Radioimmunoassay for Hepatitis B Core Antigen

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Serum hepatitis B core antigen (HBcAg) is an important marker of hepatitis B virus replication. We describe an easy, sensitive radioimmunoassay for determination of HBcAg in detergent-treated serum pellets containing Dane particles. Components of a commercial kit for anti-core determination are used, and HBcAg is measured by competitive inhibition of binding of 125I-labeled antibodies to HBcAg with HBcAg-coated beads. We assayed for HBcAg in the sera of 49 patients with hepatitis B surface antigen (HBsAg)-positive chronic hepatitis, 50 patients with HBsAg-negative chronic hepatitis, and 30 healthy volunteers. HBcAg was detected in 41% of patients with HBsAg-positive chronic hepatitis but not in patients with HBsAg-negative chronic hepatitis. Hepatitis Be antigen (an antigen closely associated with the core of Dane particles) determined in the same sera by radioimmunoassay, was not detected in 50% of HBcAg-positive sera.

Additional Keyphrases: immobilized reagents • “kit” methods • comparison with microsolid-phase radioimmunoassay

The intact hepatitis B virus, also referred to as Dane particles, is a 42-nm diameter structure seen in the sera of patients acutely or chronically infected with this virus; it is composed of an outer surface (containing hepatitis B surface antigen, HBsAg) and an inner core (containing hepatitis B core antigen, HBeAg). HBsAg is found not only on the outer surface of Dane particles but also in small serum particles (20-nm spheres and tubular particles) and in the cytoplasm of hepatocytes. HBeAg has been demonstrated in the core of Dane particles and in the nuclei of liver cells. The third antigen related to hepatitis B virus is hepatitis B antigen (HBeAg), a soluble protein seen only in HBsAg-positive sera.

Studies of these three antigenic systems of the hepatitis B virus (HBsAg, HBeAg, and HBeAg) have added considerably to our knowledge of the pathogenesis of hepatitis (1). HBsAg and HBeAg can now be radioimmunoassayed routinely in the sera of patients because of the availability of commercial kits, but determinations of HBcAg are still difficult, requiring the preparation and standardization of radio-labeled antisera.

HBcAg is a reliable marker of circulating hepatitis B virus, and its determination is important for clinical and epidemiological studies (2, 3). HBcAg in the sera of patients with chronic active hepatitis has been recently correlated with an unfavorable course of the disease (4).

As with HBeAg, which has recently been identified in the core of Dane particles (5, 6), HBcAg may also indicate infectivity. Heretofore, it has been detected mainly by solid-phase RIA, developed originally by Purcell et al. (7, 8). We describe here a simple and sensitive RIA for detecting HBcAg in the serum. Utilizing the sera of patients with chronic hepatitis, we have compared the results of this method with those of the standard solid-phase RIA and correlated the results of both methods with the presence of HBeAg.

Materials and Methods

We used sera from 99 patients with biopsy-proven chronic hepatitis; 49 of these 99 sera were HBsAg-positive. We also used sera from 30 healthy controls.

Procedures

Preparation of HBcAg-rich serum pellets. One milliliter of serum, diluted twofold with Ca2+ and Mg2+-free phosphate-buffered saline (Dulbecco’s; Gibco, Grand Island, NY 14072), was placed in 10-mL polycarbonate tubes (Nalge Co., Rochester, NY 14602). Dane particles were precipitated by centrifugation with a fixed-angle rotor (4 h, 39,000 X g, 4°C). The supernate was aspirated with a Pasteur pipette, and the internal surface of the centrifuge tubes was promptly dried with sterile cotton swabs. Each pellet was then suspended in 8 mL of phosphate-buffered saline and centrifuged again, as before. The centrifuge tubes were dried again with cotton swabs and the pellets resuspended in 50 mL of phosphate-buffered saline. This washing and thorough drying were intended to remove trace amounts of anti-HBc that may have been present in the pellets obtained after the first centrifugation. Duplicate samples of pellets were prepared for analysis by RIA.

Solid-phase RIA. The solid-phase RIA for HBcAg has been extensively described in previous papers (4, 7). Briefly, a
Table 1. Prevalence of HBcAg and Other Markers of Hepatitis B Virus in Patients with HBsAg-Positive Chronic Hepatitis and Healthy Controls

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>HBcAg</th>
<th>Solid-phase RIA assay</th>
<th>HBcAg</th>
<th>HBeAg</th>
<th>Anti-HBc</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg-positive chronic hepatitis</td>
<td>49</td>
<td>41</td>
<td>53</td>
<td>27</td>
<td>100</td>
</tr>
<tr>
<td>HBsAg-negative chronic hepatitis</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

serum with high anti-HBc titer (serum titer by RIA 1:100 000) obtained from a patient with HBsAg-positive chronic hepatitis was centrifuged at 39 000 × g. The supernate, diluted fourfold with phosphate-buffered saline, was used to coat wells of polyvinyl microtiter plates (Cook Laboratory Products, Alexandria, VA 22314). We detected anti-HBc in this serum both by RIA (Corab™; Abbott Labs., North Chicago, IL 60064) and by the immunofluorescent antibody technique, using as substrate a human liver with hepatocyte nuclei containing hepatitis B virus core particles observed by electron microscopy (9). Wells were then exposed to 10 μL of serum pellets that had previously been treated with 10 μL of a 10 mL/L solution of NP 40 detergent (Nonidet P 40; Bethesda Research Labs., Rockville, MD 20850) in distilled water at 37 °C for 30 min. NP 40 releases core antigens from the pellets that contain Dane particles (7). After incubation for 36 h at 4 °C, the wells received 50 μL of the 125I-labeled immunoglobulin fraction of the anti-HBc serum. This fraction was prepared according to Levy and Sober (10) and iodinated by a modification of the Chloramine T method (11). The wells were then cut out of the plates, and the radioactivity of each well was counted with a gamma counter. Results were expressed as S:N ratios, where S is the cpm of patients' samples and N is the average cpm with pellets of 10 healthy controls. An S:N ratio of 2.1 or greater was considered positive. This value, similar to the values considered positive in the RIA for HBeAg, was found to be a suitable indicator of positivity in this test (7).

HBcAg RIA. The HBcAg RIA method is based on the inhibitory activity of HBcAg-containing serum pellets toward binding of 125I-labeled anti-HBc to HBcAg-coated beads. We used three components of a commercial kit (Corab™) for the determination of anti-HBc: reaction trays, HBcAg-coated beads, and 125I-labeled anti-HBc.

We treated 10 μL of serum pellets with 10 μL of 10 mL/L NP 40 solution at 37 °C for 30 min in wells of a reaction tray. After adjusting the microwell contents to 100 μL with phosphate-buffered saline, we added the HBcAg-coated beads and 100 μL of 125I-labeled anti-HBc. After a 22-h incubation at room temperature, the supernate was removed; the beads were washed three times with 5 mL of phosphate-buffered saline and their radioactivity was counted with a gamma counter. Pellets were considered to contain HBcAg when the cpm was at least 3 SD below the mean cpm value for the serum pellets of 10 healthy volunteers. These results were expressed as an N:S ratio, where N and S are defined as before.

To ascertain whether the N:S ratio is proportionate to the antigen concentration, we determined HBcAg for increasing amounts of serum pellets (1, 2.5, 5, 10, and 20 μL) prepared from serum of five patients with HBcAg-positive chronic hepatitis.

HBcAg determination. Determination of HBcAg was by RIA (HBcAg; Abbott Labs.).

Experimental Variables

Specificity. In an attempt to demonstrate the specificity of the RIA method, we treated sera of four HBcAg-positive patients with anti-HBs (H BIG, Abbott Labs.) to remove Dane particles, and tested for HBcAg before and after anti-HBs treatment.

Effect of antiHBc on HBcAg RIA. To ascertain whether washing and drying with swabs removes antiHBc, 25 samples (10 μL of pellets) from 25 HBcAg-positive sera with high titers of antiHBc were not treated with NP 40. They were adjusted to a final volume of 100 μL with phosphate-buffered saline and tested for antiHBc by RIA. In addition, 10 μL of pellets obtained after the first centrifugation was also tested for antiHBc.

Statistical Analysis

Student’s t-test and linear regression functions were used for statistical evaluation.

Results

Correlation of results obtained by solid-phase RIA and by HBcAg RIA. HBcAg was detected by both RIA methods in the sera of 20 of the 49 patients (41%) with HBcAg-positive chronic hepatitis, and in six patients (12%) by HBcAg RIA only. The remaining 23 patients (47%) did not exhibit HBcAg reactivity by either method (Table 1). Serum pellets of 30 healthy controls and 50 patients with HBcAg-negative chronic hepatitis did not contain HBcAg.

Among the 20 patients whose sera were HBcAg-positive by both methods, there was a correlation (p <0.01) between the values of the two RIA procedures (Figure 1). Moreover, the N:S ratios of these 20 patients with positive sera by both methods were significantly higher (p <0.001) than those observed in the six patients who were positive only by the HBcAg RIA (mean ± SD, 15.6 ± 4.9 vs 5.8 ± 4.4). The between- and within-assay CV of both RIA methods never exceeded 5%.

Specificity. In both assays, the N:S ratio or the S:N ratio was proportionate to the antigen concentration; progressively
increasing amounts of serum pellets from five HBcAg-positive patients gave curves that were linear up to an S:N ratio of 20 or an N:S ratio of 21. Both methods were specific the S:N or N:S ratios of HBcAg in all four sera tested decreased by at least 50% after the Dane particles were removed by antiHBs treatment.

Lack of interference by antiHBc. Serum pellets of 25 HBsAg-positive patients who also had antiHBc were obtained after the centrifuge tubes had been washed and dried with swabs. Testing the pellets for antiHBc before detergent treatment showed they did not contain a detectable amount of this antibody. In contrast, 10 pellets tested before these procedures were carried out gave a positive reaction. Furthermore, HBcAg was not detected in pellets from 22 patients with HBsAg-positive chronic hepatitis or 22 patients with HBcAg-negative chronic hepatitis showing circulating antiHBc (Table 1).

Correlation between HBcAg and HBeAg. HBeAg was detected in sera of 13 of the 49 HBsAg-positive patients with chronic hepatitis (Table 1). This antigen was found only in sera that were HBcAg-positive by both methods, whereas sera of 19% of 36 HBsAg-negative patients were HBcAg-positive by both RIA procedures and 17% were positive by only the HBcAg RIA.

Discussion

The HBcAg RIA we describe is specific, reproducible, easier to perform, and more sensitive than the previously described solid-phase RIA. AntiHBc, if present in serum pellets, may interfere with the determination of HBcAg because it competes for antigen. For this reason, we were particularly careful to obtain serum pellets that were devoid of antiHBc, by thoroughly washing and drying with swabs. The use of a fixed-angle rotor is important because it allows more complete drying of the tubes. In our experience, use of swinging-bucket rotors makes drying more difficult because the pellets collect at the bottom of the tubes. Concurrent determination of antiHBc by RIA in serum pellets not exposed to detergent provides certainty that trace amounts of antiHBc do not interfere with the assay.

HBeAg, which has been characterized as a polypeptide of the core particles (5, 6), has been considered a good indication of hepatitis B virus replication and infectivity (12-14). Our study suggests that HBcAg may be a more reliable marker of infectivity than HBeAg because HBeAg was found only in HBcAg-positive sera, whereas some HBeAg-negative sera show detectable quantities of HBcAg. Thus, determination of HBcAg with the described method, which is both easy to perform and sensitive, may help in monitoring the infectivity of HBsAg-positive chronic carriers and in studying factors that favor the progression of hepatitis.

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References