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Abstract

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DETECTION OF LIVER HBc ANTIGEN AND ITS ANTIBODY IN SERA FROM VIRAL HEPATITIS BY THE IMMUNOFLUORESCENT COMPLEMENT TECHNIQUE

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Abstract: Hepatitis B core antigen (HBc Ag) and hepatitis B surface antigen (HBs Ag) were detected in the liver tissue of a patient with chronic aggressive hepatitis by the immunofluorescent complement technique. The presence of anti-HBc was examined by the same method in 67 human sera previously tested for HBs Ag, anti-HBs and s-GPT levels. HBc Ag was localized mainly in the nucleus and sometimes in the cytoplasm of the hepatic cells. HBs Ag was found only in the cytoplasm. The focal area of HBc Ag positive hepatic cells seemed to correspond to the HBs Ag positive cells. Double staining demonstrated the simultaneous presence of HBs Ag and HBc Ag in individual cells. Anti-HBc positive serum was found in 46 (68.7%) cases. Forty-eight cases (71.6%) indicated a combination of HBs Ag and anti-HBc.

It is not clear at present whether hepatitis B antigen (HB Ag) is localized in the nucleus or cytoplasm of the hepatic cell. HB Ag is separated into HBs Ag found on the surface of the Dane particle (1) and HBc Ag found in the core of the particle. The former has been found to be localized in the cytoplasm and the latter in the nucleus (2). The antibody to serum HBc Ag (anti-HBc) of patients with hepatitis B virus (HBV) infection has been investigated by the complement fixation (CF) reaction (3). The CF reaction requires a large amounts of purified HBc Ag. In practice, however, the yield from the serum of an asymptomatic carrier is quite low as the amount of Dane particles in the HB Ag particles is small (4). The present study uses the complement method of fluorescent antibody technique (5) to demonstrate the localization of HBc Ag in liver tissues and to determine anti-HBc in serum.

MATERIALS AND METHODS

Liver tissue positive for HBs Ag from one patient with chronic aggressive hepatitis (7) was frozen and sliced to 4μ thickness using a cryostat. The sections were fixed for 3 min in cold acetone and used as antigen. Acetone-fixed sections were covered by one drop of a mixture of specific anti-HBc prepared from immunized guinea pigs and complement or inactivated human serum and complement. The human serum was obtained from liver disease patients and from normal controls from the following three group: 26 cases with HBs Ag positive serum, 15 cases with anti HBs positive serum and 26 cases with HBs Ag negative and anti-HBs negative serum. The serum was diluted to 1 : 5. As complement, a drop of mixture containing the same volume of diluted serum solution at 1 : 2 obtained from guinea pigs (Kyokuto Co.) was used. The slides were incubated at 37°C for one hour. After washing with phosphate buffer saline (PBS), one drop of fluorescein isothiocyanate (FITC)-labelled anti-guinea pig γ globulin solution was added. The antibody titer was 64 or higher in 125μg of antigen protein by the micro-Ouchterlony method using immunized rabbits from our colony, and the ratio of FITC to protein was 1.5. The slides were incubated at 37°C for 40 min. After washing with PBS, the slides were covered and observed by fluorescent microscopy.

For comparative purposes, the following method were used for detection of HBs Ag and HBc Ag: (a) double staining (8) with FITC-labelled anti-HBs and tetramethyl rhodamine (TMR)-labelled anti-complement solution; (b) another complement method; (c) the direct and indirect fluorescent staining methods; and (d) the indirect fluorescent staining method. The other complement method used staining of fresh human serum complement and FITC-labelled anti-human Clq, C4 or C3 solutions (Meloy Lab.). The indirect method (6) used anti-HBc and FITC-labelled human IgG solution (Behringwerke Institute).

Serum glutamic pyruvic transaminase (s-GPT) activity was measured according to Reitman and Frankel (9). Tests for HBs Ag were performed by two methods: the immunoelectrophoretic (IEP) method described by Bussard and Huet (10) and the more sensitive immune adherence hemagglutination (IAHA) method described by Mayumi, Okochi and Nishioka (11). The anti-HBs tests were performed by IEP (10) and the passive hemagglutination (PHA) method according to the description of Vyas and Shulman (12). Purified HBs Ag and HBc Ag were prepared with the modified method of sucrose instead of cecium-chloride (13).

RESULTS

HBs Ag was detected in the cytoplasm of hepatic cells by the complement method and by both the indirect and direct methods (Fig. 1). HBc Ag stained by the complement method was granular all around the cytoplasm, near the membrane and in the nucleus of the hepatic cells (Figs. 2, 3). On staining with human complement method, HBc Ag was stained more clearly

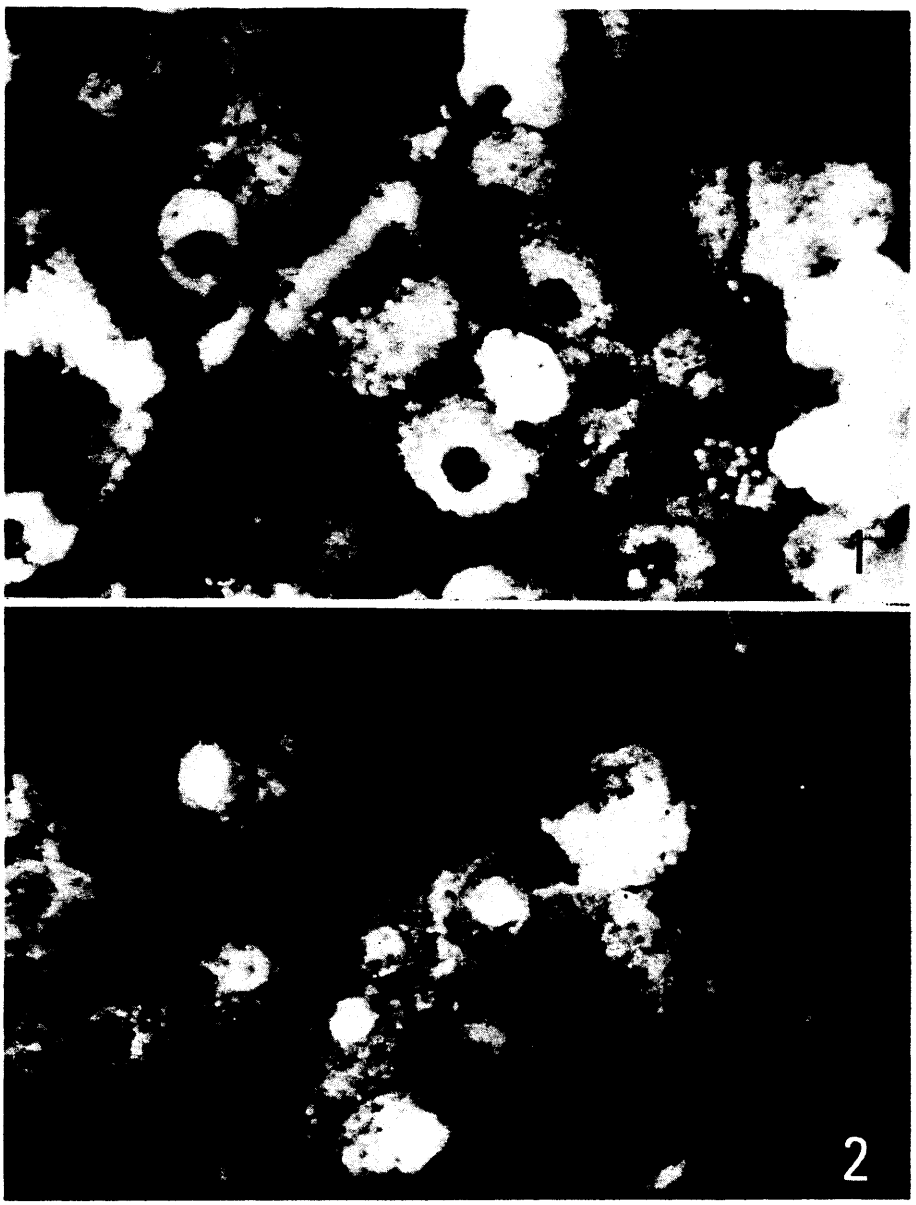

Fig. 1. Localization of HBs Ag in liver section from a case with chronic aggressive hepatitis by direct method. HBs Ag is localized only in the cytoplasm of hepatic cells and demonstrates the focal nature of the specifically stained cells. This corresponds to the characteristic focal spotty necrosis seen in viral hepatitis. ×400.

Fig. 2. The localization of HBc Ag in liver tissue from the same case using the guinea pig complement method. HBc Ag is localized mainly in the nucleus and sometimes in the cytoplasm of the hepatic cells. The focal nature of cells stained specifically corresponds to the localization of HBs Ag. ×400.

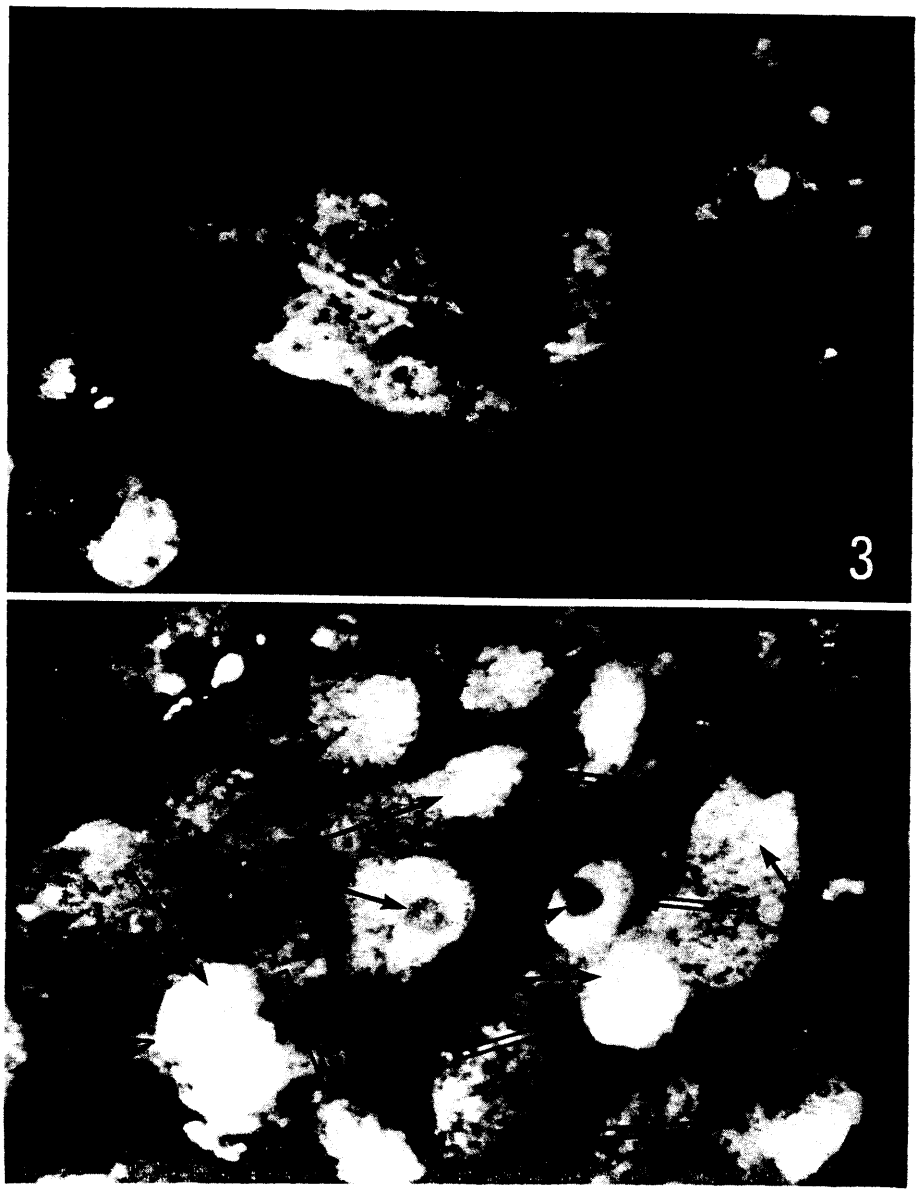

Fig. 3. This figure also demonstrates the localization of HBc Ag in another section. Hepatic cells with specifically stained nucleus are localized side by side from the peripheral to the central portions. Hepatic cells with stained nucleus are localized in the periphery, and the hepatic cells with stained nucleus and cytoplasm are in the center. ×400.

Fig. 4. HBc Ag and HBs Ag by the double staining method. The black arrows indicate reddish-brown fluorescence (HBc Ag); and the open arrows indicate yellow-green fluorescence (HBs Ag). ×400.

in the nucleus with FITC-labelled anti-C3 solution than with FITC-labelled anti-Clq or FITC-labelled anti-C4 solutions. The HBc Ag areas of the hepatic sections corresponded to those of HBs Ag positive regions. Double staining demonstrated HBs Ag and HBc Ag in the same hepatic cells (Fig. 4).

Anti-HBc positive serum cases with both HBs Ag positive and HBs Ag negative sera constituted a total of 46 (68.7%) of 67 cases (Table 1). Among

TABLE 1. HBs AG AND ANTI-HBs RELATIONSHIP TO ANTI-HBc BY THE IMMUNOFLUORESCENT COMPLEMENT METHOD

HBs Ag	Anti-HBs	Cases	Cases with anti-HBc (%)	s-GPT normal/abnormal (No. of anti-HBc)
+	−	26	26 (100.0)	20 (20) / 6 (6)
−	+	15	13 (86.7)	12 (12) / 3 (1)
−	−	26	7 (26.9)	11 (3) /15 (4)
	Total	67	46 (68.7)	43 (35) /24 (11)

HBs Ag (+): IEP and IAHA, positive; anti-HBs (+): PHA, positive

15 cases with negative HBs Ag, negative anti-HBs and abnormal s-GPT levels, four cases (26.7%) were positive for anti-HBc.

Positive complement tests were found for anti-HBc in a HBs Ag blood donor with PBS dilution titers of 2, 4, 5, 10 and 20 (+++), 40 and 80 (++) and 160 and 320 (+). The indirect method on this serum with FITC-labelled anti-human IgG yielded weak fluorescence at dilution titers of 2, 4 and 5 (+) and unclear fluorescence at dilution titers of 10 or greater (Table 2). This comparative study showed that the complement method was 100 times more sensitive than the indirect method.

TABLE 2. COMPARISON OF THE GUINEA PIG COMPLEMENT METHOD AND THE INDIRECT METHOD USING ANTI-HBc FROM SERUM OF A BLOOD DONOR WITH HBs AG AND NORMAL s-GPT LEVEL

	Dilution by PBS										
	2	4	5	10	20	40	80	160	320	640	1,280
Complement method	+++	+++	+++	+++	+++	+++	++	+	+	±	−
Indirect method	+	+	+	±	−	−	−	−	−	−	−

+++, very strong fluorescence; ++, strong fluorescence; +, weak fluorescence; ±, unclear fluorescence; −, negative fluorescence.

DISCUSSION

We first reported in Japan that HB Ag was localized mainly in the nucleus and slightly in the cytoplasm of hepatic cells using the direct method

with human anti-HB (14). It was difficult, however, to conduct experimental studies with antibody in animals. In the present follow-up study, human and guinea pig antibodies to HBc Ag were successfully demonstrated as having strong complement fixation to anti-HBc. The procedure used here is notable because it determines anti-HBc in serum rather simply.

Hoofnagle, Gerety and Barker (3) pointed out on anti-HBc with the CF reaction that cases with positive anti-HBc often had positive HBs Ag or anti-HBs. Our present findings on serum correspond closely with the results of the investigators.

However, among cases with positive anti-HBc without HBs Ag and anti-HBs, especially with abnormal s-GPT levels or so-called non-B hepatitis, 4 of 15 cases (26.7%) were found to be positive for anti-HBc. Forty-eight cases (71.6%) showed positive HBs Ag and positive anti-HBc, and all these cases probably had HBV.

On the other hand, our findings by double staining technique indicated that HBV proliferates in infected hepatic cells. The sequence of the Ag production may proceed as follows: (a) the hepatic cells are affected by HBV, (b) HBc Ag is produced in the nucleus of the hepatic cell; (c) HBs Ag is produced in the cytoplasm, and (d) HBs Ag or HBc Ag coated with HBs Ag appears in serum.

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