DETECTION AND CLINICAL SIGNIFICANCE OF ACETONE-INSOLUBLE LIVER CELL MEMBRANE ANTIGEN IN SERA OF PATIENTS WITH CHRONIC ACTIVE LIVER DISEASES

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Summary

An enzyme-linked immunosorbent assay was developed to detect insoluble liver cell membrane antigen (LMAg) which gives rise to serum LMA (anti-LM) in HBsAg-negative patients. The optical density (OD) ratio of the average LMAg level of normal subjects was less than 1.2. In HBsAg-positive cases, high LMAg levels (OD ratio >2.4) were noted in 8 of 8 patients with acute hepatitis (AH), 3 of 8 with chronic persistent hepatitis (CPH), 5 of 10 with moderate chronic aggressive hepatitis (CAH), 7 of 10 severe CAH and 4 of 8 with liver cirrhosis (LC). In HBsAg-negative cases, however, high LMAg levels were noted in only 6 of 8 patients with AH, 1 of 10 with CPH, 1 of 10 with moderate CAH, 1 of 10 with severe CAH, 0 of 8 with LC, 0 of 8 with fatty liver and 5 of 10 with alcoholic hepatitis. In micro-immunodiffusion experiments, intensively absorbed rabbit anti-rat LM precipitated two organ-specific components of rat liver homogenate, one of which was identical to liver specific protein (LSP). In immunohistochemical demonstrations of LMAg and LSP, anti-LM, prepared from the serum of a HBsAg-negative CAH patient, bound to both human and rat acetone-fixed liver cell membranes, but not to those of human or rat kidneys. Absorbed rabbit anti-rat LM also bound to liver cell membranes, but absorbed anti-rat LSP lacked organ-specificity when assayed with the immunofluorescence technique using acetone-fixed liver sections. In conclusion, the appearance of serum LMAg was associated with high-SGPT patients and HBsAg-positive CAH patients.

Key Words: Liver cell membrane antigen, LMAg, Liver specific protein, LSP, autoantibody against membrane.

Received December 5, 1983. Accepted October 20, 1984.

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This work was partly supported by a research grant from the Intractable Liver Diseases Committee of the Ministry of Health & Welfare and a grant (No. 56570285) of the Ministry of Education of Japan.

Part of this work was presented at the Basel Liver Week '82 in Basel and appeared in short paper form in Structural Carbohydrates in the Liver (ed by H. Popper et al.), MTP Press Limited, Boston, 1983, p. 684.

Introduction

In 1976, Hopf et al.1) described the detection of autoantibodies directed against liver cell membrane antigens in HBsAg-negative chronic active hepatitis (CAH) by an immunofluorescence technique. Jensen et al.5) and Kakumu et al.9) demonstrated autoantibodies against liver specific protein (LSP) in both HBsAg-negative and HBsAg-positive acute and chronic hepatitis by radioimmunoprecipitation techniques.
Further, Meyer zum Büschenfelde et al. 4) and Manns et al. 5) reported that LSP is not the only target antigen of liver membrane autoantibodies. Another target antigen, LMAg, was isolated by affinity chromatography using the gammaglobulin fraction of sera from HBsAg-negative CAH patients, and the liver membrane autoantibody (LMA or anti-LM) against LMAg was demonstrated only in HBsAg-negative CAH by a solid phase radioimmunoassay. As of writing, two different antibodies against liver cell membrane antigens have been described in Europe: 1) autoantibodies (anti-LSP) against soluble liver specific lipoprotein (LSP) in HBsAg-negative and HBsAg-positive CAH and 2) liver membrane autoantibody (LMA or anti-LM) against insoluble liver plasma membrane (LMAg) in HBsAg-negative CAH. In a previous report 6), one of the authors (T. Tsuji) and his colleagues reported that the anti-LM which Hopf et al. 1) described was associated with HBsAg-negative CAH, especially anti-HBs-positive CAH by an indirect immunofluorescence technique using acetone-fixed rat liver sections. On the contrary, in the U.S.A., the immunofluorescence, light microscopic immunoenzyme technique, immunodiffusion, and hemagglutination studies of Behrens et al. 7) and Lebwohl et al. 8) did not clearly demonstrate that LSP is a liver specific antigen. They also reported that another target antigen, i.e., an insoluble cell membrane antigen (CM), corresponds to the LMAg demonstrated by Meyer zum Büschenfelde et al., and that the antibody against CM was not associated with HBsAg-negative CAH alone. In Japan, there are many CAH patients with both persistent positive anti-HBs and positive anti-LM 6). It is still unclear whether or not the antibody against insoluble liver cell membrane antigen, anti-LM, is associated with any of the three kinds of CAH: HBsAg-positive, anti-HBs-positive and both negative (HBsAg-negative) CAHs. To obtain an answer to this problem, it is important that antigen as well as antibody be examined in various cases of CAH. In the present study we detected LMAg against anti-LM obtained from an HBsAg-negative patient with CAH in various sera by an enzyme-linked immunosorbent assay (ELISA), and tried to evaluate the clinical significance of serum LMAg.

Materials and Methods

Patient sera.

Serum samples for detection of LMAg by ELISA, were obtained from 12 normal subjects and 108 patients with various liver diseases. Of these 108, 16 were diagnosed histologically as having acute hepatitis (AH), 58 patients as having chronic hepatitis (according to the European classification, De Groote et al. 9)), 16 patients as having liver cirrhosis (LC) (excluding alcoholic liver cirrhosis), 8 patients as having fatty liver and 10 patients as having alcoholic hepatitis. Another 142 serum samples, which were examined for HBsAg and anti-HBs, from 89 patients with chronic liver diseases (CLD) and 53 symptom-free persons, were used for screening anti-LM.

Screening of anti-LM.

Screening of anti-LM was performed by the indirect immunofluorescence technique using acetone-fixed rat liver sections reported previously 6). Male Sprague-Dawley rats (Clea Japan, Inc.) were exsanguinated from femoral arteries while perfusing the liver via the portal vein with physiological saline under ether anaesthesia. The livers were cut into pieces, frozen in isopentane cooled to \(-70^\circ\text{C}\) with dry-ice acetone, and sliced to 6 \(\mu\text{m}\) thickness with a cryostat. The sections were fixed in cold-acetone for 5 minutes. Fifty \(\mu\text{l}\) of 1:10 diluted serum samples were placed on the sections and incubated at 37\(^\circ\text{C}\) in a moist chamber for 50 minutes. After incubation, the sections were