Use of Immunoglobulin M Antibody to Hepatitis B Core Antigen in Diagnosis of Viral Hepatitis

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To determine the diagnostic value of hepatitis B core (Hbc)-specific immunoglobulin M (IgM) antibody (anti-HBc IgM), the sera of six patients with known recent acute viral hepatitis B were examined for the presence of anti-HBc periodically for up to 21 months from the onset of the attack by a sensitive radioimmunoassay technique (CORAB, Abbott Laboratories). It was found that anti-HBc IgM was detectable for approximately 17 months from the onset of the illness. Hence the finding of anti-HBc IgM suggests infection by hepatitis B virus, probably within the preceding 1 to 2 years. A high level of anti-HBc IgM in the acute-phase serum of an individual with viral hepatitis is indicative of recent hepatitis B virus etiology; one patient, however, showed a low titer of anti-HBc IgM in the acute-phase serum sample, which remained virtually unchanged 15 months from onset. The diagnostic use of this serological marker is illustrated in 25 patients with viral hepatitis, in whose acute-phase sera anti-HBc was found.

Recently developed laboratory techniques facilitate the etiological diagnosis of the majority of cases of clinical hepatitis into type A, type B, or (by a process of exclusion) hepatitis non-A, non-B categories. Hepatitis A virus (HAV) is implicated in those patients possessing HAV-specific immunoglobulin M (anti-HAV IgM) in acute-phase sera, and hepatitis B virus (HBV) is implicated in patients with hepatitis B surface antigen (HBsAg) in acute-phase sera. A hepatitis non-A, non-B etiology is ascribed to those patients who do not fall into the A or B categories. Where sequential sera from patients are available, some non-A, non-B cases can be retrospectively shown to be hepatitis B by the subsequent development of antibodies to the surface antigen (anti-HBs). However, such antibodies may take months to develop, and patients do not usually remain within the diagnostic situation for sufficient time to allow this approach to be of much use.

Where HBsAg is undetected in acute-phase sera of hepatitis patients, the presence of antibody to the core antigen of HBV (core antibody, [anti-HBc]) has been advocated as indicating recent hepatitis B infection (3). However, the frequency (11%) of this antibody in a recent survey of a Sydney population with no clinical or biochemical evidence of hepatitis (1a) has shown that anti-HBc does not necessarily indicate recent hepatitis B infection.

In a recent large epidemiological study of hepatitis in Sydney, 129 patients possessed neither anti-HAV IgM nor HBsAg in acute-phase sera. Most of these did not have anti-Hbc and, thus, were fairly confidently placed in the hepatitis non-A, non-B category. However, 25 patients exhibited anti-Hbc, thus posing the problem of whether it was of long-standing duration and, thus, not associated with the current hepatitis or whether it was of recent origin, thus suggesting HBV as the cause of the current disease. This paper describes attempts made to clarify the etiological status of these patients (i.e., hepatitis B or hepatitis non-A, non-B) by use of a test for anti-Hbc IgM. The first part of the report deals with an evaluation of the technique. This is followed with results obtained from the 25 patients with hepatitis of uncertain etiology.

MATERIALS AND METHODS

Study group. (i) Hepatitis B patients used to evaluate test. Six patients with hepatitis of definite HBV etiology were bled at the time of admission to hospital and periodically for 12 to 19 months thereafter (for details see Table 1). All were anti-HBc positive in first and last sera (the sera used to evaluate the test).

(ii) Patients with hepatitis of uncertain etiology. Twenty-five hepatitis patients, negative for HBsAg and anti-HAV IgM in acute-phase sera, were tested. All had anti-HBc in acute-phase sera, and 20 also possessed anti-HBs. There were 15 males and 10 females in the group, and all but 5 were young adults aged between 18 and 30 years.

Testing for hepatitis B markers. Initially, sera were tested for HBsAg and anti-HBs by a sensitive passive hemagglutination method (7). Sera which were
negative by this method (with one exception) were retested by the radioimmunoassay kits AUSRIA II and AUSAB (Abbott Laboratories) for HBsAg and anti-HBs, respectively. Anti-HBc was detected with the radioimmunoassay kit CORAB (Abbott Laboratories) as described previously (4).

Testing for anti-HAV antibodies. Anti-HAV (total and IgM) antibodies were detected by using a minor modification of a previously described radioimmunoassay technique (5).

Fractionation of sera. Amounts of 0.25 ml of sera were centrifuged for 18 h at 117,000 × g on stepwise gradients of 10 to 40% sucrose by the method of Best et al. (1). Fractions of 1 ml were collected, and those containing IgM and IgG were identified by radial immunodiffusion in commercially available plates (Tri-partigen, Behringwerke), using low-level plates for IgM. In the early stages of the work, all fractions were tested. When it became apparent that the same fraction always yielded the peak IgM free of detectable IgG (the fraction tested for anti-HBC antibodies), radial immunodiffusion was discontinued to avoid unnecessary expense.

**RESULTS**

Evaluation of method in hepatitis B patients. In the CORAB test, the level of anti-HBc in serum is inversely related to the radioactive count obtained in the test. In the experiment set up to evaluate the method, the cutoff value, below which sera are designated as positive, was 2,692 cpm. Five of the six hepatitis B cases yielded low counts in the IgM-containing fraction of their acute-phase sera, indicating high levels of anti-HBc IgM (Table 1). Two of these, patients 5 and 6, had lost detectable anti-HBc IgM when sampled on the second occasion (17 and 21 months after onset, respectively). Another three patients (1, 2, and 3) had markedly reduced levels of anti-HBc IgM when sampled 14 months after onset. The remaining patient (patient 4) had only a low level of anti-HBc IgM in the first sample, and this remained virtually unchanged 15 months after onset.

From these results it appeared that anti-HBc IgM tended to become undetectable, as assayed by CORAB, some time between 1 and 2 years after onset of hepatitis B. The fact that five out of six hepatitis patients of known HBV etiology possessed high levels of anti-HBc IgM in sera taken early after onset and that none of the six had such levels 1 to 2 years later suggests that, for diagnostic purposes, a high level of anti-HBc IgM in the acute-phase serum of a hepatitis patient would be indicative of HBV etiology. On the other hand, the absence of anti-HBc IgM or the presence of anti-HBc IgM in low titer in acute-phase sera would tend to indicate that HBV was probably not involved in the current hepatitis. However, the fact that one of the six known HBV patients (patient 4) had low anti-HBc IgM levels in acute-phase sera means that the exclusion of low-level anti-HBc IgM patients from consideration as resulting from HBV would occasionally misdiagnose an HBV patient as non-A, non-B.

**Anti-HBc IgM in hepatitis of uncertain etiology.** The use of the test with 25 hepatitis patients negative in acute-phase sera for anti-HAV IgM, and HBsAg, but positive for anti-HBc, permitted subdivision of these subjects into three groups.

The cutoff value for the test was 2,146. Eight patients possessed high levels of anti-HBc IgM (counts between 585 and 797) and were probably HBV patients whose HBsAg antigenemia was too transient to be detected. Seven of these had anti-HBs. Ten patients possessed no anti-HBc IgM (i.e., counts above 2,146) and probably represented hepatitis non-A, non-B. Seven of these possessed anti-HBs. Seven hepatitis patients possessed low anti-HBc IgM levels (counts between 1,506 and 2,111). Six of these had anti-HBs. The etiology of their hepatitis was held to be uncertain, but was probably not HBV, although Table 1 indicates that such a possibility cannot altogether be ruled out.

Some suggestive support for the designation of the first group as HBV came from subse-
quently observing the occurrence of HBV infections in three occupants within the homes of two of the members of this group (two clinical infections in one home, one subclinical in the other). Such infections were not observed in the other two groups.

DISCUSSION

The use of anti-HBc IgM in the serodiagnosis of hepatitis B infections had recently been reported by Cohen (2). The present report is in variance with his in that the anti-HBc IgM marker persists for considerably longer periods of time than the 2 months after onset seen in that study. This is almost certainly due to the radioimmunoassay technique used here to detect anti-HBc being more sensitive than the crossover immunoelectrophoresis test used in the previous study. Persistence of specific IgM antibody for more than 1 or 2 months after viral infections appears to be unusual, but is known to occur for 6 months after HAV infection (Lehmann, personal communication) and for at least 4 months after occasional rubella infections (6).

In localities such as Sydney, experience has shown that testing for HBsAg and anti-HAV IgM is sufficient to provide a clear-cut etiological diagnosis in most hepatitis cases. In the minority of cases which do not yield a positive result by either of these tests, the finding of a negative anti-HBc (total) result implies viral hepatitis of the non-A, non-B category. In those patients where anti-HBc is positive, a strongly positive anti-HBc IgM result probably implicates HBV and a negative result excludes it. However, where the anti-HBc IgM is weakly positive, the etiology is less certain. In the bulk of infections, it probably indicates that an HBV infection has occurred within relatively recent times (1 to 2 years), but that HBV is not responsible for the current bout. Occasionally, however, the weakly positive IgM result will be due to a current HBV infection in a patient who is responding poorly in anti-HBc IgM antibodies.

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LITERATURE CITED