Serodiagnosis of Acute Hepatitis B Virus Infection by a Modified Competitive Binding Radioimmunoassay

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Using the Staphylococcus aureus absorption method, we have modified a commercial radioimmunoassay kit for the determination of total antibody to hepatitis B core antigen so that we can now determine the predominant globulin species of that antibody. One-hundred percent of acute hepatitis B sera had a predominance of immunoglobulin M antibody to hepatitis B core antigen, whereas immunoglobulin G antibody to hepatitis B core antigen was predominant in 98.2% of chronic hepatitis B surface antigen carriers. With this test and the commercially available tests for immunoglobulin M anti-hepatitis A virus, one can now reasonably differentiate acute hepatitis B from non A/non B hepatitis in a chronic hepatitis B surface antigen carrier.

The serodiagnosis of acute hepatitis B has been facilitated by the availability of radioimmunoassay (RIA) for determination of hepatitis B surface antigen (HBsAg) (5, 9). However, HBsAg positivity alone may not distinguish between acute hepatitis B or viral hepatitis of other etiology occurring in a chronic HBsAg carrier. Since it is now possible to define acute hepatitis A infection by demonstrating a predominance of anti-hepatitis A virus in the immunoglobulin M (IgM) fraction of acute illness phase serum (2, 4), we wished to determine whether specification of predominant globulin class in the hepatitis B core (HBc) antigen-antibody system might be useful in differentiating acute hepatitis B infection from the chronic HBsAg carrier state.

We modified a commercial competitive binding RIA (CORAB; Abbott Laboratories, North Chicago, Ill.) for antibody to HBc antigen (anti-HBc) (10) to determine the predominant globulin species of this antibody. Briefly, a CORAB-positive serum was diluted with phosphate-buffered saline according to its percent competition (2). The specimen was then divided, and one-half was absorbed with Staphylococcus aureus Newman D2C protein A (Sigma Chemical Co., St. Louis, Mo.). The absorbed diluted serum and the unabsorbed diluted serum were then retested by CORAB, and the ratio (R) of the unabsorbed (−A) counts per minute to absorbed (+A) counts per minute was calculated (R = +A/−A). HBsAg was measured by Ausrira-II (Abbott Laboratories). To exclude the possibility of interactions of rheumatoid factor (RF), an IgM antoglobulin, in this test system, all sera were tested for RF by a latex agglutination test (Rapi-tex-RF; Calbiochem-Behring Corp., La Jolla, Calif.), and all were negative. In addition, the RF kit positive control and two RF-positive sera which were anti-HBc negative were also tested by our modified method, and none demonstrated false positive results. An R of 2.5 or less is used in the hepatitis A virus-IgM system (2); however, we used an R of 2.0 or less to indicate IgM anti-HBc predominance.

Serial serum specimens from two chimpanzees experimentally infected with hepatitis B virus were examined for the predominance of IgM or IgG anti-HBc. Chimpanzee number 825 developed HBsAg on day 41 postinoculation and anti-HBc of predominantly IgM at day 69 (Fig. 1). By day 95, most of the anti-HBc specific immunoglobulin had shifted to IgG. Chimpanzee number 86 (Fig. 2) developed HBsAg 30 days postinoculation and a predominance of IgM anti-HBc at day 49. By day 85 most of the anti-HBc had shifted to IgG predominance. These data indicate that in the chimpanzee model, hepatitis B core IgM antibody species predominated in the acute stage of infection, and the shift to IgG species anti-HBc occurred approximately 50 days after the appearance of HBsAg.

Human sera from known acute hepatitis B infections and from documented chronic HBsAg carriers were also examined. Chronic HBsAg carriers were defined as individuals who were HBsAg positive for at least 6 months. In sera from 50 acute hepatitis B patients, the R value ranged from 0.9 to 2.0, with a mean of 1.6. In 60 convalescent-phase sera, as evidenced by antibody to HBsAg positivity, the lowest R value was 2.8, and the majority of these sera had R
values exceeding 4.6. All of the acute-phase sera had IgM anti-HBc predominance (Table 1). IgG anti-HBc was predominant in 98.2% of the chronic HBsAg carriers. The IgM response of the two chronic carriers was further documented by removing the IgM anti-HBc activity with 2-mercaptoethanol.

Our data suggest that the use of a staphylococcal protein A modified competitive binding RIA for the determination of the class of anti-HBc immunoglobulin is useful in differentiating acute hepatitis B infection from chronic HBsAg carriage. The IgG-absorbing ability of S. aureus protein A has been recognized (1). It removes all but IgG subclass 3 (7), and the percentage of protein A-reactive IgG in human sera is well over 90% (8).

It is not entirely clear why a small percentage (1.8%) of chronic HBsAg carriers continue to demonstrate a preponderance of IgM antibody. Cappel et al. (3), using Sepharose-protein A to remove IgG, reported nine patients in which IgM anti-HBc predominance could be detected for up to 2 years; all developed chronic aggressive hepatitis.

Gerlich et al. (6), using a sensitive reverse enzyme immunoassay based on \(\mu\)-chain specific antibody, followed 58 patients with acute hepatitis B. IgM anti-HBc was detected during the acute phase of all patients. However, in examination of 21 chronic HBsAg carriers, 20 (95.2%) were still positive for IgM anti-HBc after 2 years. Roggendorf et al. (11), using a similar enzyme immunoassay, demonstrated anti-HBc...
TABLE 1. Predominance of IgM to IgG anti-HBc in sera from two groups

<table>
<thead>
<tr>
<th>HBsAg status</th>
<th>No. tested</th>
<th>IgM (%)</th>
<th>IgG (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>50</td>
<td>50 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Chronic carrier</td>
<td>110</td>
<td>2 (1.8)</td>
<td>106 (98.2)</td>
</tr>
</tbody>
</table>

IgM activity of 89.4% in chronic active hepatitis as opposed to 34.9% in healthy carriers. The mean IgM titer of sera from chronic carriers was $10^{-3}$, whereas the mean IgM titer of acute hepatitis sera was $10^{-5}$. It should be emphasized, however, that these two test systems do not measure relative predominance of anti-HBc globulin species.

Our ability to differentiate acute hepatitis B cases from the chronic carrier state by using a simple modification of a commercially available competitive binding RIA should offer a reasonable approach to the diagnosis of non-B viral hepatitis in chronic HBsAg carriers.

LITERATURE CITED