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## Detection and characterization of antibody to liver cell membrane in sera from patients with chronic active liver diseases.

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# Detection and characterization of antibody to liver cell membrane in sera from patients with chronic active liver diseases.\*

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## Abstract

Sera from 84 patients with chronic liver disease [CLD] (74 chronic active) and from 53 blood donors were tested immunochemically for anti-liver cell membrane antibody (LMAb). LMAb to rat liver tested by an indirect immunofluorescent technique was positive in 53.3% of CLD patients with positive HB surface antibody (HBsAb) and 40.0% of HBsAb positive blood donors. Pepsin digestion of the sera indicated that the binding between liver cell membrane and IgG was at the Fc site on the immunoglobulin. The sera with positive LMAb from HBsAb positive blood donors had elevated Clq-binding activity (Clq-BA). The LMAb to rat liver was a macro-molecular IgG (19-22S IgG) when assayed by Sephadex G-200 column chromatography and 5-40% sucrose density gradient ultracentrifugation. The results suggest that the LMAb in serum from a patient with chronic active liver disease may be an immune complex which consists of various antigens such as HB virus and its antibodies in serum.

**KEYWORDS:** anti-liver cell membrane antibody, chronic active liver disease, Fc receptor, HB surface antibody, immune complex

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— BRIEF NOTE —

**DETECTION AND CHARACTERIZATION OF ANTIBODY  
TO LIVER CELL MEMBRANE IN SERA FROM  
PATIENTS WITH CHRONIC ACTIVE  
LIVER DISEASES**

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**Abstract.** Sera from 84 patients with chronic liver disease [CLD] (74 chronic active) and from 53 blood donors were tested immunochemically for anti-liver cell membrane antibody (LMAb). LMAb to rat liver tested by an indirect immunofluorescent technique was positive in 53.3% of CLD patients with positive HB surface antibody (HBsAb) and 40.0% of HBsAb positive blood donors. Pepsin digestion of the sera indicated that the binding between liver cell membrane and IgG was at the Fc site on the immunoglobulin. The sera with positive LMAb from HBsAb positive blood donors had elevated Clq-binding activity (Clq-BA). The LMAb to rat liver was a macro-molecular IgG (19-22S IgG) when assayed by Sephadex G-200 column chromatography and 5-40% sucrose density gradient ultracentrifugation. The results suggest that the LMAb in serum from a patient with chronic active liver disease may be an immune complex which consists of various antigens such as HB virus and its antibodies in serum.

**Key words :** anti-liver cell membrane antibody, chronic active liver disease, Fc receptor, HB surface antibody, immune complex

Important factors in the progression from chronic active hepatitis to liver cirrhosis include antibody-dependent cellular cytotoxicity (ADCC) and the cytotoxicity of immune T-cells to specific liver cell membrane (1). This paper is an immunochemical study of the clinical significance of auto-liver cell membrane antibody (LMAb) in chronic active liver disease (CALD). The relationship of this antibody to various types of CALD and to HB surface antigen (HBsAg)-antibody (HBsAb)-immune complexes was also examined.

The study group consisted of 29 patients histologically diagnosed as having chronic hepatitis according to the European classification (2) [chronic persistent

hepatitis (CPH) 10, chronic aggressive hepatitis (CAH) with moderate activity (2A) 21, CAH with severe activity (2B) 22], 17 cases of liver cirrhosis (LC), 3 cases of hepatocellular carcinoma (HCC), and 12 cases of other types. The total was 84 patients, 43 blood donors, and 10 healthy persons (137 altogether). LMAb was detected by an indirect immunofluorescent technique using rat liver acetone fixed cryostat sections as antigen. Sections of guinea pig, dog, and human liver tissue were also prepared, and suspensions of fresh patient liver cells prepared according to Hopf *et al.* (3) for comparison by direct immunofluorescence (4). Aggregated IgG (AGG) was prepared from human Cohn F-II (NBC) to confirm both the presence of receptors on liver cell membrane and the inhibitory reaction to LMAb.

Clq-binding activity (Clq-BA) of serum has been used to detect serum immune complex (IC) with Clq (Zubler *et al.*) (5). The Clq-BA of LMAb positive sera were measured to clarify any differences from the IC. LMAb bound to rat liver was treated with pepsin solution in the manner reported by Hofman *et al.* (6); namely, 4% w/v solution of pepsin in 0.2 M sodium acetate buffer pH 4.1 on the specimen was incubated at 37°C for 15 h, and then covered with rabbit anti-human IgG (Fab) or IgG (Fc) (Behringwerke Institute). Finally, the specimen was stained by FITC-labeled goat anti-rabbit IgG (Behringwerke Institute).

Sephadex G-200 column chromatography and sucrose density gradient ultracentrifugation were performed to ascertain the molecular size of LMAb. Five ml of LMAb positive serum was applied to the Sephadex column (2.0 cm in diameter and 75 cm in height). Nine drops per minute were eluted in each fraction tube. On the gradient centrifugation column, 0.2 ml of sample sera was applied to the 5–40% sucrose gradient, then centrifuged at 35,000 rpm for 18 h at 4°C in a Hitachi ultracentrifuge model 65P.

The detection rates of LMAb to rat liver in sera from various types of CALD patients are shown in Table 1. The detection rates for LMAb was 27.8% for 36 HBsAg positive CLD patients, 53.3% for 15 HBsAb positive CLD patients, and 36.4% for 33 patients with negative results for both HBsAg and HBsAb.

In blood donors, the rate was 5.5% for the HBsAg positive group, 40.0% for the HBsAb positive group, and 0% for the group with both Ag and Ab negative. In short, the rate of LMAb was significantly higher in the HBsAb positive blood donor group than in the other two blood donor groups.

The results of LMAb in sera using acetone fixed rat liver sections and an indirect immunofluorescent method were compared with the ratio of isolated liver cells bearing IgG. These cells were from liver biopsy specimens of 5 cases (Table 2). There was no significant difference. Our method with acetone-fixed

## Anti-Liver Cell Membrane Antibody in CALD

TABLE 1. DETECTIONS OF ANTI-LIVER CELL MEMBRANE ANTIBODY IN SERA FROM CHRONIC ACTIVE LIVER DISEASE PATIENTS AND HEALTHY PERSONS

|                        | HBsAg Positive <sup>a</sup> |                        | HBsAb Positive <sup>b</sup> |              | HBsAg & Ab Negative |              |
|------------------------|-----------------------------|------------------------|-----------------------------|--------------|---------------------|--------------|
|                        | No. cases                   | No. positive           | No. cases                   | No. positive | No. cases           | No. positive |
| Chronic liver diseases | 36                          | 10 (27.8) <sup>c</sup> | 15                          | 8 (53.3)     | 33                  | 12 (36.4)    |
| CH                     |                             |                        |                             |              |                     |              |
| CPH                    | 1                           | 0                      | 3                           | 2            | 6                   | 2            |
| CAH (2A)               | 10                          | 4 (40.0)               | 5                           | 3            | 6                   | 3            |
| CAH (2B)               | 18                          | 5 (27.8)               | 1                           | 0            | 3                   | 1            |
| LC                     | 6                           | 1                      | 3                           | 2            | 8                   | 3            |
| HCC                    | 0                           |                        | 1                           | 0            | 1                   | 1            |
| Other type             | 1                           | 0                      | 2                           | 1            | 9                   | 2            |
| Healthy                | 18                          | 1 (5.5)                | 25                          | 10 (40.0)    | 10                  | 0 (0)        |
| Blood type A           | 6                           | 1                      | 10                          | 4 (40.0)     | 4                   | 0            |
| B                      | 4                           | 0                      | 6                           | 3            | 3                   | 0            |
| AB                     | 3                           | 0                      | 3                           | 1            | 1                   | 0            |
| O                      | 5                           | 0                      | 6                           | 2            | 2                   | 0            |

<sup>a</sup> RPHA positive; healthy group 28 ≤<sup>b</sup> PHA positive; healthy group 28 ≤<sup>c</sup> Number in parentheses, number in percentage

TABLE 2. BEARING IgG ON AUTO-LIVER CELL MEMBRANE AND BINDING IgG TO RAT LIVER CELL MEMBRANE (LA Ab)

| Case   | Age | Sex | HBsAg or Ab in serum | Histological diagnosis | Bearing IgG on auto-liver cell membrane | Binding IgG to rat liver cell membrane (LM Ab) |
|--------|-----|-----|----------------------|------------------------|-----------------------------------------|------------------------------------------------|
| Ma, G. | 57  | M   | Ab (+)               | LC                     | + <sup>a</sup>                          | ++                                             |
| Ta, K. | 41  | M   | both (-)             | LC                     | -                                       | -                                              |
| O, S.  | 55  | M   | both (-)             | CAH (2A)               | + <sup>b</sup>                          | +                                              |
| S, T.  | 24  | M   | Ag (+)               | CAH (2A)               | + <sup>a</sup>                          | +                                              |
| O, T.  | 38  | M   | Ag (+)               | CAH (2A)               | -                                       | -                                              |

<sup>a</sup> Granular pattern, <sup>b</sup> Linear pattern

sections was a good technique for detecting LMAb in serum since it was easy to use and gave reproducible results.

The Clq-BA of LMAb positive and negative sera is shown in Fig. 1. There was no significant difference in the Clq-BA of CALD patient serum, but there were higher levels of Clq-BA in LMAb positive blood donor sera than in LMAb negative blood donor sera.

The results of a study of the binding sites of LMAb to rat liver cell membrane are shown in Table 3. The Fc portion of immunoglobulin was bound to



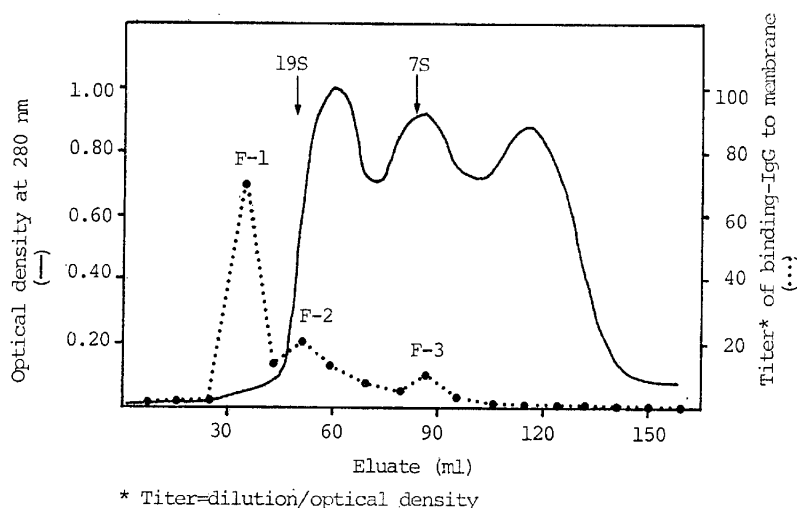


Fig. 2. Cell-filtration of anti-liver cell membrane antibody (LM Ab) positive serum on sephadex G-200. Five ml of LM Ab positive serum was applied on the column, and 9 drops per minute were eluted in each 5.0 ml fraction tube.

This LMAb was not absorbed out by specific liver membrane antigen according to Meyer zum Büschenfelde *et al.* (7).

The findings obtained in this investigation suggest that the greater part of the so-called anti-liver cell membrane antibody in CALD were derived from the Fc of IgG forming an immune complex. The detection of anti-liver cell membrane antibody (LMAb) by the indirect immunofluorescent technique may be due to a reaction of immune complexes which consist of various antigens such as HB virus, its antibodies, and liver membrane Fc receptors.

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