Localisation of immunoglobulin on the liver cell surface in primary biliary cirrhosis

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SUMMARY Direct immunofluorescence studies were performed on isolated liver cells in order to detect surface localisation of IgG in acute and chronic hepatitis and primary biliary cirrhosis. Membrane-bound IgG was demonstrated in nine patients. Six of eight patients with primary biliary cirrhosis showed granular fluorescence on their liver cell surfaces suggesting that an antibody or immune complex-mediated cytotoxicity might be involved in the pathogenesis of this disease.

Primary biliary cirrhosis (PBC) is a disease of unknown aetiology which largely affects middle-aged women. Many features of PBC such as hypergammaglobulinaemia, presence of autoantibodies, circulating immune complexes, granuloma formation and association with other autoimmune diseases suggest that immune mechanisms may have an important pathogenic role. Recent in vitro studies have indicated that lymphocytes in PBC are cytotoxic to hepatocytes. Furthermore, large and small circulating immune complexes have been demonstrated, in some series correlating with the disease activity of PBC. McFarlane et al. have now found, that many patients with PBC exhibit in vitro leucocyte migration inhibition in response to a protein fraction of normal human gall-bladder bile, and the same group of workers have also been able to detect biliary tract antigens in circulating immune complexes.

A prerequisite for immune mechanisms as the basis for the primary lesion of the liver involving cellular infiltrate and granuloma formation would be the demonstration of immune reactions taking place in the liver tissue. The present study deals with in vivo fixation of IgG to hepatocytes as a possible indicator of such reactions and compares the findings to other inflammatory liver diseases where such a binding has been shown to take place.

Patients and methods

Twenty-six patients with inflammatory liver disease were included. Diagnoses were all based on historical and clinical examination.

Ten patients had acute viral hepatitis at varying stages, five were classified as “hepatitis B” and five were “non-B.”

Five patients had chronic aggressive hepatitis (CAH) and three chronic persistent hepatitis (CPH) according to established criteria, one in each category was type B. Patients were classified as virus-B associated if they had circulating HBsAg or an anti-hepatitis B core (anti-HBC) titre above 10−3 without anti-HBs, or if acute hepatitis B initiated the present disease. Two patients with CAH were on treatment with prednisone, and one CPH patient with Arabinosid-A (500 mg/day).

Eight patients had PBC according to clinical, serological and histological criteria. Four patients were untreated at the time of investigation. One patient was receiving prednisone, two patients azathioprine and one was receiving a combination of these two drugs. Daily dosage was 10 mg of prednisone or 100 mg of azathioprine or both.

Seven subjects were included as controls. The indications for biopsy in these patients were suspicion of alcoholic liver disease, hepatitis or liver involvement in systemic diseases. None of the patients had histological signs of inflammatory liver disease, and five had only minor histological changes such as steatosis or unspecific reactive changes.

Serum samples were taken from all patients at the time of biopsy and stored at −20° until analysis.

Liver tissue

Tissue was obtained by percutaneous biopsy with the Menghini fine needle technique and the material was bisected. One part was processed for routine histological examination and the other transferred...
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BIOCHEMICAL DATA

Where data on liver biochemistry are incorporated, these were assayed at the time of biopsy.

Results

Table 1 shows the incidence of both in vivo bound IgG to the hepatocyte surface and of LMA in serum in the 26 patients with inflammatory liver disease and the seven controls. Six of eight patients with PBC had in vivo bound IgG; the pattern of staining was fine to coarse granular (Fig. 1a) except in one who had a pattern that tended to be linear. Isolated liver cells from all patients and the seven controls bound IgG from the LMA positive reference serum producing a strong linear fluorescence staining pattern (Fig. 1b) clearly distinguishable from the normal control (Fig. 1c). Furthermore, this reference serum

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cases (n)</th>
<th>Membrane IgG (n)</th>
<th>LMA* (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute hepatitis type B</td>
<td>5</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>type non-B</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chronic aggressive</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>hepatitis type B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>type non-B</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Chronic persistent</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>hepatitis type B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>type non-B</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>8</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Controls</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Liver-cell-membrane antibody.

Immunofluorescence Tests

Detection of in vivo bound IgG to the isolated hepatocytes was done by a direct immunofluorescence technique using FITC-labelled antihuman IgG specific for gamma chains (Dako, Denmark); specificity controls included blocking with unlabelled antiserum. In vitro binding by isolated hepatocytes of IgG from a serum positive for liver-cell-membrane antibody (LMA) was tested by the indirect immunofluorescence test, described by Hopf et al. In order to exclude pinocytosis or passive adsorption of IgG, routine-tests using an LMA-negative normal human serum were performed.

The patients' sera were examined under code for LMA, antinuclear antibody (ANA), smooth muscle antibody (SMA) and antimitochondrial antibody (AMA) of IgG class by the indirect immunofluorescence test and standard procedures.

The slides were read in a Leitz Ortholux 2 microscope using a Leitz fluorescence objective, fluorit FL 40/1.30, for oil immersion in combination with 6.3 × oculars.

Virology

The sera were tested for HBsAg, anti-HBs and anti-HBc by radioimmunoassay (Ausria-II, Ausab and Corab, Abbott Laboratories, Diagnostic Division, North Chicago, USA).

into Eagles minimal essential medium, pH 7.4 at 37°C and a suspension of hepatocytes was immediately prepared by a non-enzymatic technique as described in detail elsewhere. The vitality of the hepatocytes was tested by phase contrast microscopy.

Fig. 1a Photomicrograph of isolated hepatocytes from a patient with primary biliary cirrhosis showing immunofluorescent staining by FITC-antihuman immunoglobulin × 100
was found to have an LMA titre at least two doubling dilutions higher when tested with human hepatocytes as compared with rabbit hepatocytes.

In Table 2 the relation between in vivo bound IgG, and some of the serological and biochemical data are shown for the patients with PBC. Of the six patients with in vivo bound IgG, four had markedly raised alkaline phosphatase activities, whereas the two patients with no binding had only moderately raised activities. Seven of the patients had AMA of the IgG class in serum, but the titre was not related to the presence of in vivo bound IgG. The one patient without AMA had LMA, SMA and ANA all of IgG class. This autoantibody profile is usually suggestive of CAH. However, in this patient the clinical and histological features were fully compatible with PBC.

Of five patients with CAH one without virus B markers demonstrated in vivo bound IgG on the surface of isolated hepatocytes and at the same time circulating LMA and ANA. Among three patients with CPH one was virus B associated and demonstrated surface bound IgG in the absence of circulating autoantibodies. The above-mentioned two patients both had raised IgG concentrations in contrast to the remaining six patients with chronic hepatitis.

Altogether, two patients had circulating LMA, both demonstrated in vivo bound IgG.

### Discussion

In vivo fixation of IgG to the surfaces of hepatocytes was only demonstrated sporadically; however, the findings are consistent with the original report concerning fluorescence patterns and correlation to aetiology and disease activity. Of special import-

### Table 2  Serological data in eight patients with primary biliary cirrhosis investigated for the presence of membrane-bound IgG on isolated hepatocytes

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Histological stage</th>
<th>Autoantibodies</th>
<th>Alkaline phosphatase*</th>
<th>IgG*</th>
<th>IgM*</th>
<th>DIF</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>ANA (inverse titre)</td>
<td>SMA</td>
<td>AMA</td>
<td>LMA</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>III</td>
<td>256 1024 512</td>
<td>9</td>
<td>2</td>
<td></td>
<td>+</td>
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<tr>
<td>2</td>
<td>III</td>
<td>256 1024 512</td>
<td>3</td>
<td>2</td>
<td>3</td>
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</tr>
<tr>
<td>3</td>
<td>I</td>
<td>- 1024 512</td>
<td>3</td>
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<tr>
<td>4</td>
<td>III</td>
<td>- 2048 512</td>
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<tr>
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<td>II</td>
<td>- 512 1024</td>
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<tr>
<td>6</td>
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<td>7</td>
<td>II</td>
<td>- 1024 1024</td>
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<td>4</td>
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<td>8</td>
<td>II</td>
<td>- 1024 1024</td>
<td>3</td>
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<td></td>
</tr>
</tbody>
</table>

*Expresses increase × upper normal limit.

DIF = direct immunofluorescence — detection of membrane-bound IgG on hepatocytes.

ANA = antinuclear antibody.

SMA = smooth muscle antibody.

AMA = antimitochondrial antibody.

LMA = liver-cell-membrane antibody.
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In the description of the autoantibody LMA is the fact that all subjects, including the controls could bind IgG from an LMA-positive (SMA-negative) reference serum. Thus, LMA is directed against normal constituents of the liver cell membrane and the LM-antigen is obviously expressed on hepatocytes irrespective of liver disease.

The most remarkable finding was the presence of membrane IgG on the hepatocytes from six out of eight patients with PBC, something that to our knowledge has not been demonstrated before. Thomas et al. and McFarlane et al. have proposed an autoimmune reaction directed against an antigen associated with the bile canicular portion of the hepatocyte membrane as the basis for the granulomatous lesions of PBC. The in vivo fixation of IgG to isolated hepatocytes in patients with PBC, demonstrated in the present work, was not related to stage of disease (Table 2), but was also present in the one patient, where no parenchymal involvement was found. Theoretically this IgG could be an antibody directed against bile canicular antigens, or it could be part of immune complexes, thus forming the basis for an antibody-mediated cytotoxic reaction as proposed above. This point could not be further clarified due to shortage of material, but the present results do not indicate that AMA is of pathogenic influence.

In conclusion, our results extend the accumulating data suggesting the involvement of altered immune functions in the development of PBC. Biliary tract antigens might be the target of humoral responses, but proof of a causal relation must await identification of the IgG which is fixed to the hepatocyte membrane in vivo and of the putative target antigen involved.

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References