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Abstract

Immune complexes in liver specimens from 10 patients with chronic liver diseases [2 with chronic persistent hepatitis (CPH), 3 with chronic aggressive hepatitis (CAH) of moderate activity, 3 with CAH of severe activity, and 2 with liver cirrhosis] were examined by a technique of direct immunofluorescence using FITC-labelled human purified Clq (FITC-Clq). FITC-Clq bound to the nuclei of all cells in liver tissue. After DNase treatment, positive nuclei were absent, but positive staining with FITC-Clq remained in amorphous deposits and hepatic cell membranes in the areas of piecemeal necrosis of four CAH patients. Since FITC-Clq could not be demonstrated in the liver tissue of CPH and liver cirrhosis which contained no piecemeal necrosis, positive fluorescence in the liver of CAH patients was thought to indicate immune complexes bound to FITC-Clq. The fact that these positive substances, however, were few in number, may be the result of physiological mechanisms of immune clearance which rapidly eliminate immune complexes from the body.

KEYWORDS: immune complex, chronic aggressive hepatitis, Clq, DNA, Clq-binding test.

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— BRIEF NOTE —

TISSUE IMMUNE COMPLEXES DEMONSTRATED IN THE LIVER OF PATIENTS WITH CHRONIC AGGRESSIVE HEPATITIS USING FITC-LABELLED HUMAN Clq

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Abstract. Immune complexes in liver specimens from 10 patients with chronic liver diseases [2 with chronic persistent hepatitis (CPH), 3 with chronic aggressive hepatitis (CAH) of moderate activity, 3 with CAH of severe activity, and 2 with liver cirrhosis] were examined by a technique of direct immunofluorescence using FITC-labelled human purified Clq (FITC-Clq). FITC-Clq bound to the nuclei of all cells in liver tissue. After DNase treatment, positive nuclei were absent, but positive staining with FITC-Clq remained in amorphous deposits and hepatic cell membranes in the areas of piecemeal necrosis of four CAH patients. Since FITC-Clq could not be demonstrated in the liver tissue of CPH and liver cirrhosis which contained no piecemeal necrosis, positive fluorescence in the liver of CAH patients was thought to indicate immune complexes bound to FITC-Clq. The fact that these positive substances, however, were few in number, may be the result of physiological mechanisms of immune clearance which rapidly eliminate immune complexes from the body.

Key words: immune complex, chronic aggressive hepatitis, Clq, DNA, Clq-binding test.

Immune complexes circulate in a variety of chronic inflammatory and neoplastic diseases, especially chronic liver diseases (1-5), but their role is not clear. In recent years it has become possible to detect these circulating immune complexes by Raji cell assay (6), Clq-binding test (3, 7) and the technique of

immunofluorescence (8). As a results, serum factors bound to Clq are known to include IgG of 19S and about 7S (4, 8), although the antigen components of these immune complexes are not known. The localization of immune complexes in the liver tissue of HBs antigen (HBsAg) positive cases has been investigated by the staining of complement components such as Clq (9) using the technique of immunofluorescence. In this way, the localization of Clq binding substance in liver tissue specimens with chronic aggressive hepatitis has been demonstrated.

An attempt was made to detect Clq-binding substances in tissue using FITC-labelled, purified Clq. Specimens were prepared from frozen liver tissues taken from 8 patients histologically diagnosed as having chronic hepatitis according to the European classification (10) : 2 cases of chronic persistent hepatitis (CPH), 3 cases of chronic aggressive hepatitis of moderate activity and 3 cases of chronic aggressive hepatitis of severe activity, and from 2 patients of liver cirrhosis. The sera from these patients were tested for HBsAg by reversed passive hemagglutination (RPHA) (11) and for HBsAb by passive hemagglutination (PHA) (12).

Clq was purified by the method of Yonemasu and Stroud (13). FITC isomer I (BBL) was mixed with purified Clq protein in a 1 : 60 ratio and conjugated at 4°C for 6 h (14). This FITC-labelled Clq solution was eluted first in a Sephadex G-25, then in a DEAE cellulose column using 0.005 M phosphate buffer saline (pH 7.2) containing 2% W/V bovine serum albumin (BSA-PBS) for each column. Purified FITC-labelled Clq was used as the staining reagent without absorption with acetone liver powder. The reagent solution was incubated on each of the unfixed 6 μ cryostat liver sections and on the cold acetone fixed sections at 37°C for 45 min. As control materials, normal rat liver sections were used.

In order to determine the activity of purified FITC-labelled Clq solution, a solution of Cohn F-II (NBC) [heated to give aggregated gammaglobulin (AGG)] and a solution of ssDNA were prepared according to Zubler s method (7). Each of these solutions was mixed with the purified FITC-labelled Clq solution, and then incubated. The supernatant of these mixtures was assayed at an optical density of 490 m μ (Fig. 1).

As specific staining for immune complexes, each section was also treated at 37°C for 1 h with 1 drop of DNase solution containing 100 μg of DNase I (Worthington BC) in 1 ml of pH 6 9, 0.05 M phosphate, 0.003 M magnesium chroride, 0.05 M saline buffer (15). After the treatment, this section was washed three times with PBS, and stained by FITC-Clq solution. For the control experiments, heat treatment at 63°C for 10 min was used to inactivate tissue complements and 0.01% portamine sulfate (portamine-PBS) treatment

Standard AGG or DNA dissolved in normal human serum 50 μl
0.2 M EDTA, pH 7.5 100 μl

↓ Stand at 37°C for 30 min and then chilled at 0°C

Mixed with
FITC-Clq disolved in 1% BSA-VBS 50 μl
3% PEG 1,000 μl

↓ Incubated at 0°C for 60 min and then centrifuged at 1,500 × g for 20 min at 4°C

Supernatant

Photometry of FITC (optical density at 490 mμ)

Fig. 1. Procedure of FITC-Clq binding test

was also performed to remove non-specific binding of FITC-Clq, instead of the DNase treatment.

Figs. 2 and 3 showed the binding activity of FITC-Clq to AGG and ssDNA. Staining with FITC-Clq as seen in normal rats was detected in the nuclei of hepatic cells, Kupffer cells, infiltrated cells and fibrocytes in liver tissues of all 10 patients (Fig. 4). In addition to these findings, positive fluorescence in 4 CAH cases was also localized as a homogenous pattern in sinusoidal walls and

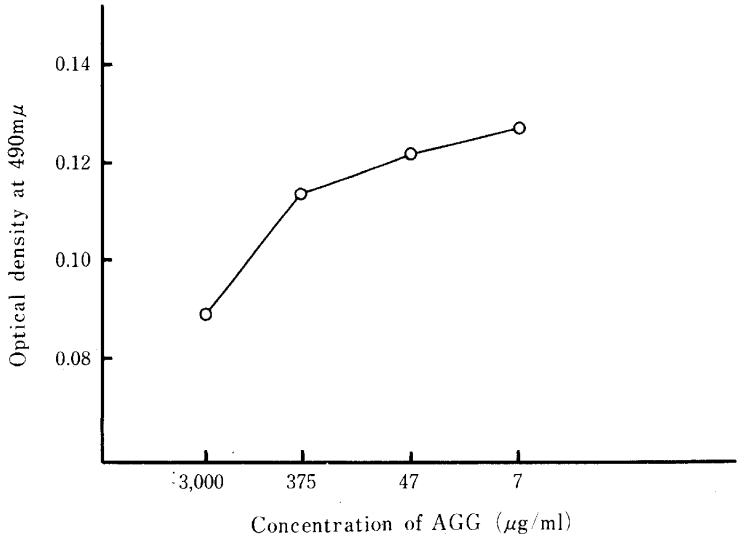

Fig. 2. AGG standard curve of FITC-Clq binding test

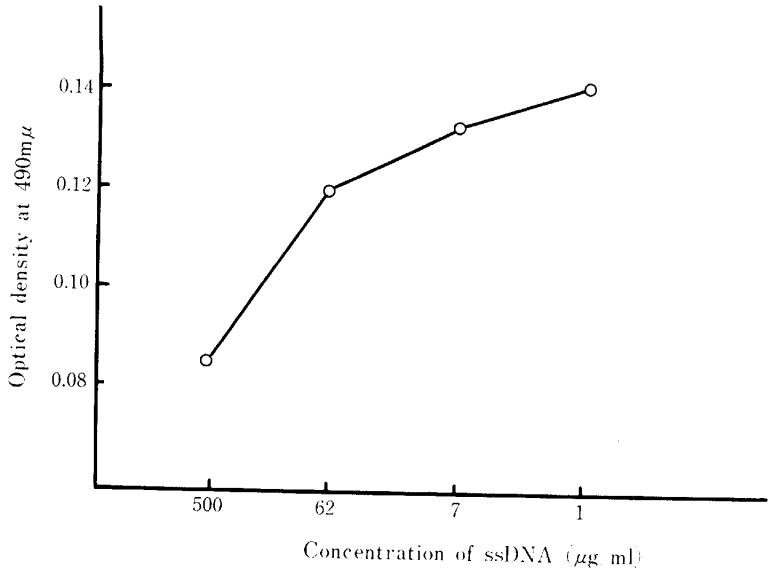

Fig. 3. ssDNA standard curve of FITC-Clq binding test

portal areas, and in areas of piecemeal necrosis. After treatment with DNase, positive fluorescence disappeared in all specimens excepting CAH cases. In the liver of CAH cases only a little fluorescence remained as a pattern of immune deposit in portal areas (Fig. 5a). A little fluorescence also remained in sinusoidal walls and areas of piecemeal necrosis in which FITC-Clq was localized to degenerated hepatic cell membranes and granular substances (Table 1 and Fig. 5b). Heat treatment and protamine-PBS treatment for the control experiments showed the same results as those on tissue sections without DNase treatment. There was no difference between unfixed and acetone fixed sections.

In the past, circulating immune complexes were detected by the Clq-binding test (3, 7), but in the present study, it was demonstrated that FITC-Clq reacted to various cell nuclei and ssDNA. Therefore, DNase treatment was performed on tissue sections for detection of immune complexes. As a result, FITC-Clq bound not only nuclei of various cells in liver tissue not treated with DNase but also denatured DNA (ssDNA). These results agree with the report that purified Clq bound to native DNA (dsDNA) and ssDNA under certain circumstances (16, 17), and the maximum specific Clq binding activity was 12% for ssDNA (60 μg/ml) (7). The fact that FITC-Clq reacted to DNA at a low level *in vitro* (Fig. 3) suggests that circulating Clq binding activity is influenced by a low level of serum DNA in liver disease, in contrast to SLE in which there is a high level of DNA (7). Fluorescence positive substance which remained in the liver tissue of CAH cases after DNase treatment was very few, suggesting

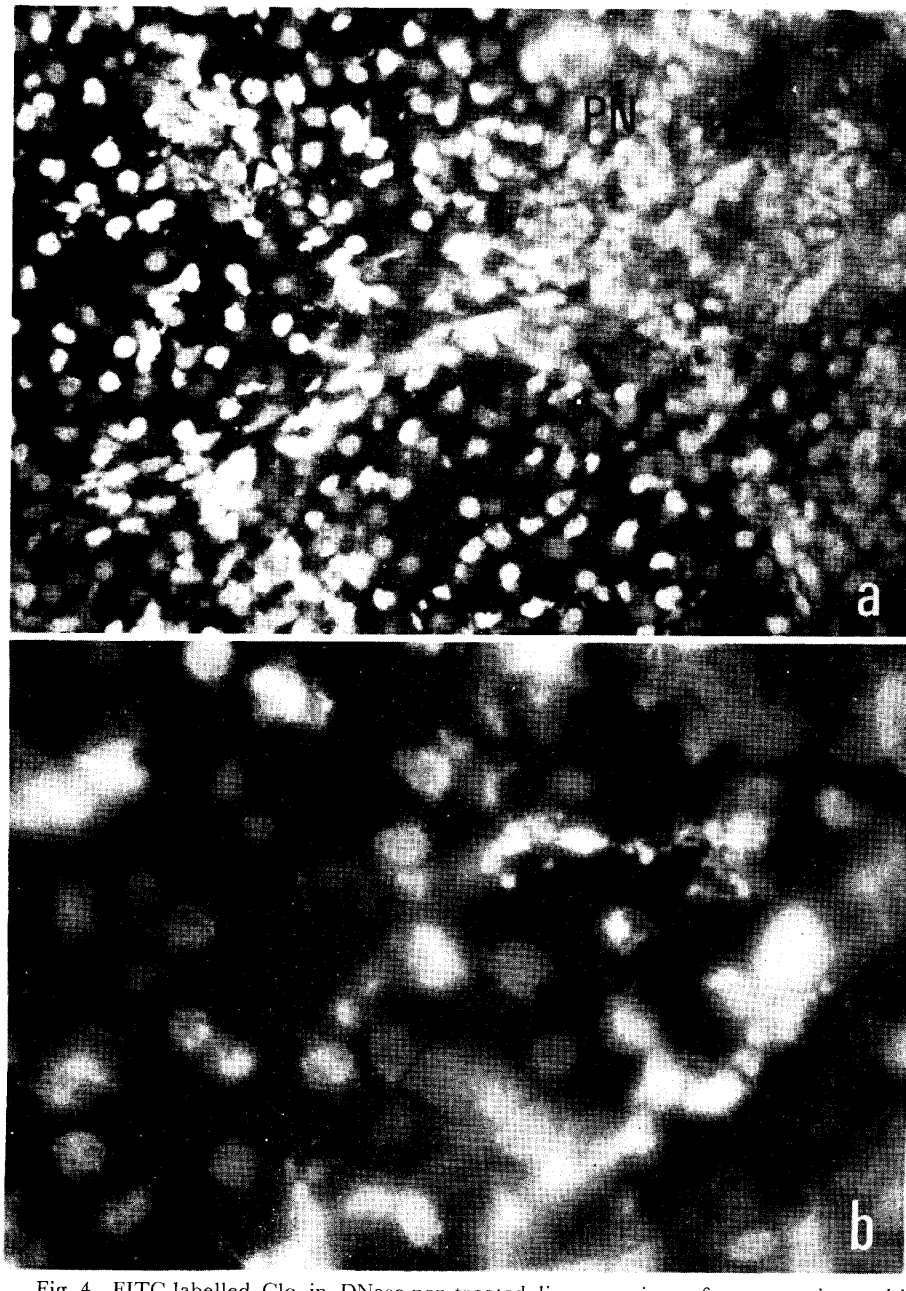

Fig. 4. FITC-labelled Clq in DNase-non-treated liver specimen from a patient with severe chronic aggressive hepatitis. a. FITC-labelled Clq is detected in the nuclei of hepatic cells, Kupffer cells, infiltrative cells and fibrocytes (160×). PN : area of piecemeal necrosis. b. High power view of the same section. Note positive Clq of a homogenous diffuse pattern in area of piecemeal necrosis (400×).

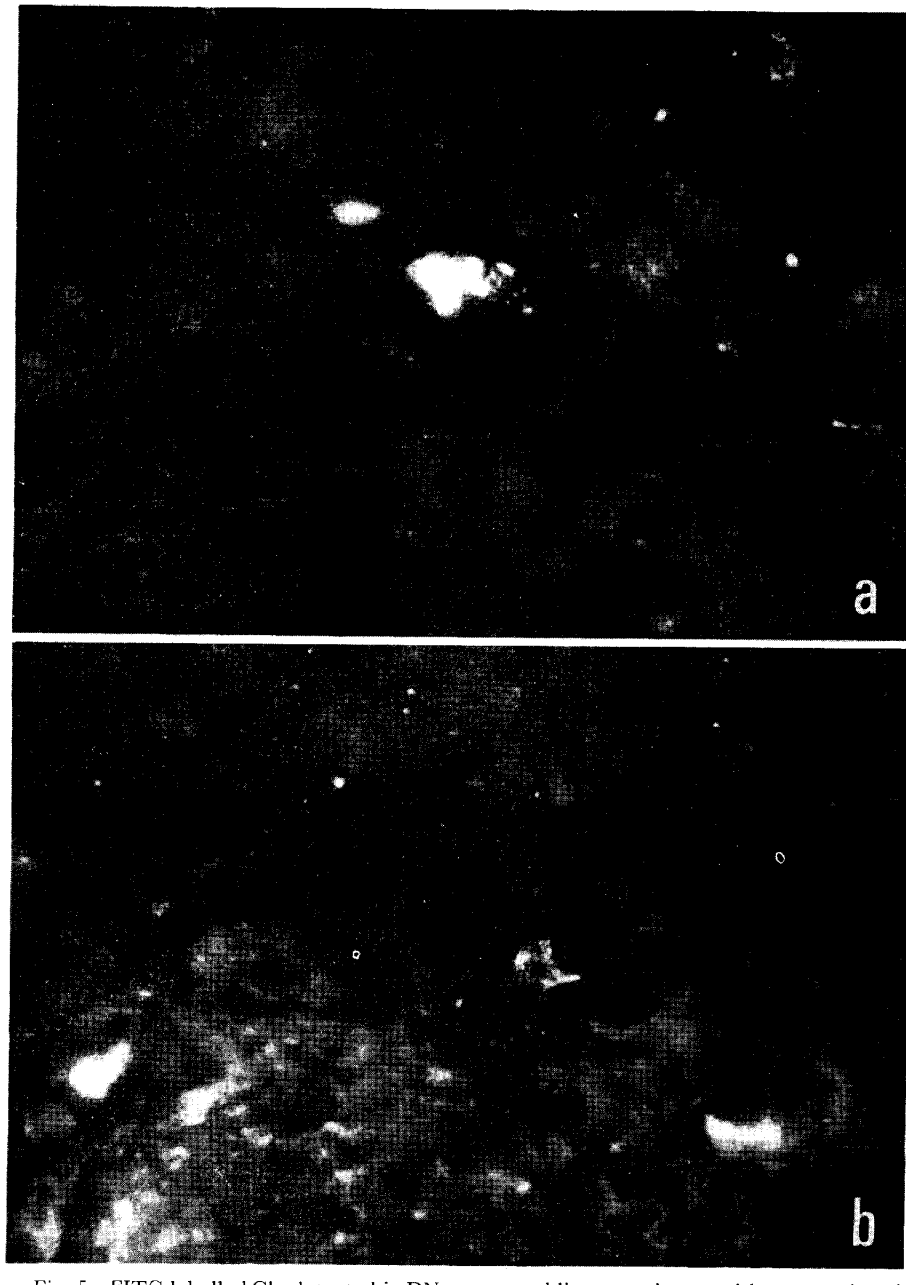

Fig. 5. FITC-labelled Clq detected in DNase-treated liver specimens with severe chronic aggressive hepatitis. a. Positive Clq is shown in the portal area as a pattern of immune deposit (400×). b. Positive Clq is localized to degenerative hepatic cell membrane and granular substances in the area of piecemeal necrosis and to the walls of sinusoidal veins (400×).

TABLE 1. DETECTION OF POSITIVE FLUORESCENCE IN LIVER TISSUE AFTER DNase TREATMENT

Diagnosis[a]	Serum HBsAg	No. cases	No. positive	Grading of fluorescence
CPH	positive	2	0	−, −
Moderate CAH	positive	2	1	−, +
	negative	1	0	−
Severe CAH	positive	2	2	+, ++
	negative	1	1	+
Liver cirrhosis	positive	2	0	−, −
Total		10	4	

a CPH=chronic persistent hepatitis, CAH=chronic aggressive hepatitis

that immune complexes formed in the body are rapidly cleared by phagocytic activity of Kupffer cells and by excretion mechanisms of the kidney as mentioned by Pernice *et al.* (18) and Thomas *et al.* (18–20).

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