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BRIEF NOTE

DETECTION AND CHARACTERIZATION OF CIRCULATING IMMUNE COMPLEXES DURING ACUTE EXACERBATION OF CHRONIC VIRAL HEPATITIS

Kiyonori ARAKI, Takao TSUJI*, Kimiaki ONOUE,
Masao TSUCHIYA, Toru SHINOHARA,
Junichi INOUE and Hideo NAGASHIMA

*The First Department of Internal Medicine, Okayama University Medical School, Okayama 700 and *Health Research Center, Okayama University, Okayama 700, Japan*

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Abstract. For the detection and characterization of circulating immune complexes (CIC) in various liver diseases, a Clq binding test was used. Though the CIC level was almost normal in HB surface antigen (HBsAg) positive asymptomatic carriers, the level increased in patients with liver diseases. During acute exacerbation of chronic viral hepatitis, the CIC level reached peaks 1 to 3 weeks before and after the hepatic cell necrosis. Study of the sedimentation rates of CIC in various liver diseases showed CIC in the 19s-22s region and in the 7s-19s region. In acid buffer, CIC was dissociated into 5 to 6 components by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). In one case of HBsAg positive severe chronic aggressive hepatitis, CIC was composed of HBsAg, IgG and another three or four undetermined components. During acute exacerbation of chronic hepatitis, minor changes of these dissociation patterns of CIC were observed.

Key words: chronic viral hepatitis, circulating immune complexes, hepatic cell necrosis, HB surface antigen, Clq.

By 1969, Shulman and Barker (1) had already showed by complement fixation tests that, in patients with post-transfusion type B acute hepatitis, the anticomplementary activity becomes high before and after the peak of the titer of HB surface antigen (HBsAg) in blood. They suggested that the high titer may be due to HBs antigen antibody immune complexes (IC).

Recently, circulating immune complexes (CIC) have been detected by various methods (2-4). CIC were also detected very frequently and in high titer in patients with acute and chronic liver diseases (1-3, 5-7), and are considered to have a close relation to the pathogenesis of hepatic cell necrosis (1).

In the present study, we measured the CIC level with a Clq binding test

(Zubler *et al.* (4)), and also immunochemically investigated the antigenic components of CIC in order to clarify the pathogenetical role of immune complexes in hepatic cell necrosis.

Studies were undertaken on 29 healthy subjects and 157 patients with various liver diseases. Fifteen patients were histologically diagnosed as acute hepatitis, 74 patients as chronic hepatitis, 56 patients as liver cirrhosis and 12 patients as hepatocellular carcinoma. Clq was purified by the method of Yonemasu and Stroud (8), and radioiodinated by a lactoperoxidase method (9). The CIC level was measured by the ^{125}I-Clq binding test of Zubler *et al.* (4). In order to determine the sedimentation rate of CIC, 150 μl of the patient's serum was fractionated by 5–40% linear sucrose density gradient ultracentrifugation (10), and each 10 drop fraction was used for Clq binding test.

CIC was isolated from 1 ml of patient's serum according to the method used in the Clq binding test, washed three times with 2.5 w/v % polyethylene glycol solution, and radioiodinated by chloramine T method (11). The resultant ^{125}I-labelled CIC was dialysed against 0.2 M isotonic borate buffer pH 8 for 48 h, and then dissociated by dialysis against 0.02 M isotonic citrate buffer pH 3.2 for 12 h (12, 13). The dissociated ^{125}I-CIC was heated at 100°C for 2 min, then fractionated with 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (14). After electrophoresis, gels were sectioned into 1 mm slices, and each slice was counted with Aloka autowell gamma system ARC-451. Each radioactive peak was obtained with preparative SDS-PAGE, dialysed against phosphate buffered saline (PBS) pH 7.4, incubated with each antiserum (containing 2% Dextran T 500) at 4°C overnight, centrifuged at 12,000 rpm for 30 min, and the radioactivities of the resultant precipitate and supernatant were measured with a Aloka gamma counter.

The Clq binding activity (Clq BA) of 166 sera of various liver diseases is shown as the level of CIC in Fig. 1. The normal level of Clq BA was less than 10%. Clq BA's of the four sera from HBsAg positive asymptomatic carriers were 11%, 13%, 14% and 20%, respectively, being almost within normal limits. The CIC level in chronic hepatitis was, to some extent, related to the chronicity of viral hepatitis or to the degree of histological progress; all of the cases with hepatocellular carcinoma were complicated by liver cirrhosis but their CIC level was relatively low. In patients with CAH (2B) and LC, the mean CIC level was higher in the HBsAg positive group than in the HBsAg negative one. This may be due to HBsAg-IC (15), rheumatoid factor (Table 1) (16) or liver membrane antigen-IC (17). No significant difference in the mean CIC level was observed between the group with SGPT greater than 200 IU, and the group with SGPT less than 200 IU (Table 2). The mean CIC level correlated with the grade of rheumatoid factor (Table 1).

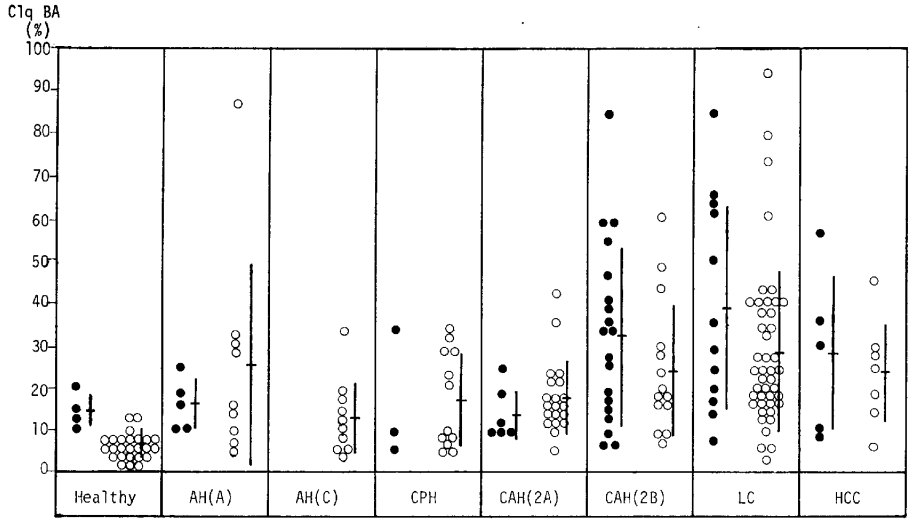

Fig. 1. Clq binding activity of sera of various liver diseases and healthy subjects. AH (A): acute hepatitis, acute stage, (C): convalescent stage, CPH: chronic persistent hepatitis, CAH (2A): chronic aggressive hepatitis, moderate, (2B): severe, LC: liver cirrhosis, HCC: hepatocellular carcinoma, ●: HBsAg positive, ○: HBsAg negative.

TABLE 1. RELATIONSHIP OF Clq BINDING ACTIVITY TO RHEUMATOID FACTOR (RF)

Grade of RF	Nnmber of Cases	Clq BA (%) (mean±SD)
−	43	20.9±12.4
±	21	23.8± 9.9
+	16	33.7±15.1
++	5	40.6±16.7

TABLE 2. RELATIONSHIP OF Clq BINDING ACTIVITY TO SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT)

SGPT Level (IU)	Number of Cases	Clq BA[a] (%) (mean±SD)
≧ 200	41	28.6±23.1
< 200	80	26.8±24.2

a. binding activity.

Changes in the level of CIC during acute exacerbation of chronic viral hepatitis are shown in Fig. 2. Two patterns of change were observed during 8 acute exacerbations in 6 cases (Fig. 3). In 6 out of 8 acute exacerbations, the CIC level reached peaks 1 to 3 weeks before and after the peaks of SGPT (Two

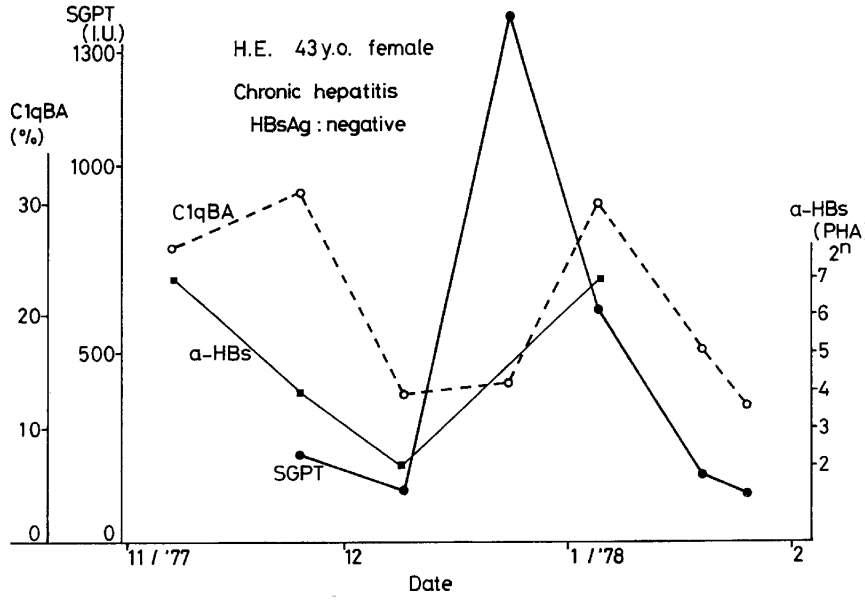

Fig. 2. Change of Clq BA in an acute exacerbation of chronic hepatitis (HBsAg negative).

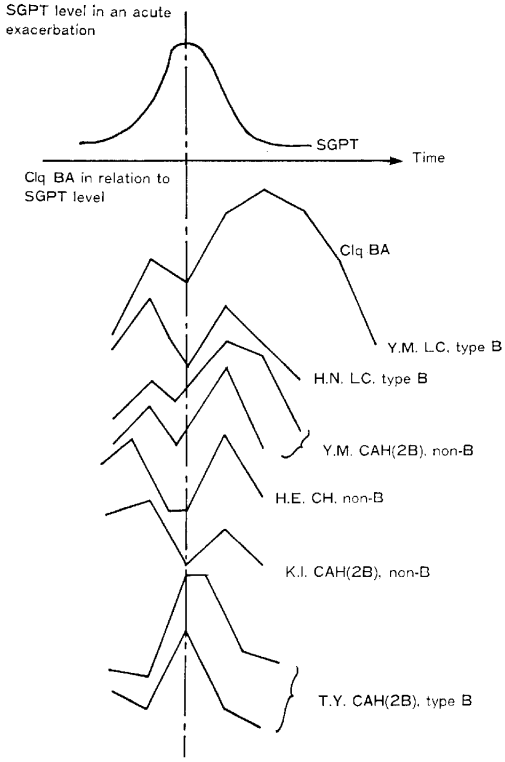

Fig. 3. Clq BA measured serially in acute exacerbations of chronic liver diseases. Y. M., H. N., Y. M., H. E., K. I. and T. Y. are patients' initials. The other abbreviations are the same as in Fig. 1.

peak pattern). In the other 2 acute exacerbations, only one peak of CIC level was observed, which coincided with the SGPT peak. The two peak pattern of CIC was common among B type and non-B type cases.

Clq reactive immune complexes were detected in two regions by sucrose density gradient examination, *i.e.* with sedimentation rates of 19s–22s and 7s–19s (Fig. 4) (6).

The clinical course of an acute exacerbation of HBsAg positive severe chronic aggressive hepatitis is illustrated in Fig. 5. Clp BA increased to 80% 2 weeks after the peak of SGPT (451 IU). The titer of HBsAg decreased rapidly at the same time. In the figure, the upper graphs [1], [2], [3] and [4] show the

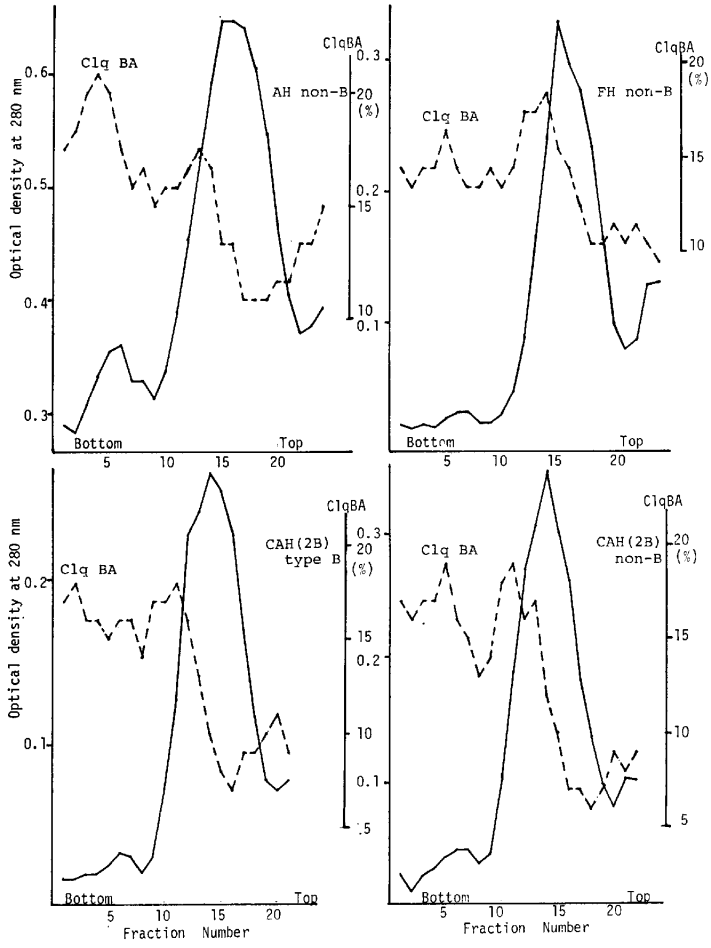

Fig. 4. Sucrose density gradient ultracentrifugal analyses of various hepatitis sera. (——) O. D., (- - -) Clq BA. FH: fulminant hepatitis.

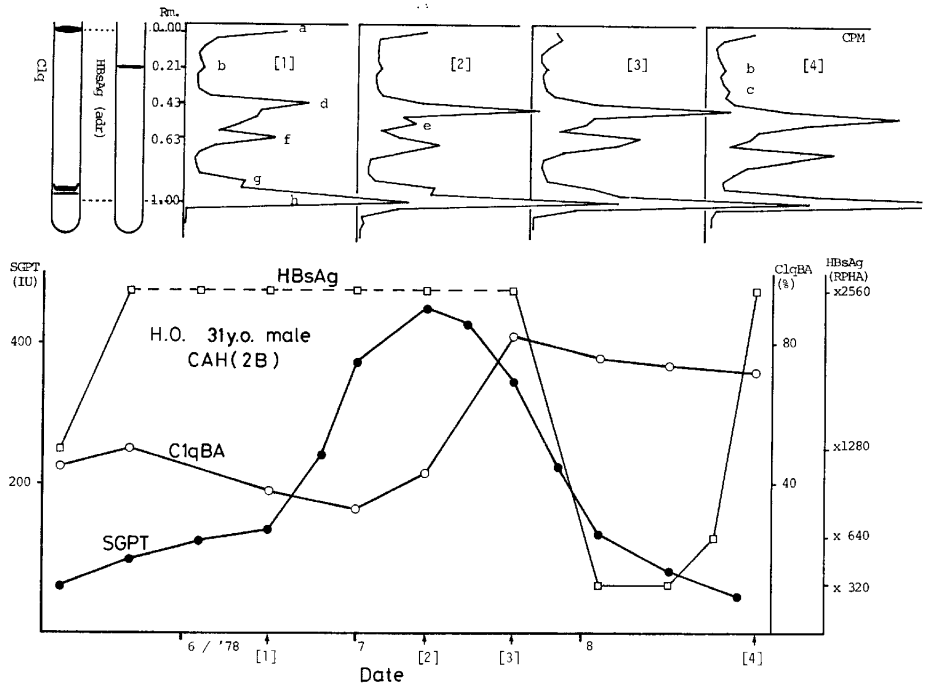

Fig. 5. Dissociation analysis (with SDS-PAGE) of circulating immune complexes in the course of an acute exacerbation of HBsAg positive severe chronic aggressive hepatitis. The upper graphs [1], [2], [3] and [4] show the results of dissociation analyses of circulating immune complexes at the stages of [1], [2], [3] and [4], respectively.

TABLE 3. DETERMINATION OF THE COMPONENTS OF CIRCULATING IMMUNE COMPLEXES

Antiserum	Fraction (Ppt. %)						
	a	b	d	e	f	g	h
Anti-IgG	8.3	5.0	3.4	11.8	44.1	5.6	1.7
Anti-HBsAg	17.0	14.5	6.8	7.3	8.5	14.4	2.1
Control (PBS)	3.7	2.6	1.6	2.1	1.8	1.1	1.5

Each fraction of ^{125}I-CIC was obtained with preparative SDS-PAGE, dialysed against PBS pH 7.4, incubated with antiserum (containing 2% Dextran T 500) at 4°C overnight, and centrifuged at 12,000 rpm for 30 min, and the radioactivities of the resultant precipitate and the radioactivities of the resultant precipitate and the supernatant were measured with a gamma counter.

$$\text{Ppt. } \% = \frac{\text{count of Ppt.}}{\text{total count}} \times 100$$

results of CIC dissociation analyses at the stages of [1], [2], [3] and [4], respectively. CIC dissociated in acid buffer was fractionated into 5 or 6 fractions with SDS-PAGE. Fraction (Frac.) a. was the undissociated CIC which could not

enter gel, Frac. h. being free ^{125}I. Purified Clq and purified HBsAg (adr) are also shown in the same figure, making their relative mobilities equal to each other in SDS-PAGE under the same conditions. The relative mobility of Frac. b. was the same as that of purified HBsAg (adr). Each fraction obtained with preparative SDS-PAGE was identified with the corresponding antiserum (Table 3). Frac. f. was a polypeptide fraction having an antigenic determinant of IgG, and anti-HBs antibody reacted with Frac. a., Frac. b. and Frac. g.. Frac. g also may be a polypeptide fraction having an antigenic determinant of HBsAg.

It has been thus shown that, at any stage of acute exacerbation of HBsAg positive chronic viral hepatitis, HBsAg is one of the antigens of which CIC is composed. Other antigens could not be identified, but minor changes in the CIC antigens were observed during an acute exacerbation.

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