yet been established. However, a commercially available ELISA kit for detecting antibodies against F-actin was recently developed. The detection of anti-F-actin using this kit had almost the same specificity but higher sensitivity in the diagnosis of type 1 AIH, compared with the IIF technique [75, 76].

Anti-F-actin are likely to recognize a specific epitope corresponding to the α-actinin-binding domain. Gueguen and colleagues emphasized the importance of analyzing both anti-actin and anti-α-actinin antibodies in patients with type 1 AIH [77]. One-third of sera with type 1 AIH reacted simultaneously with anti-F-actin and anti-α-actinin. Moreover, antibodies to α-actinin were frequently associated with antibodies to single stranded DNA [77, 78].

10–20 % of patients with HCV-related CLD also had SMA [41–44]. However, SMA in most sera of patients with HCV-related CLD lacked in the reactivities with F-actin [43, 59]. Non-muscle myosin seems to be a candidate for a target antigen of SMA in those patients [79].

Clinical significance in predicting disease activity

AIH patients with anti-F-actin had the clinical characteristics of younger onset than those without anti-F-actin [80]. However, the emergence of anti-F-actin did not necessarily reflect more advanced staging. In contrast, double reactivity with F-actin and α-actinin defined a severe form of type 1 AIH [77].

Approximately, 30 % of patients with AIH/PBC overlap syndrome had SMA [51, 81]. Seropositivity for SMA is one of the components for the Paris criteria, which is an international diagnostic criterion for AIH/PBC overlap syndrome proposed by Chazouillerres et al. [82].

Patients with HCV-related CLD seropositive for SMA had more severe interface hepatitis than those seronegative for SMA [83]. However, the emergence of non-organ-specific autoantibodies (NOSA), including ANA, SMA and anti-LKM1, did not necessarily affect the anti-viral treatment in patients with CH-C [43, 84].

Prognostic value

Czaja and his colleagues [80] elucidated that type 1 AIH patients seropositive for anti-F-actin developed liver failure and required liver transplantation more frequently than

those seronegative for anti-F-actin. In fact, we recently revealed that anti-F-actin were associated with relapse in AIH patients after withdrawal of treatment with corticosteroid [85]. On the other hand, anti-α-actinin can be recognized as an independent predictor of a poor responder to corticosteroid [86].

Antibodies to liver kidney microsomes (anti-LKM)

Diagnostic value

Autoantibodies to liver kidney microsomal proteins (anti-LKM) are closely associated with several kinds of liver diseases including type 2 AIH, drug-induced hepatitis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), HCV and HDV infections [87]. Anti-LKM are directed against the proximal renal tubules and cytoplasms of hepatocytes [88].

The antibody to LKM type 1(anti-LKM1) is a major serological hallmark of type 2 AIH [89], but is also found in some sera of patients with chronic hepatitis C (CH-C) [90]. The major target antigen of anti-LKM1 was identified as cytochrome monooxygenase p450 IID6 (CYP2D6) [91]. The most immunodominant epitope of CYP2D6 was the C-terminal portion of the molecule [92]. The amino acid (aa) sequence between 316 and 327, which was exposed on the surface of the molecule, appeared to be the region capable of distinguishing type 2 AIH from HCV infection [93].

Recently, molecular mimicry between the aa sequence 252–271 of CYP2D6 and HCV E1 was shown [94]. The cross-reactivity took place in patients with chronic hepatitis C, who possessed the HLA B51 allotype [94]. Moreover, CYP2A6, CYP2E1 and CYP3A4 were identified as novel target autoantigens of anti-LKM1 in patients with chronic hepatitis C seropositive for anti-LKM1, respectively [95]. Therefore, a heterogeneous autoimmune reaction may take place in patients with chronic hepatitis C seropositive for anti-LKM1.

Antibodies to LKM type 2 (anti-LKM2) have been detected in the sera of patients with tienilic acid-induced hepatitis [96]. CYP2C9 has been identified as the target antigen of anti-LKM2. The binding of an active metabolite to CYP2C9 becomes antigenic and eventually leads to the production of these autoantibodies. Antibodies to LKM type 3 (Anti-LKM3) are primarily present in the sera of patients with HDV infection, and are directed against uridine diphosphate glucuronosyltransferase (UGT) [96].

Clinical significance in predicting disease activity and concurrent disease

The clinical characteristics of type 2 AIH are quite different from those of type 1 AIH. AIH patients with anti-LKM1 are younger at disease onset, and show more severe necroinflammation of the liver including higher levels of serum bilirubin and transaminases [89].

A recent article elucidated that the emergence of anti-LKM1 reduced the CYP2D6 metabolic activity in patients with CH-C seropositive for anti-LKM1 [97]. On the other hand, it is remarkable that CH-C patients with anti-LKM1 were frequently associated with autoimmune thyroiditis [98, 99].

Antibodies to soluble liver antigen (anti-SLA)

Diagnostic value

Antibodies to soluble liver antigen (anti-SLA) were originally identified as a serological marker for type 3 AIH [100]. The molecular target of anti-SLA has been established as a 422 amino acid protein, which is a transfer ribonucleoprotein involved in selenocysteine insertion (tRNP(ser)sec) and recently renamed SEPSECS [Sep (O-phosphor-serine) tRNA:Sec (selenocysteine) tRNA synthase] by the Nomencluster Commission of the Human Genome Organization [20]. Anti-SLA turned out to be identical to the antibody to liver pancreas (anti-LP) [101]. The isotypes of anti-LP were previously investigated among patients with AIH. A predominant type of anti-LP in patients with AIH was the IgG1 type. None of the patients with AIH had IgG3 and IgG4 types of anti-LP [102].

Clinical, serological, and genetic studies have elucidated that patients with AIH seropositive for anti-SLA did not define a distinct subgroup of AIH, but, rather belonged to type 1 AIH [103, 104]. It is of importance that the presence of anti-SLA is highly specific to type 1 AIH, although anti-SLA are detected in the sera of only 15 % of North American patients with type 1 AIH [16]. Furthermore, a strong association of DRB1*0301 with anti-SLA has been reported as the genetic background in patients with AIH [105].

Anti-SLA was rarely detected in patients with CH-C, type 2 AIH and PSC [106, 107]. Vitozzi and colleagues [108] revealed that the prevalence of anti-SLA was increased when anti-LKM1 was present in patients with HCV-related CLD.

Clinical significance in predicting disease activity

Several articles confirmed that AIH patients with anti-SLA displayed a more severe clinical course, and they required longer duration of treatment or had higher frequency of liver transplantation than AIH patients without anti-SLA [106, 109].

Prognostic value

Anti-SLA can be the most promising serological markers to identify patients with AIH who will experience a relapse after withdrawal of the treatment with corticosteroid [16, 110]. Therefore, patients with AIH seropositive for anti-SLA at presentation may have difficulties in the immunosuppressive treatment.

Antimitochondrial antibodies (AMA)

Diagnostic value

AMA is considered to be the diagnostic hallmark of PBC, because the autoantibodies are detected in up to 90–95 % of patients with PBC [111]. AMA recognizes the highly conserved regions of 2-oxoacid dehydrogenase complexes (2-OADC), which exist in the inner membrane of mitochondria. The major autoantigen of 2-OADC turns out to be the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2) [112]. AMA selectively reacts with the E2 subunit of the branched chain 2-oxoacid dehydrogenase complex (BCOADC-E2), the E2 component of the 2-oxoglutarate dehydrogenase complex (OGDC-E2), the dihydrolipoamide dehydrogenase (E3)-binding protein (E3BP), and the E1α subunit of the pyruvate dehydrogenase complex (PDC-E1α) [113]. Therefore, analysis of the IgA and IgG types of AMA by the ELISA technique using recombinant MIT3 antigen, which contains the three immunodominant epitopes PDC-E2, BCOADC-E2 and OGDC-E2 increases the specificity of PBC [114].

Mucosal immunity contributes to the pathogenesis of PBC. Tanaka and colleagues [115] revealed that the IgA type of AMA was detected in 57 (69 %) of 83 patients with PBC, and that most of these AMA belonged to secretory-type IgA. Positive correlation of IgA class anti-OGDC with histopathological stage was also shown [116]. Another report documented that transcytosis of IgA was mediated by the polymeric immunoglobulin receptor (pIgR), and that co-localization of PDH-E2 and IgA was observed in epithelial cells transfected with pIgR [117]. These data support the hypothesis that IgA-PDC-E2 immune complexes are transported to the biliary epithelial cells by the way of pIgR. Furthermore, Matsumura and colleagues [118] provided evidence that transcytosis of AMA-IgA caused caspase activation, resulting in apoptosis of bile duct epithelia.

AMA is also present in the sera of patients with AIH. Nezu and colleagues [119] documented that AMA was found in 14 of 41 (34 %) patients with type 1 AIH by immunoblotting using beef heart mitochondria proteins as the antigens. However, serological parameters including ANA profile and biochemistry, and histological findings such as bile duct lesions were almost similar regardless of AMA status.

According to the report by Ramos-Casals' group, AMA was detected in 18 (8 %) of 237 patients with HCV-related CLD [120]. However, only 5 (28 %) of 18 patients exhibited histological findings compatible with PBC. Twelve (67 %) of these 18 patients had concomitant autoimmune diseases including Sjögren's syndrome, systemic sclerosis and systemic lupus erythematosus (SLE). Four (22 %) of 18 patients proceeded with neoplasia including HCC.

Clinical significance in predicting disease activity

Titers of AMA do not appear to be correlated with disease severity in patients with PBC [121]. There was an interesting article on the comparison of histological activities between AMA-positive and AMA-negative patients with PBC. AMA-negative patients with PBC exhibited more severe bile duct damages. This damage was associated with the infiltration of CD20-positive B cells into the liver [122]. On the other hand, ductular invasion of CD5-positive cells was more frequently observed in the portal area of the liver from AMA-negative patients with PBC [122].

Aric and his colleagues [123] investigated the clinical significance of AMA in patients with AIH/PBC overlap syndrome. Nine of 21 patients with AIH/PBC overlap syndrome had AMA. ANA and/or SMA were present in the sera of those patients without AMA. Those patients without AMA had more severe bile ductular damage and hepatic fibrosis.

IgG subclasses of AMA were also analyzed in the sera from patients with PBC. The IgG3 subtype of AMA defined more advanced disease activity and more frequent liver cirrhosis [124]. Kawai and his colleagues documented the correlation between the IgG2 subclass of AMA and the innate immune response against bacterial particles [125].

Strikingly, Leung et al. [126] revealed the presence of autoantibodies against PDC-E2, BCOAD-E2, and OGDC-E2 during acute liver failure. However, these autoantibodies immediately disappeared after the recovery of hepatic reserve. These findings support the hypothesis that liver damage caused by oxidative stress may trigger the production of AMA.

Antineutrophil cytoplasmic antibodies (ANCA)

Diagnostic value

ANCA are directed against the cytoplasmic constituents of neutrophilic granulocytes. These autoantibodies are largely divided into two groups by the staining pattern on ethanol-fixed granulocytes as substrates by the IIF method:

perinuclear pattern (pANCA) and cytoplasmic pattern (cANCA) [127]. Classical pANCA were often detected in the sera of patients with microscopic polyangiitis, and myeloperoxidase has been identified as their target antigen. On the other hand, cANCA are commonly found in the sera of patients with Wegener's granulomatosis, and primarily recognize proteinase 3.

pANCA are present in patients with PSC and type 1 AIH [128]. Actin [129] or high mobility group (HMG) non-histone proteins [130] were regarded as candidates for the target antigens of pANCA in patients with type 1 AIH. pANCA in patients with PSC exhibits an atypical staining pattern that has heterogeneous rim-like staining of the nuclear periphery and multiple intra-nuclear spots [131]. Terjung and colleagues [132] identified a 50-kD nuclear envelope protein of myeloid cells as a novel target antigen of pANCA which showed atypical perinuclear staining.

A recent study documented that the IgG subclasses of pANCA in patients with PSC were IgG1 and IgG3 isotypes, while that in patients with type 1 AIH was IgG1-dominant [133]. pANCA in patients with PSC was neutrophil-specific, whereas that in patients with type 1 AIH reacted with neutrophils and monocytes [133] The IgA type of pANCA was observed in autoimmune liver disease [134]. The IgA type of pANCA had no disease-specificity, although the prevalence of IgA class pANCA was higher in patients with type 1 AIH than in patients with PSC. Recently, Terjung et al. [135] elucidated that p-ANCA in autoimmune liver disease were directed against human beta tubulin isotype 5 as well as bacterial precursor protein FtsZ, which has proposed a novel concept on the potential role of microorganisms in the autoimmune liver disease.

Clinical significance in predicting disease activity

It is of interest that Roozendaal and his colleagues [136] elucidated that ANCA were associated with decreased serum albumin and increased alkaline phosphatase levels in patients with PSC, suggesting that the presence of ANCA in patients with PSC may predict severe disease activity.

Autoantibodies to asialoglycoprotein receptors (anti-ASGPR)

Diagnostic value

Asialoglycoprotein receptor (ASGPR) is a transmembrane glycoprotein, which was previously identified as a liver-specific autoantigen [137]. Antibodies to ASGPR (anti-ASGPR) are frequently found in the sera of patients with type 1 AIH, although some patients with PBC, viral hepatitis and alcoholic liver disease have these autoantibodies, indicating that these antibodies lack in the specificity for

type 1 AIH [138, 139]. However, anti-ASGPR have diagnostic value in patients with AIH, who are seronegative for conventionally tested autoantibodies including ANA, SMA, anti-LKM1, and anti-SLA [140]. These antibodies are mainly detected by an EIA, a RIA or an immunoblot method. However, a standardized assay for detection of anti-ASGPR has not yet been determined [141]. Recently, a commercially available ELISA kit using purified rabbit ASGPR as a target antigen was developed, and the efficacy of the kit was shown by Hausdorf et al. [142].

Clinical significance in predicting disease activity

AIH patients with anti-ASGPR had higher IgG levels and more severe histological activity than those without anti-ASGPR [141, 143]. A recent study also revealed that the titers of anti-ASGPR were associated with serum ALT levels, suggesting that these antibodies can be monitored for a disease activity [142].

Prognostic value

The presence of anti-ASGPR at presentation might predict relapse after drug withdrawal in patients with AIH [139, 144]. The persistence or disappearance of these antibodies often reflected the adequacy of the treatment response in those patients.

Autoantibodies to tumor-associated antigens (anti-TAA)

Diagnostic value

Numerous tumor-associated antigens (TAAs), including p53, c-myc, cyclin B1, IGF-II mRNA binding proteins (IMPs) and survivin, have been identified from the patients with HCC (Table 3) [145]. Most of these TAAs are primarily engaged in essential cellular function including DNA replication, DNA transcription, and pre-mRNA splicing and translation [146].

We previously examined the prevalence of antibodies to p53, c-myc, survivin and IMPs in the sera of patients with HCC. Eight of 86 (9.3 %) sera of HCC patients had one or more of these autoantibodies [147]. It is of interest that the serum level of alpha-fetoprotein (AFP), the tumor marker for HCC, remained within normal limits in 7 of 8 HCC patients with anti-TAA, suggesting that the antibodies to TAA are complimentary serological markers for HCC. It is notable that HCC patients with circulating anti-IMPs had overexpression of IMPs in the tumor cells, indicating that these autoantibodies to TAAs were produced by an antigen-driven immune system [148]. Moreover, the titers of anti-TAA were often increased by the development of

Table 3 Tumor-associated antigens identified from patients with HCC and their functions

Intracellular component	Autoantigens	Biological functions
Nucleus	DNA topoisomerase II	DNA replication/transcription
	CENP-F	Mitotic function
	HCC-1	mRNA splicing
	Cyclin B1	Cell-cycle progression
	Fibrillarin	rRNA processing
	NOR-90/hUBF	RNA pol I transcription
	B23	Ribosome maturation
	Ku86	DNA doule-strand break repair
Cytoplasm	p62/IMP2	Regulation of IGF-II mRNA
	Koc/IMP3	Regulation of IGF-II mRNA
	Golgi	Processing, transporting and sorting of intracellular proteins

HCC [149]. We also revealed that antibodies to p53 and IMP3 in a patient with HCC were detected prior to the clinical diagnosis of HCC [147].

A recent article by Nomura and colleagues [150] demonstrated that circulating antibodies to Ku 86, a DNA-binding nuclear autoantigen, were potential biomarkers for HCV-related HCC. The titers of anti-Ku86 had a tendency to be associated with the severity of Ku 86 expression in the tumor cells. Furthermore, the titers of anti-Ku86 seemed to be independent of the serum AFP levels in patients with HCC.

Antibodies to p53 (anti-p53) are also found in patients who are suffering from autoimmune disease including SLE, type 1 diabetes mellitus, and chronic thyroiditis [151, 152]. The efficacy of anti-p53 was shown in discriminating AIH patients from PBC patients [153, 154]. Interestingly, anti-p53 was associated with anti-dsDNA, suggesting that DNA damage may trigger the production of anti-p53 [154].

Antibodies to stress proteins

Clinical significance in predicting disease activity

Degenerated proteins initiated by oxidative stress can be immunogenic. Recently, oxidatively modified autoantigens, including oxidized low-density lipoprotein (ox-LDL) and 8-oxodeoxyguanine, were identified from patients with SLE [155].

Hepatic steatosis is one of the common metabolic abnormalities caused by HCV-induced oxidative stress [156]. We recently elucidated that titers of antibodies to ox-LDL were significantly associated with the severity of hepatic steatosis in patients with CH-C [157], indicating that the autoimmune response was involved in the process of hepatic steatosis (Table 4). However, we failed to show the correlations between titers of anti-ox-LDL and the severity of insulin resistance in patients with HCV-related CLD. Vidali and his colleagues also demonstrated that titers of antibodies to malondialdehyde (MDA) in severe steatosis were significantly higher than those with mild or no steatosis among patients with CH-C [158].

CYP 2E1 is an endoplasmic monooxygenase and major source of oxidative stress in microsomes [159]. Overexpression of CYP 2E1 is well recognized in the liver of patients with NASH [160] and CH-C [161] as well as alcoholic liver disease [162]. The hepatic expression of CYP 2E1 is related to the severity of hepatic steatosis in those patients [161]. Recently, approximately 40 % of patients with CH-C had antibodies to CYP2E1 [163]. The molecular mimicry between HCV NS5B and CYP2E1 may trigger the production of these autoantibodies in patients with CH-C [164]. It is of interest that the emergence of anti-CYP 2E1 was independent of hepatic steatosis in CH-C patients [163]. However, CH-C patients with antibodies to CYP 2E1 had more severe necroinflammation in the liver than those without anti-CYP2E1 [163]. Another study revealed the efficacy of antibodies to CYP 2E1 in patients with drug-induced patients. Njoku and his colleagues [165]

Table 4 Autoantibodies to stress proteins in liver disease

Autoantibodies	Molecular target	Associated liver disease	Clinical significance
Anti-ox-LDL	ox-LDL	HCV	Correlation with hepatic steatosis
Anti-MDA	MDA	HCV, NASH	Correlation with hepatic steatosis
Anti-CYP2E1	CYP2E1	Type 1 AIH, HCV, alcohol, halothane-induced hepatitis	
Anti-cardiolipin	Cardiolipin	Type 1 AIH, PBC, NASH, alcohol, HCV HCV	IgG4 type of anti-CYP2E1 correlation with hepatic fibrosis
Anti-SOD	SOD	AIH, HCV	Correlation with anti-HSP65
Anti-HSP	HSP65	AIH	
	HSP70	HCV	Inverse correlation with disease-activity

elucidated that the IgG4 type of antibodies to CYP 2E1 were specific to the patients with anesthetic-induced hepatitis.

Antibodies to cardiolipin (anti-cardiolipin), the serological hallmark of antiphospholipid antibodies syndrome, are detected in the sera of patients with type 1 AIH, PBC, PSC, HBV- and HCV-related CLD, and alcoholic liver disease [166–169]. However, most of those patients with anti-cardiolipin lacked in the category for the antiphospholipid antibody syndrome [168].

The association between anti-cardiolipin and oxidative stress was previously shown in patients with CH-C [169]. Patients with HCV infection seropositive for anti-cardiolipin developed more advanced hepatic fibrosis than those seronegative for anti-cardiolipin [169], as patients with AIH seropositive for anti-cardiolipin did [168]. The emergence of anti-cardiolipin might imply concurrent oral lichen planus in patients with CH-C [170], although it remains controversial.

Miyata and his colleagues [171] exhibited that patients with AIH had significantly higher titers of antibodies to superoxide dismutase (SOD) than patients with CH-C, or SLE. They suggested that the autoimmune response derived from molecular mimicry between human SOD and mycobacterial heat shock protein (HSP) 65, because titers of anti-SOD were correlated with titers of anti-HSP65. Another study reported that antibodies to HSP70 were also detected in 14 (24 %) of 59 patients with CH-C [172]. The article showed that titers of anti-HSP70 were inversely correlated with serum ALT levels and loads of HCV-RNA.

Interferon-induced autoantibodies

Diagnostic value

It has been well recognized that treatment with interferon-α (IFN-α) or pegylated IFN-α initiates various kinds of autoimmune diseases [173]. Therefore, CH-C patients treated with IFN-α occasionally develop autoimmune diseases including autoimmune thyroid disease (Graves' disease and Hashimoto's thyroiditis), type 1 diabetes mellitus (DM) and type 1 AIH [174]. Approximately 10 % of CH-C patients had thyroid autoantibodies including thyroid microsome antibodies, thyroglobulin antibodies and antibodies to thyroid peroxidase during treatment with IFN-α/pegylated IFN-α [175, 176]. Antibodies to glutamic acid decarboxylase (GAD) were also detected in 1–3 % of CH-C patients during treatment with IFN-α/Pegylated IFN-α, respectively [176]. The emergence of these autoantibodies was restricted to the specific HLA haplotypes. Patients who developed Type 1 DM were genetically restricted to HLA DRB1-DQB1 haplotype [177], while IFN-induced autoimmune thyroiditis had genetic susceptibility to HLA A2 in the Japanese population [178], or HLA DRB1*11 in the Caucasian population [179].

Parana and his colleagues [180] documented that antibodies to the Golgi complex appeared in three patients with CH-C during treatment with pegylated IFN. The authors paid attention to the progressive disease in those patients with anti-Golgi complex.

Recently, a novel autoantibody induced by pegylated IFN and ribavirin was found in sera of patients with CH-C. This antibody was named antibodies to cytoplasmic rods and rings, which consisted of rod-like cytoplasmic structures and rings [181, 182]. However, the clinical relevance of this autoantibody remains uncertain. Vaquez-Del Mercado and his colleagues [183] speculated that antibodies to argonaute 2 (Su antigen), a miRNA-binding protein, were also induced by IFN.

Clinical significance in predicting the outcome of anti-viral treatment

NOSA including ANA, SMA and anti-LKM1 at base line did not generally affect the outcome of anti-viral treatment in patients with CH-C [43, 84]. However, ANA at the end of treatment, or an increase in titers of SMA during the treatment can predict the unfavorable outcome in CH-C patients [184].

The emergence of antibodies to thyroid peroxidase at base line can predict the progression to IFN-induced thyroid disease in patients with CH-C [185].

Conflict of interest Takashi Himoto and Mikio Nishioka have no conflict of interest to declare.

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