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CLINICAL, BIOLOGICAL AND PHARMACOLOGICAL KEYNOTES

Auto-antibodies in autoimmune hepatitis: Anti-smooth muscle antibodies (ASMA)

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Anti-smooth muscle antibodies (ASMA) with F-actin specificity are commonly regarded as specific markers of type 1 autoimmune hepatitis (AIH-1). They were included in the revised criteria of the International Autoimmune Hepatitis Group (IAIHG) [1] and then in the simplified diagnostic score in 2008 [2]. ASMA are present in 85% of patients with AIH-1 [3]. Unfortunately, they are found in a variety of liver and non-liver diseases and their usefulness as diagnostic markers depends on their actin or non-actin specificity [4]. Indirect immunofluorescence (IIF) on frozen sections of rat liver, kidney and stomach represents the standard technique to detect ASMA. However, a gold standard method for the detection of anti-F-actin specificity is not yet available. The scoring system for the diagnosis of AIH-1 does not included anti-F-actin antibodies [5].

In this keynote, we provide a synthesis of the main findings concerning ASMA.

Background and anti-smooth muscle antibodies (ASMA) detection

ASMA were first described by Johnson et al. in 1965 [6]. They belong to a heterogeneous group of antibodies that react with protein subunits of different types of cytoplasmic filaments (microfilaments, microtubules or intermediate filaments). An association between ASMA and antibodies to F-actin microfilaments in a context of autoimmune hepatitis (AIH) was first established in 1973 [7]. The predominant epitope has been identified as the α-actinin-binding domain on the carboxy-terminal part of F-actin [8].

IIF on rat tissue sections is the best screening method for ASMA. The classic pattern as described in 1976 by Bottazzo [9] is shown in Fig. 1a. In the stomach, the muscle layer and vascular axis of the lamina propria of the gastric mucosa were stained; in the liver, the submembrane actin of hepatocytes was stained according to the classic ''honeycomb'' pattern, while in the kidney, three types of IIF were defined: ASMA-V (vessels) when sera stained the vessels walls exclusively, ASMA-G (glomeruli), and ASMA-T (peritubular) when the sera recognized the vessels walls, the mesangial cells of the glomerulus and peritubular fibrils. ASMA staining patterns more specific to type 1 autoimmune hepatitis (AIH-1) are ASMA-G and ASMA-T. However, in cases of ASMA positivity, it is necessary to confirm F-actin specificity using a specific method such as Elisa, dot blot or IIF on different cell lines. When compared to IIF, Elisa seems to be less specific, false positive results being reported in patients with pathologies other than AIH-1. By IIF, anti-F-actin can be detected using cultured human fibroblasts, HEp-2 cells, colchicine-treated HEp-2 cells (Fig. 1b), vascular smooth muscle (VSM 47) cell lines and, more recently, a rat intestinal epithelial cell line (Fig. 1c) [5,10—13].

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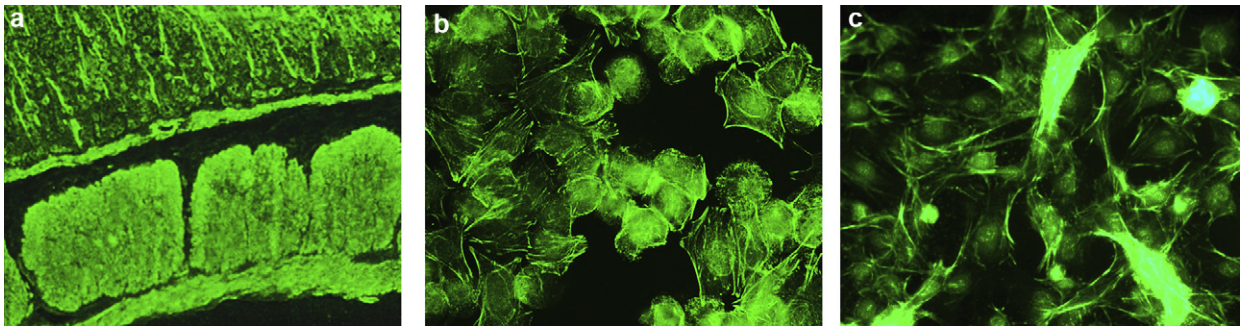

Figure 1 Immunofluorescence pattern of anti-smooth muscle antibodies (ASMA); a: in the rat stomach (× 100); b: in colchicine-treated Hep2 cells (× 400); c: rat intestinal epithelial cell line (× 400).

A diagnostic marker of type 1 autoimmune hepatitis (AIH-1)

In AIH-1, ASMA are detected in up to 85% of patients, either alone or in conjunction with antinuclear antibodies (ANA) [3,14]. The ASMA titre can contribute to IAIHG diagnostic score in patients with a probable or definite diagnosis of AIH [1,2]. These antibodies have also been reported in 33% to 65% of cases of PBC/AIH overlap syndrome [5,15], the concomitant presence of ASMA and AMA being highly suggestive of this setting

This high sensitivity is, however, associated with low specificity. ASMA have been reported in the context of other liver diseases (viral hepatitis, drug-induced hepatitis, alcohol-induced liver disease, hepatocellular carcinoma), and also in non-liver diseases [14].

Anti-F-actin sensitivity (43—82%) has been shown to be lower than that of ASMA, but its specificity for AIH-1 (76—95%) was higher [5,10,16], the highest specificity being observed among young patients [4]. However, anti-F-actin antibodies may be found in numerous disease settings, including viral infection, connective tissue diseases or celiac disease, etc. [4].

Prognostic value of anti-smooth muscle antibodies (ASMA)

The prevalence of ASMA has not been shown to be correlated with the clinical or histological severity of AIH-1 at diagnosis [17]. Only one study [18] suggested that anti-F-actin reactivity was strongly associated with HLA-B8 and DR3 alleles, and with a worse prognosis. However, in paediatric AIH-1 cases, Gregorio et al. found a positive correlation between the ASMA titre and AST levels, suggesting a potential use of ASMA to monitor disease activity [19]. In adult and paediatric AIH-1 patients, Granito et al. showed that anti-F-actin was associated with higher γ-globulin and IgG levels, but the presence of these antibodies did not correlate with any other clinical, biochemical, histological or immunogenetic parameters, suggesting that anti-F-actin does not appear to identify a particular subset of the disease among AIH-1 patients [10].

Anti-smooth muscle antibodies (ASMA) and the response to treatment

No correlation has been demonstrated between antibody status and response to immunosuppressive therapy [17]. A disappearance of ASMA is observed during immunosuppressive therapy in most patients with AIH-1. However, neither their titre at diagnosis, nor any fluctuations in their levels during the disease, are thought to predict disease course and outcome after the withdrawal of corticosteroid therapy [14,20].

Anti-smooth muscle antibodies (ASMA) in the aetiopathogenesis of autoimmune hepatitis

The role of auto-antibodies in the pathogenesis of autoimmune liver damage has been suggested by the finding that hepatocytes isolated from AIH patients are coated with immunoglobulins and are the target of antibody-dependent, cell-mediated cytotoxic attack [21]. Interestingly, during early studies, actin closely associated with the liver plasma cell membrane was demonstrated to be responsible for the *in vitro* binding of IgG to the surface of isolated hepatocytes [19]. However, ASMA are a diagnostic rather than a prognostic marker, so this finding indicates that ASMA do not appear to be involved in the pathogenesis of liver injury in a context of AIH [20].

In summary

ASMA with F-actin specificity alone or associated with ANA and/or anti-SLA, are of major diagnostic importance in AIH-1, particularly in young female patients and those suffering from overlap syndromes with cholestatic diseases. However, ASMA may also be found in other diseases, but mostly at low titres.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References

[1] Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. International autoimmune hepatitis group

report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999;31:929—38.
[2] Hennes EM, Zeniya M, Czaja AJ, Parés A, Dalekos GN, Krawitt EL, et al. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology 2008;48:169—76.
[3] Obermayer-Straub P, Strassburg CP, Manns MP. Autoimmune hepatitis. J Hepatol 2000;32:181—97.
[4] Chretien-Leprince P, Ballot E, Andre C, Olsson NO, Fabien N, Escande A, et al. Diagnostic value of anti-F-actin antibodies in a French multicenter study. Ann N Y Acad Sci 2005;1050:266—73.
[5] Villalta D, Bizzaro N, Da Re M, Tozzoli R, Komorowski L, Tonutti E. Diagnostic accuracy of four different immunological methods for the detection of anti-F-actin auto-antibodies in type 1 autoimmune hepatitis and other liver-related disorders. Autoimmunity 2008;41:105—10.
[6] Johnson GD, Holborow EJ, Glynn LE. Antibody to smooth muscle in patients with liver disease. Lancet 1965;2:878—9.
[7] Gabbiani G, Graeme BR, Lamelin JP, Vassalli P, Majno G, Bouvier CA, et al. Human smooth muscle auto-antibody. Its identification as anti-actin antibody and a study of its binding to nonmuscular cells. Am J Pathol 1973;72:473—88.
[8] Gueguen P, Dalekos G, Nousbaum JB, Zachou K, Putterman C, Youinou P, et al. Double reactivity against actin and alpha-actinin defines a severe form of autoimmune hepatitis type 1. J Clin Immunol 2006;26:495—505.
[9] Bottazzo GF, Florin Christensen A, Failax A, Iwana G, Doniach D, Groeschel-Stevart U. Classification of smooth muscle auto-antibodies detected by immunofluorescence. J Clin Pathol 1976;29:403—10.
[10] Granito A, Muratori L, Muratori P, Pappas G, Guidi M, Cassani F, et al. Antibodies to filamentous actin (F-actin) in type 1 autoimmune hepatitis. J Clin Pathol 2006;59:280—4.
[11] Soares A, Cunha R, Rodrigues F, Ribeiro H. Smooth muscle auto-antibodies with F-actin specificity. Autoimmun Rev 2009;8:713—6.
[12] Muratori L, Muratori P, Granito A, Pappas G, Cassani F, Lenzi M. Current topics in autoimmune hepatitis. Dig Liver Dis 2010;42:757—64.
[13] Toh BH, Taylor R, Pollock W, Dearden S, Gill CC, Buchner C, et al. Actin-reactive discriminated from non-actin-reactive smooth muscle auto-antibody by immunofluorescence reactivity with rat epithelial cell line. Pathology 2010;42: 463—9.
[14] Bogdanos DP, Invernizzi P, Mackay IR, Vergani D. Autoimmune liver serology: current diagnostic and clinical challenges. World J Gastroenterol 2008;14:3374—87.
[15] Muratori P, Granito A, Pappas G, Pendino GM, Quarneti C, Cicola R, et al. The serological profile of the autoimmune hepatitis/primary biliary cirrhosis overlap syndrome. Am J Gastroenterol 2009;104:1420—5.
[16] Frenzel C, Herkel J, Lüth S, Galle PR, Schramm C, Lohse AW. Evaluation of F-actin Elisa for the diagnosis of autoimmune hepatitis. Am J Gastroenterol 2006;101:2731—6.
[17] Mehendiratta V, Mitroo P, Bombonati A, Navarro VJ, Rossi S, Rubin R, et al. Serologic markers do not predict histologic severity or response to treatment in patients with autoimmune hepatitis. Clin Gastroenterol Hepatol 2009;7:98—103.
[18] Czaja AJ, Cassani F, Cataleta M, Valentini P, Bianchi FB. Frequency and significance of antibodies to actin in type 1 autoimmune hepatitis. Hepatology 1996;24:1068—73.
[19] Gregorio GV, McFarlane B, Bracken P, Vergani D, Mieli-Vergani G. Organ and non-organ specific auto-antibody titres and IgG levels as markers of disease activity: a longitudinal study in childhood autoimmune liver disease. Autoimmunity 2002;35:515—9.
[20] Dalekos GN, Zachou K, Liaskos C, Gatselis N. Auto-antibodies and defined target auto-antigens in autoimmune hepatitis: an overwiew. Eur J Int Med 2002;13:293—303.
[21] Longhi MS, Ma Y, Mieli-Vergani G, Vergani D. Aetiopathogenesis of autoimmune hepatitis. J Autoimmun 2010;34:7—14.