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IMMUNOGLOBULIN M ANTIBODY TO HEPATITIS B CORE ANTIGEN (IgM ANTI-HBc) AS A MARKER OF INTERFERON THERAPY IN PATIENTS WITH PERSISTENT HEPATITIS B VIRUS INFECTION

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SUMMARY Immunoglobulin M antibody to hepatitis B core antigen (IgM anti-HBc) was measured by radioimmunoassay in the sera of 96 HBV carriers. IgM anti-HBc was detected in 17 of 66 patients with chronic active hepatitis and in 4 of 11 with liver cirrhosis. This antibody was not present in asymptomatic carriers or in patients with chronic persistent hepatitis. Testing of sequential samples revealed that the presence of IgM anti-HBc indicated active replication of HBV and at the same time an immune response to the virus. The relationship between IgM anti-HBc and the response to interferon (IFN) therapy was also studied. Results showed that IgM anti-HBc is a useful marker of the efficacy of interferon therapy.

INTRODUCTION

Antibody responses to acute hepatities B virus (HBV) infections are characterized by an early immunoglobulin M antibody response to hepatitis B core antigen (IgM anti-HBc) (Gelich et al., 1980; Lemon et al., 1981). Even in persistent infections with HBV, however, IgM anti-HBc is detectable by a sensitive radioimmunoassay (Sjogren et al., 1983). The clinical significance of this antibody in persistent HBV infection is not fully understood. IFN-α has been used for treatment of HBV infection, but many patients do not respond to this therapy. In this study we measured IgM anti-HBc in patients with persistatent HBV infection and examined the relation of the IgM anti-HBc level to the response to IFN therapy.

TABLE 1. *Immunoglobulin M antibody to hepatitis B core antigen in 96 serum samples of patients with persistent hepatitis B virus infection.*

Disease group	No. of patients	No. (percentage) of patients with positive IgM anti-HBc
ASC	10	0 (0%)
CPH	9	0 (0%)
CAH	66	17 (25%)
LC	11	4 (36%)
Total	96	21 (20%)

ASC: asymptomatic carrier
CPH: chronic persistent hepatitis
CAH: chronic active hepatitis
LC: liver cirrhosis
IgM anti-HBc: immunoglobulin M antibody to hepatitis B core antigen

MATERIALS AND METHODS

1. *Patients*

Studies were made on 96 patients. All patients had had HBsAg in their serum for at least one year before this study. The patients were classified into four groups. Ten patients, who had normal serum alanine transaminase (ALT) levels, were classified as asymptomatic HBV carriers. The other 86 patients underwent liver biopsy and were classified according to histologic findings as having chornic persistent heaptitis (CPH), chronic active hepatitis (CAH) or liver cirrhosis (LC).

2. *Interferons*

Human leukocyte interferon (HuIFN-α) was supplied by Kyoto Red Cross Blood Center, Kyoto, Japan. The specific activity of the preparation was more than 1×10^7 international units (IU)/mg protein. HuIFN-α was administered by intramuscular injection.

3. *Serological tests*

Hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs), hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe) were assayed by radioimmunoassay (Dinabot Laboratories, North Chicago, IL). Hepatitis B virus-DNA polymerase activity (DNAP) was assayed by determining ^{3}H-thymidine incorporation by the method of Fang et al. (1981). Immunoglobulin M antibody to hepatitis B core antigen (IgM anti-HBc) was measured with a commercial radioimmunoassay kit (ANTI HBc-M·RIAKIT®: Dinabot Laboratories, North Chicago, IL). In brief, the serum samples to be tested were diluted 100-fold in buffer and 100 μl of the diluted samples were incubated with anti-IgM-coated beads for 3 h at room temperature. The washed beads were incubated with hepatitis B core antigen (HBcAg) for 20 h at 15 C. The beads were then washed and ^{125}I-labeled human IgG antibody to HBcAg (anti-HBc) was added to each well. The plate was incubated for 3 h at 15 C. The beads were then washed again and transferred to tubes and radioactivity was counted in a gamma counter. The average counts (cpm) of three samples of positive controls and of negative controls were used for judgment of the cut off induces in the radioimmunoassay test for IgM-anti-HBc. The cut off index was calculated as follows:

$$\frac{\text{cpm of sample}}{\text{cpm of negative control} + 0.1\times\text{cpm of positive control}}$$

A ratio of more than 1.0 was considered to be positive.

RESULTS

IgM anti-HBc was detected in 17 of 66 patients with chronic active hepatitis and in 4 of 11 with liver cirrhosis. But it was not detected in asymptomatic carriers or in patients with chronic persistent hepatitis (Table 1).

IgM anti-HBc was detected in both HBeAg-positive sera and anti-HBe-positive sera. The cut off indices in most of the cases were less

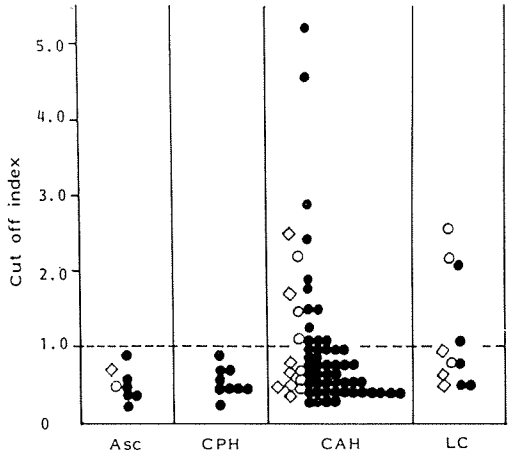

FIGURE 1. Analysis of IgM-anti-HBc by RIA and cut off indices in serum samples in four groups of liver diseases.

●; HBeAg$^+$/anti-HBe$^-$

○; HBeAg$^-$/anti-HBe$^+$

◇; Judgement not determined

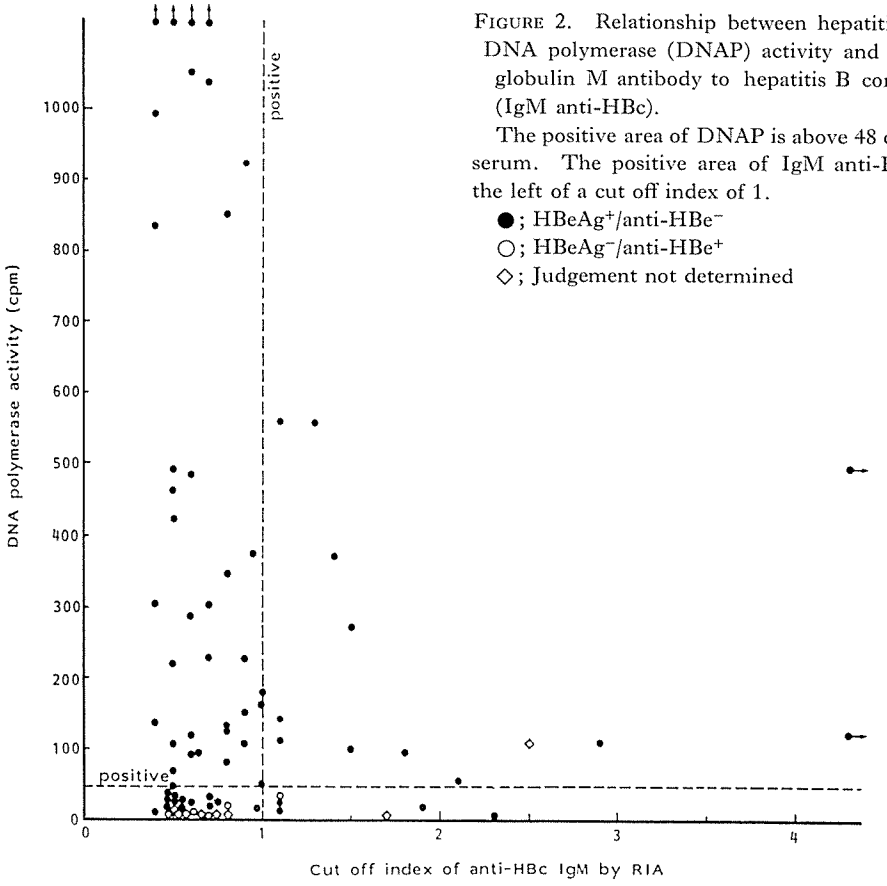

FIGURE 2. Relationship between hepatitis B virus DNA polymerase (DNAP) activity and immunoglobulin M antibody to hepatitis B core antigen (IgM anti-HBc).

The positive area of DNAP is above 48 cpm/ml of serum. The positive area of IgM anti-HBc is to the left of a cut off index of 1.

●; HBeAg$^+$/anti-HBe$^-$

○; HBeAg$^-$/anti-HBe$^+$

◇; Judgement not determined

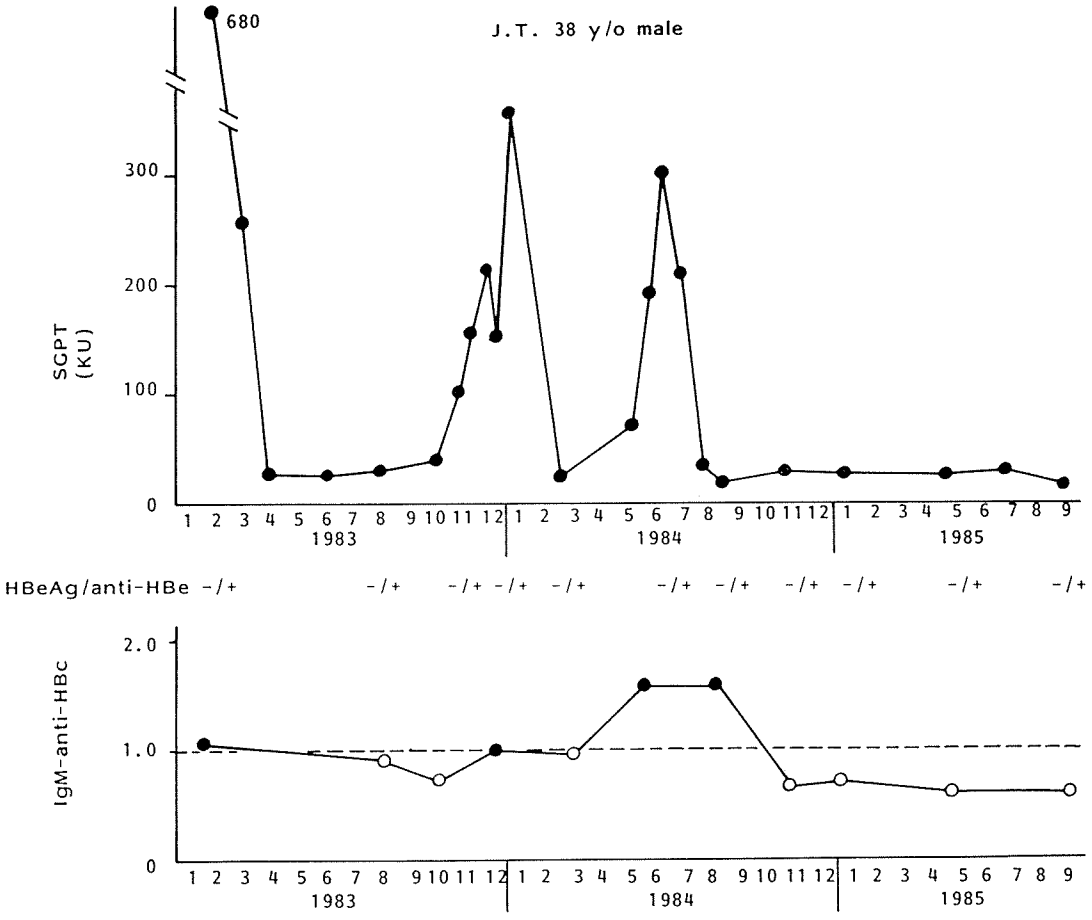

FIGURE 3. Results of serial tests for serum glutamic pyruvic transaminase (SGPT), hepatitis B e antigen (HBeAg) and antibody and immunoglobulin M antibody to hepatitis B core antigen (IgM anti-HBc) in a patient with chronic hepatitis B.

than 3.0 (Fig. 1).

The relationship between IgM anti-HBc and DNAP was examined (Fig. 2). IgM anti-HBc positive sera had less than 600 cpm of DNAP, whereas sera containing more than 800 cpm of DNAP had no detectable IgM anti-HBc.

IgM anti-HBc was measured in serial specimens from 11 patients with chronic active hepatitis. Three of these patients, who remained anti-HBe positive, exhibited temporary elevations of serum ALT during periods of exacerbation and IgM anti-HBc became seropositive during these periods (Fig. 3). In a HBeAg-positive patient who received Hu-IFN-α intramusculary at 1×10^7 IU initially and then at 1×10^6 IU once a week as a maintenance dose for 13 months, HBeAg, DNAP and IgM anti-HBc became undetectable during IFN therapy and remained negative for about 7 months after the end of the therapy. But with elevations of ALT and the reappearance of DNAP, IgM anti-HBc increased or became seropositive again (Fig. 4).

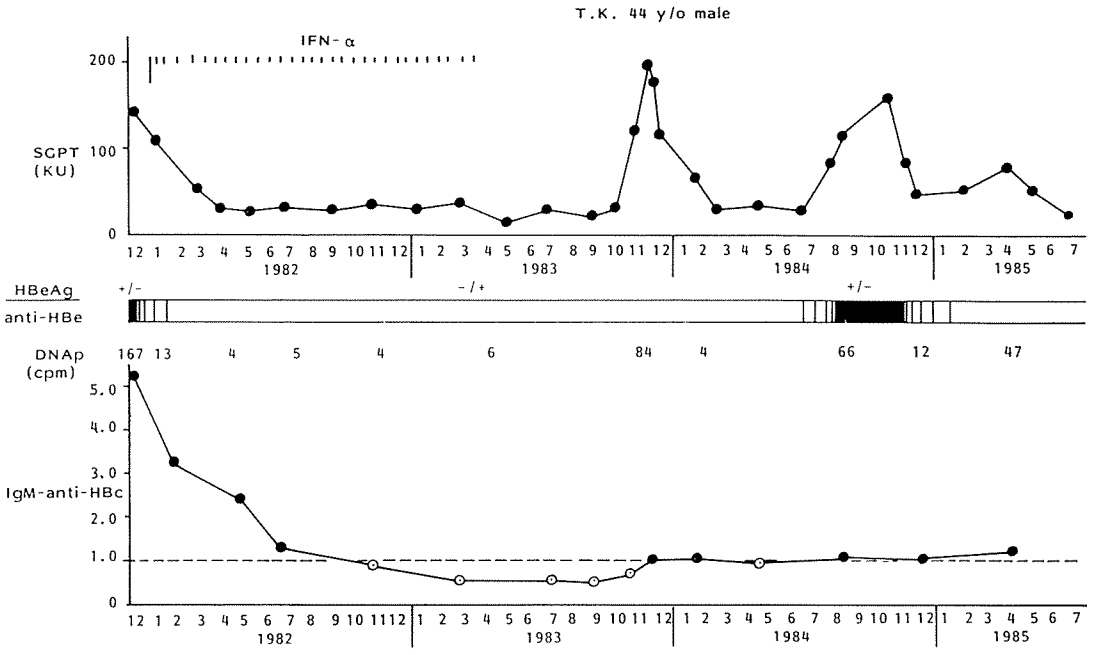

FIGURE 4. Results of serial tests for serum glutamic pyruvic transaminase (SGPT), hepatitis B e antigen and antibody, hepatitis B virus DNA polymerase (DNAP) activity and immunoglobulin M antibody to hepatitis B core antigen (IgM anti-HBc) in a patient with chronic hepatitis B, treated with HuIFN-α for 13 months. A DNAP value of more than 48 cpm is judged as positive and closed circules in the lower panel indicate positive values.

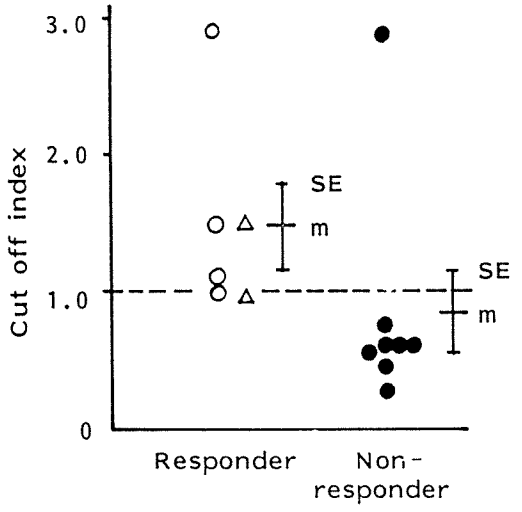

FIGURE 5. Immunoglobulin M antibody to hepatitis B core antigen (IgM anti-HBc) of responders and non-responders to antiviral therapy with IFN.

○; patients who showed seroconversion from hepatitis B e antigen (HBeAg) to anti-HBe within a year after IFN therapy.

△; patients with no HBeAg or antibody to HBeAg in the serum in a year after IFN therapy.

●; patients who remained HBeAg positive after IFN therapy.

The patients who responded to IFN therapy mostly had detectable IgM anti-HBc in the serum before IFN treatment. All but one of the patients who responded to IFN therapy, who showed seroconversion from HBeAg to anti-HBe or whose HBeAg and anti-HBe became negative, were seropositive for IgM anti-HBc before IFN therapy. On the contrary, all but one of the patients with cirrhotic change, who showed no response to IFN therapy, did not have IgM anti-HBc before IFN therapy (Fig. 5).

DISCUSSION

As shown in Table 1 and Fig. 1, IgM anti-HBc was detected only in the sera of some patients with chronic active hepatitis and with liver cirrhosis. This antibody was detected not only in HBeAg-positive sera but also in anti-HBe-positive sera. In one patient, the serum was IgM anti-HBc-positive with little or no DNAP, but no patient had IgM anti-HBc-positive serum with high DNAP.

Bianchi and Gudat (1979) reported that among patients with persistent HBV infection, those with chronic active hepatitis, liver cirrhosis, the anti-HBe-positive state or a low value of DNAP, show higher immune responses to HBV than asymptomatic carriers, or patients with chronic persistent hepatitis, the HBe-Ag-positive-state or a high value of DNAP. These facts indicate that during persistent HBV infection, IgM anti-HBc is detected only in conditions in which some immune response to HBV develops. Moreover the presence of this antibody in the serum can indicate histologically active liver disease. In the present study, measurements of sequential samples demonstrated a close relation between the presence of IgM anti-HBc and elevation of ALT. In anti-HBe-positive patients such as those shown in Fig. 3, the biochemical changes and the extent of HBV replication could be estimated by assaying IgM anti-HBc instead of direct viral markers such as DNAP activity or HBV-DNA. During suppression of active replication of HBV by HuIFN-α, the IgM anti-HBc level gradually decreased and became undetectable after DNAP activity became negative. IgM anti-HBc became detectable again when there was evidence of viral replication and spontaneous exacerbation of chronic hepatitis (Fig. 4). As Bianchi and Gudat (1979) reported, even when patients show an active immune response to HBV, their sera are IgM anti-HBc negative unless viral replication is active. Thus, IgM anti-HBc can be used as a serological marker to indicate active replication of HBV with a concomitant immune response to viral replication (Nowicki et al., 1984; Sjogren and Hoofnagle, 1985). The continuous presence of IgM anti-HBc for a long period suggests that the liver disease is progressive. Such cases, which require effective therapy, are likely to respond to the therapy, because of their high immune response to HBV. Indeed, a retrospective study on the effectiveness of IFN therapy indicated that IgM anti-HBc can also be a useful indicator when IFN therapy is applied (Fig. 5).

We conclude that IgM anti-HBc is a good marker of the immune response to HBV while replication of HBV is active. Furthermore, in patients with IgM anti-HBc before treatment, IFN therapy is likely to be effective. So, this antibody is useful as an indicator of when to apply IFN therapy.

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REFERENCES

Bianchi, L., Gudat, F. Immunopathology of hepatitis B. 1979. p. 371–392. *In* H. Popper, F.

Schaffner [ed.] Progress in Liver Disease Vol. VI, Grune & Stratton, New York.

Fang, C. T., Nath, N., Pielech, M., Dodd, R. Y. 1981. A modified technique for the detection of hepatitis B virus-specific DNA polymerase. J. Virol. Methods. 2: 349–356.

Gerlich, W. H., Luer, W., Thomssen, W., The study group for viral hepatitis of the Deutsche Forschungsgemeinschaft. 1980. Diagnosis of acute and inapparent hepatitis B virus infections by measurement of IgM antibody to hepatitis B core antigen. J. Infect. Dis. 142: 95–101.

Lemon, S. M., Gates, N. L., Simms, T. E., Bancroft, W. H. 1981. IgM antibody to hepatitis B core antigen as a diagnostic parameter of acute infection with hepatitis B virus. J. Infect. Dis. 143: 803–809.

Nowicki, M. J., Tong, M. J., Nair, P. V., Stevenson, D. 1984. Detection of anti-HBc IgM following predonisone treatment in patients with chronic active hepatitis B virus infection. Hepatology 4: 1129–1133.

Sjogren, M. H., Hoofnagle, J. H. 1985. Immunoglobulin M antibody to hepatitis B core antigen in patients with chronic type B hepatitis. Gastroenterology 89: 252–258.

Sjogren, M. H., Lemon, S. M. 1983. Low-molecular-weight IgM antibody to hepatitis B core antigen in chronic infections with hepatitis B virus. J. Infect. Dis. 148: 445–451.