Serum complement in chronic liver disease

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SUMMARY Total serum haemolytic complement activity (CH_{50}) and the serum concentrations of both the third and fourth components of the complement system (C3 and C4) have been measured in 29 control subjects, 92 patients with chronic hepatocellular disease, and eight patients with large duct biliary tract obstruction. The mean C4 concentration was reduced in all types of chronic liver disease studied. However, the mean CH_{50} and C3 values were increased in compensated primary biliary cirrhosis, were relatively normal in non-cirrhotic chronic active hepatitis, and were decreased in cryptogenic cirrhosis, particularly when ascites was present. There was a significant correlation between CH_{50} and C3 in patients with chronic liver disease but no correlation between CH_{50} and C4 or between C3 and C4. Raised values for CH_{50} and C3 in primary biliary cirrhosis may be due at least in part to concomitant cholestasis since these values tend to be raised in patients with large duct biliary tract obstruction. Although primary biliary cirrhosis, chronic active hepatitis, and cryptogenic cirrhosis are considered to be part of a spectrum of chronic liver disease associated with disturbed immunity, the results of this study emphasize that there are clearly definable differences between these diseases in terms of the pattern of changes in serum complement.

Although some types of chronic liver disease, such as chronic active hepatitis, primary biliary cirrhosis, and cryptogenic cirrhosis are usually associated with evidence of disturbed immunity, so far no immune mechanism has been demonstrated to be of fundamental importance in the pathogenesis of these diseases. One well established cause of cell lysis and death involves the complement system, classically activated by antigen-antibody interaction on a cell surface (Rapp and Borsos, 1970). If this immune process is responsible for liver cell injury in patients with chronic liver disease, there may be associated changes in serum complement. However, changes in the complement system may also arise as a consequence of the disease process itself.

It has previously been shown that patients with chronic liver disease tend to have a reduced total serum complement haemolytic activity (Goldner, 1929; Jordan, 1953; Asherson, 1960; Townes, 1967; Inai, Fujikawa, Naguki, Takahashi, Ozono, and Ishida, 1967; Farini, Gambari, Fagiolo *et al*, 1970; Pagaltos, Smith, Eddleston, and Williams, 1971; De Meo and Anderson, 1972; Torisu, Yokoyama, Kohler, Durst, Martineau, Schroter, Amemiya, Groth, and Starzl, 1972) and a reduced serum concentration of the third component of comple-

ment (C3), (West, Northway, and Davis, 1964; MacLachlan, Rodnan, Cooper, and Fennell, 1965; Grob, Jemelka, and Muller, 1971; Deo Meo and Anderson, 1972; Torisu *et al*, 1972) but the values obtained for the two estimates in these studies were correlated neither with the patients' clinical state nor with the results of liver function tests. A comparison has been made between total serum complement haemolytic activity and the serum concentration of individual components of the complement system in patients with acute hepatitis (Alpert, Isselbacher, and Schur, 1971) and serial measurements of the serum concentration of C3 have been made in patients with acute infectious hepatitis and serum hepatitis (Kosmidis and Leader-Williams, 1972). We report here the results of measurements of the total serum complement haemolytic activity (CH_{50}) and the serum concentrations of both the third and fourth components of the complement system (C3 and C4) in control subjects and patients with chronic liver disease, including, in particular, patients with chronic active hepatitis, primary biliary cirrhosis, and cryptogenic cirrhosis. The data have been correlated both with the patients' clinical state and with the results of routine tests of liver function. Although chronic active hepatitis, primary biliary cirrhosis, and cryptogenic cirrhosis are considered to represent part of a spectrum of immunological

liver disease (Read, 1971), the results of the present studies indicate that there are strikingly different patterns of change in the complement system in each of these disorders.

Patients and Methods

CONTROL SUBJECTS AND PATIENTS

The subjects investigated comprised 29 normal healthy controls, 92 patients with chronic hepatocellular disease, and eight patients with cholestasis unassociated with chronic liver disease. The patients with chronic liver disease included 30 patients with cryptogenic cirrhosis, 22 patients with chronic active hepatitis, 32 patients with primary biliary cirrhosis, and eight patients with cirrhosis of the alcoholic. The diagnosis in all of the patients with liver disease had been confirmed by needle biopsy of the liver. The distribution of age in all groups was similar with the exception of the group of patients with chronic active hepatitis who had a younger mean age distribution. There was a predominance of females in the groups of patients with primary biliary cirrhosis and chronic active hepatitis.

METHODS

Ten ml of clotted peripheral blood was obtained from each subject. The serum was separated and stored at -20°C . Estimations of CH_{50} and C3 and C4 concentrations were made within 10 days of the sample being taken.

Whole serum complement activity

Duplicate determinations of the whole serum complement activity were made by the method of Kabat and Mayer (1961) as modified by Rapp and Borsos (1970). Sheep red blood cells in Alsever's solution (Burrough's Wellcome Ltd), after sensitization with rabbit haemolysin (Burrough's Wellcome Ltd), were incubated for one hour at 37°C with varying dilutions of the test serum, made up to a total volume of 7.5 ml. The 50% lysis point was then determined by means of a Van Krogh plot, using values (determined spectrophotometrically) between 20 and 80% lysis. The serum volume for 50% lysis was then expressed as a reciprocal value to give CH_{50} units. All samples were processed in parallel with a standard guinea-pig complement source (Burrough's Wellcome Ltd). The reproducibility of the method was better than $\pm 10\%$.

The third component of complement (C3)

The serum C3 concentration was measured using the single radial immunodiffusion method of Mancini, Carbonara, and Heremans (1955) and antihuman C3 prepared in rabbits (Hyland immunoplates). Three reference sera of known C3 concentration (50, 100, 300

mg/100 ml) (Hyland Laboratories) were incubated at room temperature for 16 to 24 hours, together with each set of test sera. All estimates of C3 concentration were made in duplicate. The reproducibility of the method was $\pm 10\%$ which is in satisfactory agreement with the data of Kohler and Muller-Eberhard (1967).

The fourth component of complement (C4)

The serum C4 concentration was measured using the single radial immunodiffusion method of Mancini (Mancini *et al*, 1965) and an antihuman C4 anti-serum prepared in rabbits (Behringwerke). A reference serum of known C4 concentration (18 mg/100 ml) (Behringwerke) was diluted to give several solutions containing different concentrations of C4. These solutions were incubated for 24 hours at room temperature with each set of appropriately diluted test sera. The concentration of C4 in diluted test sera always fell within the linear portions of the standard curve. All estimates of C4 concentration were made in duplicate. The reproducibility of the method was $\pm 15\%$.

Results

The table gives the mean, SEM, and the number of estimates of CH_{50} , C3, and C4 respectively for each group of subjects studied. The values for each of the three estimates obtained in normal subjects agree satisfactorily with other similar data on normal subjects obtained by others (Klemperer, Gotoff, Alper, Levin, and Rosen, 1965; Kohler and Muller-Eberhard, 1967; Alpert *et al*, 1971).

DIFFERENCES IN SERUM COMPLEMENT IN DIFFERENT DISEASE GROUPS

The mean C4 concentration was significantly reduced in all groups of patients with chronic liver disease: in primary biliary cirrhosis ($P < 0.0005$), in chronic active hepatitis ($P < 0.0005$), in cryptogenic cirrhosis ($P < 0.0005$), and in alcoholic cirrhosis ($P < 0.0005$).

There were clearly defined differences in the mean values for CH_{50} and C3 concentration in different groups of patients.

Primary biliary cirrhosis

In patients with well compensated primary biliary cirrhosis, whereas the mean C4 concentration was significantly reduced ($P < 0.05$), the mean CH_{50} value was increased ($P < 0.0025$), and the mean C3 concentration was also increased ($P < 0.0005$) (fig 1). However, in patients with primary biliary cirrhosis who had developed ascites both the mean CH_{50} ($P < 0.005$) and the mean C3 concentration ($P < 0.0005$) were decreased.

		<i>CH</i> ₅₀	<i>C3</i> (mg/100 ml)	<i>C4</i> (mg/100 ml)
Normals	Mean	34.9	137	31.6
	SEM	1.5	5.0	1.3
	N	29	29	28
Cirrhosis	Mean	22.1	84.7	17.2
	SEM	1.7	7.1	1.6
	N	42	42	41
Primary biliary cirrhosis	Mean	36.3	170	23.5
	SEM	2.5	11.3	1.9
	N	32	32	30
Alcoholic cirrhosis	Mean	33.8	134	14.7
	SEM	5.3	23.0	3.1
	N	8	8	8
Chronic active hepatitis without cirrhosis	Mean	29.6	147	20.4
	SEM	3.8	13.6	3.0
	N	10	10	8
Chronic active hepatitis with cirrhosis	Mean	21.9	64.6	10.8
	SEM	3.5	10.1	8.1
	N	12	12	12
Cirrhosis without ascites	Mean	25.9	105	18.2
	SEM	2.5	9.7	2.3
	N	18	18	17
Cirrhosis with ascites	Mean	19.4	67.6	16.5
	SEM	2.2	8.7	2.2
	N	24	24	24
Primary biliary cirrhosis without ascites	Mean	43.7	208.6	26.7
	SEM	2.5	9.9	2.2
	N	23	22	25
Primary biliary cirrhosis with ascites	Mean	24.0	91.8	15.2
	SEM	3.8	9.0	3.0
	N	9	10	5
Biliary tract obstruction	Mean	33.0	205	42.6
	SEM	4.3	25.2	4.9
	N	8	8	8

Table Serum *CH*₅₀ and *C3* and *C4* concentrations in chronic liver disease

Fig 1 Serum *CH*₅₀ and *C3* and *C4* concentrations in normal control subjects and patients with compensated primary biliary cirrhosis.

Chronic active hepatitis

In contrast to patients with primary biliary cirrhosis, patients who had chronic active hepatitis that had not progressed to cirrhosis had values for CH_{50} and C3 concentration that were little changed from those in normal subjects. However, in those patients with chronic active hepatitis that had progressed to cirrhosis, the mean CH_{50} was significantly decreased ($P < 0.0005$) and the mean C3 concentration was also decreased ($P < 0.0005$).

Cryptogenic cirrhosis

In the group of patients with cryptogenic cirrhosis, the mean values for both CH_{50} ($P < 0.0005$) and C3 concentration ($P < 0.0005$) were reduced. The mean values for CH_{50} and C3 were particularly low once the patients had developed ascites (see table).

Cirrhosis of the alcoholic

The CH_{50} and C3 values in the small group of patients with alcoholic cirrhosis were similar to those in normal subjects.

Large duct biliary tract obstruction

In patients with cholestasis due to large duct biliary tract obstruction, the mean CH_{50} was little changed but the mean C3 concentration was elevated ($P < 0.0005$), as was the mean C4 concentration ($P < 0.0025$).

CORRELATIONS BETWEEN CH_{50} , C3, AND C4

There was a significant correlation between CH_{50} and C3 in patients with chronic liver disease ($r =$

0.672; $P < 0.001$) (fig 2). In contrast there was no significant correlation between CH_{50} and C4 or between C3 and C4.

RELATIONSHIP OF SERUM COMPLEMENT TO BIOCHEMICAL TESTS OF LIVER FUNCTION

The relationships between values of CH_{50} , C3, and C4, and the serum levels of bilirubin, alkaline phosphatase, aspartate transaminase, gamma globulin, and albumin were examined. There were slight but significant correlations between CH_{50} and serum albumin ($r = 0.434$, $P < 0.001$) and between C3 and serum albumin ($r = 0.277$, $P < 0.05$) in the whole group of patients with chronic liver disease.

There was a negative correlation between C4 and serum gamma globulin in the whole group of patients with chronic liver disease ($r = 0.398$, $P < 0.001$). Thus a low C4 concentration tended to be associated with a high gamma globulin concentration.

No other significant correlations were found. In particular values for CH_{50} and C3 concentration in patients with primary biliary cirrhosis and large duct biliary tract obstruction did not correlate closely with the degree of cholestasis as measured by the serum levels of bilirubin and alkaline phosphatase.

OTHER OBSERVATIONS

No relationship was found between values of CH_{50} and C3 and C4 concentration and the titres of anti-mitochondrial antibody, smooth muscle antibody, and antinuclear factor.

Only four patients' sera were positive for hepatitis-associated antigen and the complement levels in these patients were not obviously different from those in

Fig 2 Correlation between serum CH_{50} and C3 concentration in patients with chronic hepatocellular disease.

similar patients whose sera were negative for this antigen.

No appreciable differences were apparent in values of CH_{50} , C3, and C4 in patients who were receiving prednisolone and/or azothiaprime, compared with those in patients who were not receiving immunosuppressive therapy.

Discussion

The studies reported here provide results of three different measurements of serum complement in a group of patients with different types of chronic liver disease. Our studies supplement and extend the data of Finlayson, Krohn, Fauconnet, and Anderson (1972). Of particular interest is the finding that although chronic active hepatitis, primary biliary cirrhosis, and cryptogenic cirrhosis are considered to be part of a spectrum of chronic liver disease associated with disturbed immunity (Read, 1971), the results of the current studies indicate that there are clearly definable differences between these diseases in terms of changes in serum complement. In some of the diseases studied, values for CH_{50} , C3, and C4 did not change in parallel. This observation is highlighted by the lack of correlation between CH_{50} and C4 and between C3 and C4.

The pattern of values of serum complement in patients with compensated primary biliary cirrhosis was distinct. Whereas both the mean CH_{50} and the mean C3 concentration were increased, the mean C4 concentration was reduced. These results are at variance with those of Pagaltsos *et al* (1971), who found consistently low values for CH_{50} in patients with primary biliary cirrhosis. However, these authors did not state whether their patients were in a well compensated phase of the disease. The values for CH_{50} and C3 concentration did not correlate closely with the degree of cholestasis as measured by the serum levels of alkaline phosphatase and bilirubin. However, raised values for total haemolytic complement activity have been found in patients with cholestasis due to large duct biliary tract obstruction (Jordan, 1953; Mandel and Lange, 1955; Asherson, 1960; Farini *et al*, 1970; Pagaltsos *et al*, 1971). The value for CH_{50} was also raised in a patient with cholestasis due to chlorpromazine (Asherson, 1960). It would appear, therefore, that cholestasis could be a non-immunological cause of an increase in the total haemolytic complement activity.

The increased serum concentration of C3 in patients with primary biliary cirrhosis is probably associated with an increased synthetic rate of this protein, and hence a higher catabolic rate. In one patient with primary biliary cirrhosis and a raised serum concentration of C3, highly purified radio-

iodinated C3 was injected intravenously and the plasma disappearance curve of radioactivity was defined. The calculated turnover rate of the protein was 9.99% of the intravascular pool per hour in the patient, compared with 1.87% in a control subject who had received some of the same labelled preparation (Potter, Trueman, and Jones, 1973).

An increased value for CH_{50} may be associated with an increased serum concentration of one or more of the components of the complement system. That an increased mean value for CH_{50} is associated with a decreased mean C4 concentration in primary biliary cirrhosis suggests that C4 is normally present in excess in the serum for normal activation of the complement system. Indeed, it has been suggested that other components, C2 and C5, are rate limiting in the complement cascade (Cooper and Muller-Eberhard, 1970). The association of increased concentrations of C3 with decreased concentrations of C4 in primary biliary cirrhosis implies that there are fundamentally different mechanisms involved in the control of the serum concentrations of these two proteins.

In contrast to CH_{50} and C3, the mean C4 concentration was reduced in all groups of patients with chronic liver disease. The observation that low C4 concentration tended to be associated with high serum gamma globulin values suggests an association with abnormal immunological activity but does not necessarily imply the utilization of C4 in an immune process.

There are a number of possible mechanisms which could account for low values for serum complement in patients with chronic liver disease. (1) If some complement components are synthesized by the hepatic parenchymal cells (Alper, Johnson, Birtch, and Moore, 1969; Colten, 1972), their synthetic rate may be reduced as a direct consequence of injury and death of these cells. (2) If some complement components are synthesized at extrahepatic sites (Colten, Borsos, and Rapp, 1966; Littleton, Kessler, and Burkholder, 1970), then their synthetic rate may be reduced by the effect of metabolic disturbances associated with liver failure on the synthetic mechanisms. (3) There may be circulating inactivators of complement components (Spitzer, Vallota, Forristal, Sudor, Stitzel, Davis, and West, 1969; Peters, Martin, Weinstein, Cameron, Barratt, Ogg, and Lachmann, 1972). (4) There may be increased consumption by antigen-antibody complexes and consequently increased activation of complement (Lachmann, Muller-Eberhard, Kunkel, and Paronetto, 1961; Wilson and Dixon, 1970; Kohler and Ten Bonsel, 1969). (5) There may be increased endogenous catabolism or increased loss of these

components into the urinary or gastrointestinal tracts. (6) Finally, the tendency for the plasma volume to be increased in these patients could be a contributory factor (Lieberman and Reynolds, 1967).

Further insight into the mechanisms responsible for low values for serum complement in chronic liver disease may come from studies of the metabolic behaviour *in vivo* of purified radioiodinated complement components and the screening of sera from these patients for conversion products of individual components. In the present series the values for serum complement tended to be particularly low when there was evidence of severe hepatic decompensation, such as ascites. Even in these patients or in patients with massive hepatic necrosis (Fox, Dudley, and Sherlock, 1971) it cannot be assumed that low levels of serum complement can be attributed wholly to a reduced synthetic rate by the liver. The sites of synthesis of the various complement components are not established with certainty and even if their synthetic rate is reduced, there may be concomitant increased consumption of these components.

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