Clq and Immune Complexes in Liver Cirrhosis Sera

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YOSHIDA, H., SATO, M., WATANABE, S., NISHIMAKI, T., MORITO, T., KASUKAWA, R. and NAKAI, M. Clq and Immune Complexes in Liver Cirrhosis Sera. Tohoku J. exp. Med., 1983, 140 (1), 73-79 — The highest mean value of the serum Clq concentrations among chronic liver diseases was obtained in patients with liver cirrhosis (LC). Clq serum levels seemed to increase along the progression of liver damage. Serum levels of Clq and CH50 in patients with LC and systemic lupus erythematosus (SLE) were simultaneously estimated. No correlation between Clq and CH50 levels was observed in LC sera, while a significant correlation was demonstrated in SLE sera. It could be suggested that the mechanism for the low CH50 in LC sera seemed different from that in SLE sera, and that the activation of classical complement pathway was not the predominant cause in LC sera. Correlations of serum levels between immune complexes and Clq were examined in patients with LC and SLE. It could be deduced from the results of a positive correlation in LC sera and the reverse tendency in SLE sera that the significances of Clq and immune complexes in LC sera differed from those in SLE sera. — liver cirrhosis; complement; Clq; immune complex

Clq, one of the subcomponents of the first complement component, is known to react with the Fc portion of IgG and IgM and the reaction initiates the activation of classical complement pathway (Müller-Eberhard 1975). Low levels of serum Clq were reported in patients with systemic lupus erythematosus (SLE), glomerulonephritis and immunodeficiencies (Hanauer and Christian 1967; O’Connell et al. 1967; Kohler and Bensel 1969; Kohler and Müller-Eberhard 1972; Stroud et al. 1970). In SLE sera, low levels of serum Clq as well as hemolytic complement activity (CH50) were known to be the result induced predominantly by the activation of complement through binding with immune complexes (Kohler and Bensel 1969; Kohler and Müller-Eberhard 1972).

In liver diseases, low serum CH50 levels have been observed in patients in chronic and/or advanced stages. Since the liver is the major site of synthesis of most of the complement components, the low serum complement level has been proposed to be induced by the defective synthesis of the components (Fox et al.

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1971; Finlayson et al. 1972; Torisu et al. 1972; Kourilsky et al. 1973). However, another possibility of activation or consumption of complement during immune processes was suggested in such cases as acute viral hepatitis (Alpert et al. 1971; Onion et al. 1971) and chronic liver diseases (Inai et al. 1976; Kondo et al. 1976; Kitamura et al. 1977).

In our previous studies (Morito et al. 1977; Yoshida et al. 1980), high levels of serum Clq were demonstrated in the advanced liver diseases though they had lower serum CH50 titers. In this communication, Clq in sera of patients with various liver diseases was estimated and the significance of the higher levels of Clq in liver cirrhosis sera were discussed in reference to the amounts of immune complex.

**MATERIALS AND METHODS**

**Sera and plasmas.** Sera and plasmas were obtained from patients with various liver diseases and collagen diseases in our clinic and from healthy staff members of the clinic. They were separated after incubating the blood samples at room temperature for 1 hr and were stored at −70°C until used. The diagnosis of the liver diseases was made on the basis of histologic findings of the biopsy samples.

**Assay of hemolytic complement activity (CH50).** This was followed according to the procedure described by Mayer (1961).

**Estimation of Clq protein concentration.** This was performed by means of the single radial immunodiffusion described previously (Yoshida et al. 1980). Briefly, 1% of agarose (Sigma Chem. Co., St. Louis, USA) solution dissolved in a physiologic saline containing 0.01 M EDTA·Na₂ (Wako Chem. Co., Tokyo) was mixed with rabbit antiserum to Clq (Behring-Werke Ltd., Marburg, West Germany) at 47°C to make a final concentration of the antiserum at 1:80. This was poured into plastic plates and solidified at room temperature, and wells with a diameter of 3 mm were prepared, into which 7 μl of the samples were placed. Clq concentrations were estimated by measuring the diameters of precipitation rings after incubation at room temperature for 3 days. In each experiment, the pooled serum taken from 12 healthy staff members was employed as the standard. It contained Clq at 34.5 μl/ml. The purified Clq was obtained according to the procedure described by Yonemasu and Stroud (1971). The protein amount was estimated by the method of Lowry et al. (1951). Since Clq seemed more labile in the purified form than in whole serum even though samples were divided and stored at −70°C, the pooled native serum was preferably employed as the standard of 100%. The concentrations of Clq in sera were expressed as percent of the standard.

**Detection of immune complexes.** Clq solid phase radioimmunoassay was employed following the procedures described by Hay et al. (1976), and the amount of immune complex in sera was expressed as an equivalent amount of aggregated IgG which was obtained by heating (62°C for 20 min) the pooled 7 S fractions purified from human Cohn’s fraction II (Sigma Chem. Co.) through DEAE (Nakarai Chem. Ltd., Osaka)-column chromatography followed by Sephadex G-200 (Pharmacia, Uppsala) gel filtration.

**RESULTS**

Clq concentrations in sera of patients with various liver diseases are summarized in Fig. 1. In 35 sera of patients with nonalcoholic liver cirrhosis, Clq concentrations ranged widely from 86.0 to 225.0%, and the mean value was 146.8%. Only one serum contained a lower amount of Clq than that of the pooled serum and no particular clinical difference was noticed between this case and those with
higher amounts of Clq. Seven sera of patients with hepatoma, all of which were associated with nonalcoholic liver cirrhosis, showed a mean value of 131.5%. In chronic hepatitis, the mean value of 25 sera of patients in active stages and that of 10 sera of patients in inactive stages were 129.6% and 104.4%, respectively. Sera of patients with nonspecific reactive hepatitis, which was diagnosed according to the criteria by Chung et al. (1964), contained Clq at a mean value of 102% which was close to the value of pooled healthy serum. Acute hepatitis sera, in which transaminase levels were abnormal ranging from 60 to 1000, showed the highest mean value of 161.7%. From these results, it could be speculated that serum Clq concentrations in chronic liver diseases seemed to increase along the progression of the liver damages.

Serum levels of Clq and CH50 were simultaneously estimated in liver cirrhosis and SLE, and the results are summarized in Fig. 2. In 24 sera of patients with nonalcoholic liver cirrhosis including 2 primary biliary cirrhosis (PBC), wide ranges of Clq and CH50 levels were observed and higher CH50 levels of more than 50 units were shown in cases of PBC. No significant correlation ($r=0.09$) between Clq and CH50 levels was observed. In SLE sera, however, a significant correlation ($r=0.67, p<0.02$) between Clq and CH50 levels was demonstrated. It was

![Fig. 1. Clq levels in various liver disease sera. The concentrations were estimated by means of a single radial immunodiffusion. The pooled healthy serum was taken as 100%. LC, liver cirrhosis; CAH, chronic active hepatitis; CIH, chronic inactive hepatitis; NSRH, nonspecific reactive hepatitis; AH, acute hepatitis.](image-url)
Fig. 2. Correlations of serum Clq concentrations and CH50 titers in patients with liver cirrhosis and SLE. •, primary biliary cirrhosis.

Fig. 3. Correlations of serum immune complex levels and Clq concentrations in patients with SLE (o) and liver cirrhosis (△). The correlations were described as follows: 

SLE: \[ y = -5.9x + 678 \ (r = -0.44, 0.2 < p < 0.3) \]

Liver cirrhosis: \[ y = 0.81x - 15.0 \ (r = 0.77, p < 0.01) \]
suggested that the mechanism for the low levels of CH50 in liver cirrhosis sera is different from that in SLE sera. In SLE, the lower serum Clq levels have been considered to result from the activation of classical complement pathway.

Immune complex titers in sera were compared with the serum Clq concentrations. Thirty-five nonalcoholic liver cirrhosis sera and 25 SLE sera were chosen randomly. Nine of 35 liver cirrhosis sera (26%) and 10 of 25 SLE sera (40%) were shown to be positive for immune complexes and their concentrations in relation to the amount of Clq are shown in Fig. 3. A positive correlation ($r=0.77$, $p<0.01$) was demonstrated in the liver cirrhosis sera, while the reverse tendency between them was suggested in SLE sera. These results indicate that the significances of Clq and immune complexes in liver cirrhosis sera differed from those in SLE sera.

**DISCUSSION**

Participation of the immunologic mechanism in the decrease of serum complement activity was reported in cases of acute viral hepatitis accompanied with extrahepatic manifestations (Alpert et al. 1971; Onion et al. 1971) and in some cases of chronic liver diseases (Kondo et al. 1976; Kitamura et al. 1977). In the latter, the activation of classical complement pathway was proposed on the basis of the findings that serum CH50 as well as early components of complement were reduced by incubation of the sera around 4°C (Kitamura et al. 1977). In our study (Yoshida et al. 1980), however, serum Clq concentrations were shown to be higher in cases of chronic liver diseases, though their serum complement activities were lower and sometimes undetectable. It was shown in in vitro study that the serum Clq activity of the chronic liver diseases was not affected by the cold incubation, although CH50 was markedly reduced. Among chronic cases, the Clq level was the highest in liver cirrhosis sera and its serum levels, contrary to the serum CH50 levels, seemed to increase with the progression of liver damages. The Clq levels in sera of patients with liver cirrhosis did not correlate significantly with CH50 levels, although a significant correlation was obtained in SLE. In SLE, Clq and CH50 were significantly and correlatively decreased as the result of immunological activation by binding with immune complexes (Kohler and Bensel, 1969; Kohler and Müller-Eberhard 1972). It could be speculated that the low complement activity in liver cirrhosis sera might not be brought on by the activation of classical complement pathway.

A positive correlation between the amounts of immune complexes and Clq concentrations in liver cirrhosis sera and the reverse tendency between them in SLE sera would also support this hypothesis. The mechanism of high serum Clq concentrations in liver cirrhosis is still unknown. However, it seems plausible to consider such a phenomenon as Clq-bypath activation, and the hypothesis could be employed in cases not only of chronic liver diseases but of Clq deficiency (Wara et al. 1975; Berkel et al. 1979). Potter et al. (1980) reported a marked increase of fractional catabolic rates of Clq in PBC and HBs-Ag positive chronic liver
diseases. PBC patients contained higher Clq concentrations and showed increased catabolic rates, and both positive and negative cases of HBs-Ag showed lower serum Clq concentrations, while higher catabolic rates were observed only in HBs-Ag positive group. In this study, however, 22 out of 25 cases of chronic active hepatitis (CAH) were shown to contain higher serum Clq concentrations than that of healthy pooled serum. The average value of whole CAH sera was 129.6%. Further studies including metabolism of Clq seem necessary to clarify its role in the mechanism of the decrease in the hemolytic complement activity.

Although numerous studies including ours (Morito et al. 1977) have been performed on the detection of immune complexes, the significance of their participation in the development of hepatic lesions is still obscure. In order to explain the increased levels of circulating immune complexes in advanced liver diseases, two possibilities should be considered; increased formation and decreased hepatic clearance, that is, the disturbance of function of the reticuloendothelial system (Mannik et al. 1971; Thomas et al. 1978). The presence of serum Clq in higher concentrations might suggest the exemption of its consumption by binding with immune complexes in vivo, although immune complexes were detected by means of Clq radioimmunoassay. Another possibility to explain the discrepancy, high Clq concentrations and low CH50 titers, is that only a small amount of Clq might have the capacity to react with immune complexes.

It seems to be hesitating to determine the pathogenetic significance of immune complexes in liver diseases from the standpoint of serum Clq levels, and further metabolic studies may offer more information on the significance of Clq in LC.

References


