Editorial

Liver Membrane Autoantibodies: Methods for Their Detection and Clinical Significance

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There is a growing evidence suggesting that immunological reactions against liver membrane antigens are involved in the process of ongoing hepatic injury in chronic active liver diseases (CALD). Liver specific lipoprotein (LSP) has been described as one of the target antigens of the liver cell membrane. Autoantibodies against LSP (anti-LSP) are predominantly directed against organ-specific determinants of a low density LSP subfraction (1.08-1.10 g/ccm). Anti-LSP detected in acute viral hepatitis are mainly directed against species-specific, whereas those in chronic active hepatitis are mainly directed against non-species-specific determinants of the LSP complex. Further liver membrane autoantibodies were found to react with additional antigens of the hepatocellular membrane (LM-Ag). These liver membrane autoantibodies (LMA) are disease-specific for autoimmune type CALD. The detection of liver membrane autoantibodies by indirect immunofluorescence on isolated rabbit hepatocytes (LMA-test) is clinically important since this subgroup of CALD does well respond to immunosuppressive therapy. It is still unknown, however, whether these liver membrane autoantibodies are really involved in the immunopathogenesis of CALD via antibody dependent cellular cytoxicity (ADCC) or whether they are a phenomenon secondary to liver cell destruction. Nevertheless they represent diagnostic markers by which one can classify subgroups of CALD with different etiologies. A further characterization of liver membrane antibody-antigen systems could lead to a more precise evaluation of the clinical relevance of liver membrane autoantibodies.

(Key Words: Hepatitis, Liver Cell Membrane, Autoantibodies, Liver Specific Protein)

INTRODUCTION

It is well known that several autoantibodies are present in the sera of patients with chronic inflammatory liver diseases. Antinuclear antibodies (ANA) are very frequently found in autoimmune type chronic active hepatitis (CAH) (34). Furthermore smooth muscle antibodies (SMA), antimitochondrial antibodies (AMA), antibodies to a microsomal antigen of liver and kidney (57), antiribosomal antibodies (8) and bile canalicular antibodies (5) are also found in chronic hepatitis. Of interest is the finding that AMA are characteristically found in primary biliary cirrhosis (PBC) and also in the cholestatic subtype of CAH (2).

These autoantibodies, except some types of AMA, are neither organ- nor disease-specific. It is conceivable that these autoantibodies are not involved in the pathogenesis of inflammatory liver diseases. They are not
directed against plasma cell membrane antigens, a criterion that should be fulfilled by target antigens of pathogenetic significance. A great attention has been paid to the liver specific lipoprotein (LSP) (30). LSP is a macromolecular protein present on the surface of the liver cell membrane. It was found to have at least one organ-specific membrane-associated determinant (9). Although autoantibodies against liver cell membrane antigens initially proved to be difficult to demonstrate, recent technical progress has enabled some laboratories to detect these antibodies in patient sera. These autoantibodies may be involved in the immunological process of ongoing hepatic destruction in inflammatory liver diseases (35).

This review deals with the clinical and immunopathological significance of autoantibodies against liver cell membrane antigens.

1. Characterization of liver membrane antigens

Before discussing the autoantibodies against liver membrane antigens we would like to mention the properties of liver membrane antigens so far reported in the literature.

1.1 The liver specific lipoprotein (LSP)

The liver specific lipoprotein (LSP) is a macromolecular protein present on the liver cell membrane. LSP shows organ-specificity and incomplete species-specificity. Human LSP demonstrates antigenic crossreactivity with LSP similarily prepared from livers of other mammalian species. It was originally prepared from fresh liver by homogenization, followed by ultracentrifugation, gel filtration on Sephadex G 100 and finally on Sephadex G 200. Concentrated first peak of Sephadex G 200 was used for immunization of rabbits. After the absorption of the antisera with human kidney homogenate, human plasma and blood cells these antisera reacted only with the macromolecular protein fraction from liver but not with equivalent fractions from other organs (30). Studies with antisera prepared in different species led to the conclusion that the macromolecular protein fraction contains a protein with an organ-specific determinant which was originally termed LP I. In later studies this determinant was found to be membrane-associated (9). Mc Farlane et al (27) described a further purification of the LP I containing liver protein fraction by Sepharose 6B chromatography, using a Tris buffer system containing 1mM EDTA. In this buffer, the antigen was stable and could be used for further investigations. Since then the term liver specific membrane lipoprotein (LSP) has been used for this antigen preparation.

It is difficult to determine the molecular weight of LSP by gel filtration techniques, because of its lipoprotein nature. The molecular weight, however, is suggested between 4×10^6 and 20×10^6 daltons since LSP appears in the void volume of a Sepharose 4B. So far it is not known whether LSP is composed of different subunits or is an aggregate of identical subunits. Lipid and apoprotein moieties were partially characterized after separation on LH-20 column chromatography. Thin layer chromatography of the three different fractions showed that LSP contains large amounts of phosphatides and triglycerides. A further analysis of the phosphatides revealed cephalin, sphingomyelin, lecithin, and lyssolecithin.
The antigenicity of LSP proved to be dependent on its lipid contents since delipitated LSP did not react with antisera against LSP when tested by double immunodiffusion (16). LSP could be subdivided into several subcomponents. By the analysis of SDS-polyacrylamide gel electrophoresis at least five major and several minor components could be distinguished (16, 27, 31). LSP isolated from human, rat, rabbit, and guinea pig livers showed a similar mobility of the macrolipoprotein when tested with polyacrylamide gel electrophoresis (31).

In recent studies, however, species-specific and non-species-specific determinants of the LSP complex could be identified. Antihuman LSP serum prepared in a sheep reacted with two precipitin peaks in crossed immunoelectrophoresis (CIE) (24). Rabbit, rat, mouse, and swine LSP revealed one precipitin peak, but no reaction was seen with sheep and bovine LSP. Therefore human LSP complex has both species-specific and non-species-specific determinants.

By fused rocket immunoelectrophoresis the non-species-specific determinant of LSP complex was demonstrated to be identical in different species (Fig. 1).

Preliminary studies suggest that LSP is neither a component of the surface of the hepatitis B virus (Dane particles) nor HBsAg or HBcAg. No identity was shown between LSP and HBeAg by immunodiffusion analysis. In addition LSP and a HBsAg vaccine had no antigenic relationship (Hess et al, unpublished data).

![Fig. 1](image_url) LSP of seven species tested against sheep anti human LSP serum by fused rocket immunoelectrophoresis. 1. human, 2. rabbit, 3. rat, 4. swine, 5. mouse, 6. sheep, 7. bovine LSP.

1.2 The liver membrane antigen (LM-Ag)

Liver membrane autoantibodies can be detected by a linear immunofluorescence pattern on isolated rabbit hepatocytes (LMA-test). These LMA are closely associated with autoimmune type CALD (13). The finding
that the LMA immunofluorescence pattern could not be completely absorbed with purified LSP led to the suggestion that LSP may not be the only target antigen in liver diseases (32). By affinity chromatography with insolubilized serum of patients with HBsAg-negative CAH a protein was isolated which revealed to react in crossed immunoelectrophoresis with HBsAg-negative, LMA-positive serum (33). The LM-Ag prepared from rabbit and human soluble liver proteins demonstrated a sharp precipitin pattern only with LMA but not with sheep anti-human-LSP serum, sera from HBsAg-positive CAH, normal human serum, normal sheep serum and anti human plasma protein serum. The precipitin pattern of LSP purified by Sepharose 6B chromatography differed from that of LM-Ag. In tandem-crossed immunoelectrophoresis the immunological identity of LM-Ag in different species could be demonstrated.

2. Characterization of liver membrane autoantibodies

2.1 Autoantibodies against liver specific lipoprotein (LSP)

Initially autoantibodies against liver specific lipoprotein (LSP) were difficult to detect. Jensen et al (18) first reported a sensitive radioimmunoprecipitation technique for the detection of circulating antibodies to the LSP antigen fraction in patient sera. In this test system LSP was radio-labeled with $^{125}$I by the Bolton-Hunter technique (3) and LSP-anti LSP complexes were precipitated by Cowan I staphylococcal cells which contain protein A in their cell walls. Protein A avidly binds the Fc region of IgG. Ninety seven percent (29/30) of patients with untreated CAH were positive for anti-LSP (Table 1). The mean titer was higher in HBsAg-negative cases when compared to HBsAg-positive cases. The difference, however, was not significant. Ninety five percent (20/21) of patients with acute viral hepatitis (AVH) had anti-LSP within two weeks from the onset of jaundice. In uncomplicated AVH anti-LSP disappeared within 12 weeks. Anti-LSP were found in 60% (10/17) of patients with chronic persistent hepatitis (CPH). In contrast to AVH a highly significant correlation was observed in CAH between antibody titers and histological as well as biochemical parameters of disease activity. No correlation was found between anti-LSP titers and non-organ-specific autoantibodies such as ANA, SMA, or AMA. Furthermore this group reported that they detected anti-LSP only in later stages of primary biliary cirrhosis when piece meal necrosis had developed (39) and that 27% (15/55) of patients with alcohol-related liver lesions had anti-LSP. In alcohol induced liver disease the presence of anti-LSP was closely related to the degree of a lymphocytic infiltrate in the portal tracts together with piece meal necrosis of periportal hepatocytes (36).

A similar technique was used by Gerber et al (7), who detected anti-LSP in 68% (38/60) of patients with CAH irrespective of the presence of non-organ-specific autoantibodies (ANA, SMS, AMA) or of HBsAg-status. The incidence of anti-LSP was significantly higher in untreated patients with CAH. Anti-LSP were also found in patients with primary biliary cirrhosis (PBC), CPH and AVH but not in patients with alcohol induced liver diseases. Interestingly anti-LSP were detected in 18% (3/17) of patients with glomerulonephritis. Kakumu et al (19) used a different
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<th>Table 1 Frequency of anti-LSP autoantibodies in patient sera</th>
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<td>Chronic active hepatitis</td>
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<td>HBsAg -</td>
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<td>Chronic persistent hepatitis</td>
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<td>HBsAg -</td>
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<td>type NANB</td>
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<td>Acute viral hepatitis</td>
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<td>HBsAg +</td>
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<td>HBsAg -</td>
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<tr>
<td>type A</td>
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<tr>
<td>type NANB</td>
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<tr>
<td>Alcohol induced liver diseases</td>
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<td>Miscellaneous liver diseases</td>
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<tr>
<td>Cirrhosis of the liver</td>
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<td>Primary biliary cirrhosis</td>
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<td>Drug induced liver disease</td>
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<td>Primary non-hepatic autoimmune diseases</td>
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radioimmunoprecipitation technique to detect circulating anti-LSP in patient sera. In their study LSP was labelled by the chloramine T method (17) and rabbit anti-human IgG serum was used as second antibody. These authors found anti-LSP in 57% (25/44) of the patients with CAH irrespective of HBsAg-status. Furthermore anti-LSP were detected in 22% of patients with liver cirrhosis and in 40% of patients with AVH. In patients with uncomplicated AVH anti-LSP disappeared in every case within two months from onset of illness. On the other hand anti-LSP remained positive in cases which progressed to CAH. Elevated anti-LSP titers were detected before histological diagnosis of CAH was made. In contrast to the results of Jensen et al, the frequency of anti-LSP was significantly correlated with the presence of non-organ-specific autoantibodies (ANA, SMA, AMA). No correlation was observed with other biochemical data. Manns et al (23) developed a radioimmunoprecipitation test for anti-LSP similar to the one described by Kakumu et al (19). Anti-LSP were detected in 44% of patients with CALD, 56% with CPH, 42% with AVH, and 20% with inactive cirrhosis, again irrespective of HBsAg-status. Anti-LSP were further found in 29% of patients with alcohol-induced liver diseases, 14% with primary non-hepatic autoimmune diseases (Table 1). Anti-LSP were not found in 31 healthy blood donors. In this study within the group of CALD, no correlation was found between anti-LSP titers and sex, age, HBsAg-status, gamma globulin levels, serum glutamic-oxaloacetic transaminase (SGOT) levels, or non-organ-specific autoantibodies (ANA, SMA, AMA). Using the same method Manns et al (25) studied the prevalence of anti-LSP in patients with acute and chronic non-B hepatitis. Anti-LSP were detected in five of eight patients with type A and in two of 18 patients with non-A, non-B (NANB) type acute hepatitis. None of 27 patients with chronic NANB hepatitis, displaying the morphology of CPH, were anti-LSP positive. These findings indicate that anti-LSP autoantibodies unlikely play an important role in the development of chronic NANB hepatitis. Similar data have been reported by Meliconi et al (29) recently.

Human LSP was used as test antigen in the assays described by the authors above and anti-human LSP activity was absorbed with human as well as rat LSP, which indicated that non-species-specific determinants of LSP were targets for anti-LSP auto-antibodies in the sera tested.

Since species-specific and non-species-specific determinants of LSP could be demonstrated by heterologous antisera (24), all groups of patients were tested for anti-human and anti-rabbit LSP (23). Only 9% of patients with AVH reacted with rabbit LSP whereas 42% had antibodies against human LSP. As previously mentioned, anti-LSP were found to be transient in uncomplicated AVH (18, 19). It is important to note that antibodies against human LSP as well as against rabbit LSP were found in rabbits which had developed histological signs of CAH after long-term immunization with human LSP. On the other hand rabbits after short-term immunization with human LSP had only antibodies against human-LSP (24). These rabbits had developed antibodies against species-specific determinants. These data suggest that antibodies against non-species-specific determinants of LSP reflect a self perpetuating state of CALD. LSP at
its present stage of purification also contains non-organ-specific determinants (1). Therefore the question may be raised as to whether these non-organ-specific determinants are targets for circulating anti-LSP. In order to answer that we prepared a kidney protein fraction (HKP) in the same way as LSP and used HKP as antigen in place of LSP in the radioimmunoprecipitation assay (23). Antibodies against HKP, the kidney equivalent of LSP, were only found in 10% (6/62) of patients with CALD, whereas anti-LSP were found in 44% (27/62) in those cases. This finding indicates that naturally occurring anti-LSP autoantibodies are predominantly directed against organ-specific determinants of the LSP preparation. A further study using LSP subfractions prepared by CsCl density gradient centrifugation (26) revealed that organ-specific and non-organ-specific determinants were localized in the low density subfraction (1.08—1.10g/ccm). These organ-specific and non-organ-specific determinants of the low density LSP subfraction are targets of circulating anti-LSP in CALD.

The data described above indicate that autoantibodies against the antigen fraction LSP are possibly important in terms of diagnosis and prognosis of inflammatory liver diseases. It was supposed that immune reactions against the membrane antigen-LSP represent a final common pathway in the immunopathogenesis of inflammatory liver diseases of different etiologies (6). At present all test systems use an antigen of partial purification, a further purification of the LSP antigen complex and its antigenic determinants seems to be necessary, especially a further separation of organ- and non-organ-specific determinants. A further approach would be the development of monoclonal antibodies against the organ-specific determinant of LSP.

2.2 Liver membrane autoantibodies detected by immunofluorescence

In vivo membrane-fixed IgG could be detected on isolated hepatocytes from rabbits with experimentally induced CAH by direct immunofluorescence (10). Furthermore Hopf et al (11) reported membrane-fixed IgG on hepatocytes isolated from biopsy specimen obtained from patients with CAH. The mechanical isolation of hepatocytes either from rabbit liver or from human liver biopsy specimen without enzymes has been described in detail elsewhere (9). In this study a positive membrane staining was found in five of six untreated patients with HBsAg-negative CAH and in two of 27 others on immunosuppressive therapy. In patients with acute hepatitis A, CPH and other liver diseases, no in vivo bound IgG was observed. The fluorescence pattern on hepatocyte membranes from patients with HBsAg-negative CAH was linear (Fig. 2), while in those with HBsAg-positive CAH it appeared as a granular pattern (12) (Fig. 3). Since the liver membrane antigens showed no species-specificity, isolated rabbit hepatocytes seemed appropriate for the detection of circulating antibodies against hepatocyte membrane antigens in patient sera. These liver membrane autoantibodies (LMA) are detected in vitro by indirect immunofluorescence on isolated rabbit hepatocytes (13). When isolated rabbit hepatocytes are incubated with patient serum, circulating liver membrane autoantibodies are observed by a characteristic linear membrane staining (LMA-test)
In this original study LMA were detected in seven of ten patients with HBsAg-negative CAH but not in patients with AVH, CPH, HBsAg-positive CAH, healthy HBsAg carriers, alcoholic liver disease and healthy controls. The first study was followed by a larger multicentre trial (38).

**Fig. 2** Typical linear membrane fluorescence pattern of an isolated rabbit hepatocyte incubated with a serum from a patient with autoimmune type chronic active hepatitis.

**Fig. 3** Typical granular fluorescence pattern of an isolated hepatocyte from a patient with HBsAg positive chronic active hepatitis.
LMA were found in 38% of patients with HBsAg-negative CAH and 61% of patients with HBsAg-negative cirrhosis. LMA were only rarely detected in other liver diseases. Kawanishi and Mac Dermott (20) also detected liver membrane antibodies by indirect immunofluorescence. Human and rabbit hepatocytes were isolated after liver perfusion in the presence of 0.1% collagenase. In all ten patients with CALD antibodies were detected against the surface membrane of human and rabbit hepatocytes. These sera had been absorbed with human kidney tissues. They also found that after pretreatment of rabbit hepatocytes with sera from patients with CALD, marked cytotoxicity was observed in a K-cell-enriched population of normal lymphocytes, suggesting that antibody dependent cellular cytotoxicity mediated by liver membrane autoantibodies may be an effector mechanism in CALD. The detected antibodies in this study proved to be of IgG class. Two different immunofluorescence pattern were observed: linear for HBsAg-negative and granular for HBsAg-positive CALD.

The interpretation of the granular in vivo IgG binding in HBsAg-positive CAH is still speculative. Whether the IgG binding represents an antibody against a normal liver membrane component, or an antibody to viral antigens is open for discussion. Circulating immune complexes are present in most patients with HBsAg-positive CAH (15) and these immune complexes are possibly bound to the hepatocyte membrane via IgG Fc or C₃ receptors. These receptors were demonstrated on hepatocytes (14).

It was shown that LMA is not species-specific (16). Isolated hepatocytes from mice, rats and rabbits react with liver membrane antibodies in the LMA-test. A low percentage of binding was observed with hepatocytes isolated from mouse and rat livers; in contrast hepatocytes from human and rabbit livers showed a higher percentage of binding. For routine testing of liver membrane antibodies rabbit hepatocytes should be used.

2.3 Heterogeneity of liver membrane autoantibodies

Initially it was suggested that LMA resemble antibodies against LSP (12, 18). Absorption studies revealed that LMA activity was completely absorbed by rabbit hepatocytes. But many of the LMA positive sera could not be absorbed by LSP (33). Recently, testing 231 patient sera for LMA and anti-LSP (23), it was found that the LMA-test detects liver membrane autoantibodies against further membrane antigens beside LSP. The LMA activity detected by immunofluorescence could be absorbed by 100,000g supernatants of liver homogenate. The corresponding antigens had therefore to be searched for in this protein fraction.

Very recently a solid phase radioimmunoassay (RIA) (22) was used to further characterize liver membrane autoantibodies. The IgG fractions of 38 patient sera, prepared by ammonium sulfate precipitation, were labelled with I¹²⁵ using the chloramine T method (17). The IgG fraction was used to coat the assay tubes and as labelled antibody. The 100,000g supernatants of liver homogenates were used as test antigen. Autoantibodies detected by RIA were found in 10 of 14 patients with HBsAg-negative CAH. Nine of these 10 RIA-positive patients had LMA detectable by indirect immunofluorescence (Table 2). Liver membrane antibodies were not detected by RIA or LMA immunofluorescence test in sera from patients with
HBsAg-positive acute and chronic liver diseases, patients with miscellaneous liver diseases including two PBC patients, and in seven patients with primary non-hepatic autoimmune diseases. Autoantibodies detected by RIA did not correlate with ANA, AMA or SMA. The antibody activity could be absorbed by preincubation with isolated plasma cell membrane fractions prepared from rabbit livers. Liver membrane autoantibodies detected by RIA were directed against three different antigen fractions obtained from Sepharose 6B chromatography, including LSP and LM-Ag (Fig. 1 a-c). Three of ten positive sera reacted with fractions containing LSP and six of ten with fractions containing LM-Ag: Three of ten sera had their corresponding antigen peak at fractions 15–19. Seven of ten positive patients had species-cross-reacting autoantibodies; other three patients had autoantibodies which could not be absorbed by rabbit liver proteins. Only the antibody to LSP was organ-specific, all other antibodies cross-reacted with equivalent kidney protein fractions. Ferritin, human serum albumin and human plasma lipoprotein were excluded as target antigens.

These data demonstrate a heterogeneity of liver membrane autoantibodies and it is noteworthy that so far as inflammatory liver diseases are concerned we are not dealing with one membrane-associated target antigen-antibody system in autoimmune type liver disease.

Table 2 Detection of liver membrane autoantibodies by solid phase radioimmunoassay (RIA) and by the LMA immunofluorescence test (LMA) in various hepatic and non-hepatic diseases (Manns et al. (22).)

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<tr>
<th></th>
<th>No. of cases</th>
<th>RIA</th>
<th>LMA</th>
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<tbody>
<tr>
<td>AVH B</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-B</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CPH HBsAg +</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CAH HBsAg +</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HBsAg –</td>
<td>14</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Miscellaneous liver diseases</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-hepatic autoimmune diseases</td>
<td>7</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Healthy blood donors</td>
<td>2</td>
<td>0</td>
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Fig. 4 Sepharose 6B chromatography of human soluble liver proteins. Fraction 1 represents the first fraction after a void volume of 124 ml (-----). RIA for detecting liver membrane antigens; results are presented as counts per minute (-----). Protein content is indicated as percent optical transmission at 280 nm (%).
(a). serum from a patient reacting with the LSP and LM-Ag fractions. (b). serum from a patient reacting with the third antigen fraction. (c). patient with antibody to the LM-Ag fraction.
With rabbit liver proteins similar results were obtained for all patients tested.
(From: Manns et al, Clinical and Experimental Immunology, 1980; 42: 263—272).
CONCLUSION

The best described antigen remains the membrane antigen complex LSP. The LSP antigen complex contains at least one organ-specific determinant. Autoantibodies against LSP (anti-LSP) are predominantly directed against organ-specific-determinants. Anti-LSP antibodies detected in AVH are in the majority directed against species-specific determinants, whereas in CALD anti-LSP are mainly directed against non-species-specific determinants.

In addition to immune reactions against LSP, liver membrane autoantibodies were found to react with further antigens of the hepatocellular membrane. The detection of liver membrane autoantibodies by indirect immunofluorescence on isolated rabbit hepatocytes (LMA-test) (Fig. 2) is a good diagnostic marker for autoimmune type CALD. A diagnostic test for this subgroup of CALD is clinically important since this subgroup well responds to immunosuppressive therapy. Further studies have to be focussed on a better characterization and purification of these liver membrane antigens and of possible neoantigens (28). Such studies will lead to a more precise evaluation of the clinical relevance of liver membrane autoantibodies. It should also be clarified whether special types of liver membrane autoantibodies are characteristic for subgroups of chronic active liver diseases. In this sense the application of the recently developed monoclonal (hybridoma) antibody technique seems to be a valuable approach since monospecific antibodies would react only with a single antigenic site of a whole antigen-complex (4).

In autoimmune type CAH a defect in suppressor T cell function is suggested. It cannot be concluded, however, whether in autoimmune type CAH the primary event is an autoimmune attack. Unknown viruses and even drugs could lead a loss of immune tolerance against liver membrane antigens, possibly mediated by a genetic predisposition (21).

So far it is not yet known whether these liver membrane autoantibodies are really involved in the immunopathogenesis of CALD via ADCC. Possibly they are a phenomenon secondary to cell destruction. At least they represent diagnostic markers identifying subgroups of CALD with different etiologies.

REFERENCES

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