Circulating immune complexes and complement concentrations in patients with alcoholic liver disease

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SUMMARY A prospective evaluation of circulating immune complexes (CIC) and the activity of the complement system was undertaken in 53 alcoholic patients just before diagnostic liver biopsy. Circulating immune complexes were detected in 39% of patients with alcoholic steatosis ($n = 26$), 58% of patients with alcoholic hepatitis ($n = 12$), and 60% of patients with alcoholic cirrhosis ($n = 15$). No significant difference was found between the three groups of patients. The activity of the complement system was within reference limits in the majority of patients and only slight differences were detected between the three groups. No significant differences were observed in liver biochemistry and complement concentrations in CIC-positive and CIC-negative patients. Detection of CIC in patients with alcoholic liver disease does not seem to be of any diagnostic value or play any pathogenic role. The high prevalence of CIC in these patients may be due to a depressing effect of ethanol on clearance of CIC or to increased immunological reactivity, or to both.

Alcoholic hepatitis and alcoholic cirrhosis have been associated with an increased prevalence of circulating immune complexes (CIC).¹⁻³ Patients with alcoholic steatosis, however, have been reported CIC-negative in most studies.¹⁻³

Circulating alcoholic hyaline and antibody to alcoholic hyalin have been demonstrated in patients with alcoholic hepatitis, but not in patients with alcoholic steatosis and cirrhosis.⁴ It has been suggested that alcoholic hyalin (or other immunogenic material), being released into circulation as a consequence of tissue destruction, led to the formation of immune complexes.² Furthermore, severe liver-cell damage is supposed to cause a reduction in concentration of certain complement components.⁵

Detection of CIC and estimation of complement concentrations and activity may therefore have diagnostic and pathogenic implications in patients with alcoholic liver diseases. In order to examine the relation between presence of CIC, the complement activity in serum and degree of liver damage, we studied chronic alcoholics just before diagnostic liver biopsy.

Patients and methods

PATIENTS

After informed consent 59 consecutive patients admitting a consumption of more than 50 g ethanol per day for at least two years and suspected of alcoholic liver disease were studied. Four patients with clinical signs of infection, one with rheumatoid arthritis and one with gout were excluded to avoid any possible effects of these diseases on the generation of CIC. The remaining 53 patients then formed the study group. Their age ranged from 25 to 66 yr (median 50 yr), and all were men. Liver biopsy showed steatosis in 26, alcoholic hepatitis in 12 (with concomitant cirrhosis in 10), and alcoholic cirrhosis without hepatitis in 15.

LABORATORY INVESTIGATION

Routine liver test included serum bilirubin, serum aspartate-aminotransferase (ASAT), serum alkaline phosphatase, serum albumin, plasma factor II + VII + X, plasma IgG, plasma IgA and plasma IgM.

CIC DETECTION AND COMPLEMENT ANALYSIS

After fasting overnight serum and plasma were obtained by venepuncture just before diagnostic

liver biopsy. The samples were stored at -80°C . Detection of CIC was performed by means of the complement consumption test (CCT) performed as a modification of the method described by Johnson *et al.*,⁶ and in the continuous flow system previously described,⁷ using a 3.4% suspension of sheep red blood cells, optimally sensitised with rabbit haemolytic serum (Behringwerke AG). Heat-inactivated serum (56°C , 60 min) was diluted 1/10 and 1/18 and incubated with one CH 90 unit of guinea pig complement (Behringwerke AG) for 30 min at 37°C just before analysis. The reduction in degree of haemolysis in the samples containing serum was expressed as a percentage of a control containing barbital-buffered saline instead of serum. The CCT was registered as positive if inhibition of haemolysis in patient serum dilutions were greater than the inhibition in corresponding serum dilutions obtained from 100 healthy volunteer blood donors.⁸ The total haemolytic activity of the complement system in serum (CH 50) was estimated in the same auto-analyser system.⁷ Quantification of the complement components C1q, C4, C3 activator, C5 and C9 was performed by means of rocket immunoelectrophoresis,⁹ employing monospecific antisera (Behringwerke AG). The results were expressed as percentages of a pooled standard. Complement conversion products (split products) of C4 and C3 were detected in EDTA-plasma by means of crossed immunoelectrophoresis with monospecific antisera.

STATISTICAL ANALYSIS

Non-parametric Mann-Whitney test, Spearman rank correlation test, and Fischer's exact test were used in statistical analysis. The type I error was fixed at the 5% level.

Results

LIVER BIOCHEMISTRY

Patients with alcoholic hepatitis and patients with alcoholic cirrhosis had significantly ($p < 0.05$) higher concentrations of serum ASAT, serum alkaline phosphatase, and plasma immunoglobulins and significantly ($p < 0.05$) lower concentrations of serum albumin than patients with alcoholic steatosis (Table 1). Patients with alcoholic hepatitis also had significantly ($p < 0.05$) higher concentrations of serum bilirubin than patients with steatosis. No significant differences were found in liver biochemistry of patients with alcoholic hepatitis and alcoholic cirrhosis.

CIRCULATING IMMUNE COMPLEXES

The prevalence of CIC was 39%, 58%, and 60% in patients with alcoholic steatosis, hepatitis and

Table 1 *Liver biochemistry in patients with histologically verified alcoholic steatosis (n = 26), alcoholic hepatitis (n = 12), and alcoholic cirrhosis (n = 15). Figures are medians and range*

Variable (reference limits)	Alcoholic steatosis	Alcoholic hepatitis	Alcoholic cirrhosis
S-bilirubin (5-17 $\mu\text{mol/l}$)	9 (6-52)	18* (9-56)	14 (5-130)
S-ASAT (10-40 U/l)	39 (12-226)	85* (35-184)	58 (20-105)
S-alkaline phosphatase (50-275 U/l)	205 (112-741)	285* (150-860)	315* (91-853)
S-albumin (540-800 $\mu\text{mol/l}$)	578 (425-676)	412* (309-516)	521* (323-604)
P-factor II + VII + X (0.7-1.3 arbitrary U)	1.2 (0.6-2.0)	0.8* (0.5-1.3)	0.6* (0.4-1.2)
P-IgG (80-185 KIU/l)	117 (69-225)	232* (160-278)	184* (78-303)
P-IgA (30-210 KIU/l)	122 (156-210)	408* (191-561)	262* (67-670)
P-IgM (25-179 KIU/l)	68 (36-165)	134* (59-205)	115* (36-369)

* $p < 0.05$ when compared to patients with alcoholic steatosis.
S = serum, P = plasma.

cirrhosis, respectively (Table 2). No significant difference could be found between the three groups, but when compared to controls all groups had a significantly ($p < 0.05$) raised prevalence of CIC. In the total group of patients 49% (95% confidence limits: 35-63%) were CIC-positive.

Table 2 *The prevalence of circulating immune complexes in controls and patients with alcoholic liver diseases*

Group	Number tested	Number positive	% positive	95% confidence limits (%)
Controls	100	0	0	0-4
Alcoholic steatosis	26	10*	39	20-59
Alcoholic hepatitis	12	7*	58	28-85
Alcoholic cirrhosis	15	9*	60	32-84

* $p < 0.05$ when compared to controls.

COMPLEMENT CONCENTRATIONS

The Figure shows the complement concentrations in the three groups of patients with alcoholic liver disease. No significant differences were observed between the three groups, apart from patients with alcoholic hepatitis and cirrhosis having significantly ($p < 0.05$) lower concentrations of C3 activator than patients with alcoholic steatosis. Further, patients with alcoholic hepatitis had significantly ($p < 0.05$) higher levels of C5 than patients with steatosis. No significant changes were observed between patients with alcoholic hepatitis and alcoholic cirrhosis. All the median values of complement components were within the reference limits of normal controls. However, a number of patients in all groups had values either above or below reference limits.

Complement concentrations (C1q, C4, C3, C3 activator (C3A), C5, and C9) in patients with alcoholic steatosis (●), alcoholic hepatitis (▲), and alcoholic cirrhosis (■). The horizontal non-broken lines represent median values and 95% limits of normal controls. Broken horizontal lines represent median values of patients. The stars represent $p < 0.05$ when compared to patients with steatosis. Ordinate: percentage of standards.

Correlating C3 and C9 concentrations to serum albumin and plasma factor II + VII + X values in the total groups of patients, showed that C3 correlated significantly with plasma factor II + VII + X ($r = +0.39$, $p < 0.01$). No other significant correlations were found.

In the total group of patients with alcoholic liver disease 16 patients had both C4 and C3 split products (30%, 95% confidence limits: 18-44%) while six patients had either C4 or C3 split products alone (11%, 95% confidence limits: 4-23%). No significant differences were found between the three groups of patients concerning C4 and C3 split products, and occurrence of split products were not significantly correlated to low concentrations of C4 and C3.

LIVER BIOCHEMISTRY AND COMPLEMENT CONCENTRATIONS IN CIC-POSITIVE AND -NEGATIVE PATIENTS

Comparing CIC-positive patients ($n = 26$) with those without CIC ($n = 27$) showed no significant differences in liver biochemistry, including plasma immunoglobulin and serum albumin concentrations, and concentrations of complement components. Nine patients with detectable CIC had simultaneous activation of both C4 and C3, suggesting *in vivo* activation of the complement system via the classical pathway. However, seven patients without detectable CIC also had activation of both C4 and C3.

Discussion

The present study confirms and extends previous

findings of a high prevalence of CIC in patients with alcoholic liver disease. All patients were examined just before diagnostic liver biopsy. The patients were referred to the department because of symptoms attributable to liver disease, and patients who exhibited symptoms of other diseases which could cause CIC were excluded from this study. A prevalence of CIC in 60% of patients with alcoholic cirrhosis are in agreement with earlier studies. Penner *et al*² found CIC in 50% of patients with alcoholic cirrhosis using the Raji-cell assay. In a recent study, Abrass *et al*¹⁰ found CIC in 63 and 73% of patients with alcoholic liver disease (mostly alcoholic hepatitis and cirrhosis) using solid-phase C1q-binding assay and fluid-phase C1q-binding assay.

Only limited and conflicting data are available concerning the prevalence of CIC in patients with alcoholic steatosis. Sodomann *et al*³ and Penner *et al*² found no CIC-positive cases in patients with alcoholic steatosis using C1q deviation and precipitation methods and the Raji-cell assay. Thomas *et al*¹ reached the same conclusion using C1q-binding to detect CIC, but in five patients with steatosis they found an increased level of anticomplementary activity. In this study the prevalence of CIC was 39% in patients with alcoholic steatosis which is significantly increased compared to controls. The reason for these discrepancies is not known, but earlier studies concerned only a limited number of subjects and, probably most importantly, different methods of detection of CIC have been employed. Different methods for detection of CIC is based on various biological properties of the complexes. Therefore, different techniques may show varying results.¹¹ The complement consumption test has proved its ability to detect IgG aggregates of both intermediate size and sizes > 19S, whereas other techniques such as the C1q precipitation and the platelet aggregation test only detects large CIC (> 19S).¹² The complement consumption test can give rise to false positive results in patients with alcoholic liver disease due to aggregated globulin or the presence of activators of the complement system such as lipopolysaccharide. Aggregation of immunoglobulin may occur because of either low serum albumin or high plasma immunoglobulin. However, we found no significant difference in the concentration of these proteins between CIC-positive and -negative patients. Earlier studies have reported an increased prevalence of endotoxin, the lipopolysaccharide component of the cell wall of Gram-negative bacteria, in patients with cirrhosis. However, a recent study, in which inadvertent contamination of specimens by endotoxin was avoided, was not able to detect any endotoxin.¹³ It should also be stressed that the complement consumption test may

give false-negative results as only complement-fixing immune complexes are detected.

The nature of the antigen or antigens involved in CIC in patients with alcoholic liver disease is not known. Several possibilities exist. Kanagasundaram *et al*⁴ demonstrated circulating alcoholic hyalin and its antibody in patients with alcoholic hepatitis. This antigen-antibody system could not be detected in patients with alcoholic steatosis or alcoholic cirrhosis. In our study, the prevalence of CIC in patients with alcoholic hepatitis was not significantly different from the prevalence in patients with alcoholic steatosis or cirrhosis. Kehl *et al*¹⁴ were not able to confirm the findings of Kanagasundaram *et al*.⁴ Our findings do not seem to support the hypothesis that CIC in patients with alcoholic liver disease should be composed of alcoholic hyalin and antialcoholic hyalin as the prevalence of CIC was not increased in patients with alcoholic hepatitis. In accordance with this assumption is the fact that we could find no significant differences in liver biochemistry in CIC-positive and -negative patients. However, attempts should be made to identify the antigen(s) of CIC before this question can be settled.

Studies in patients with primary biliary cirrhosis have revealed a significant correlation between concentrations of CIC and titres of antimitochondrial antibodies.¹⁵ An association between CIC and autoantibodies has also been described in patients with insulin-dependent diabetes mellitus and in normal controls.¹⁶ Patients with alcoholic cirrhosis have a high prevalence of autoantibodies,¹⁷⁻¹⁹ and it is suggested that autoantibodies are involved in at least some of the CIC found in patients with alcoholic liver disease.

In patients with liver disease a high prevalence of CIC could be due to increased formation, but may also be caused by a decreased clearance. The hepatic reticuloendothelial system is known to clear the circulation of immune complexes.²⁰ A decreased hepatic clearance of antigen has been described in animals with experimental cirrhosis,²¹ but this would not explain the high prevalence of CIC in patients with steatosis. However, alcohol is known to depress the function of the reticuloendothelial system.²² Such an effect might explain the high prevalence of CIC found in alcoholics irrespective of degree of liver damage. Further, recent observations have clearly shown that parenchymal as well as non-parenchymal liver cells take up immune complexes.²³ The uptake of CIC by parenchymal liver cells may also be depressed by alcohol.

Although many of our patients had complement concentrations either above or below normal reference limits, all median values fell within the normal range, and only minor differences were

observed between the three groups of patients with alcoholic liver disease. Only a significant correlation was found between liver-cell damage, expressed by plasma factor II + VII + X, and the concentration of C3. The occurrence of C4 and C3 split products, indicating in vivo activation of the complement system, did not explain the different concentrations of the complement components either. These findings are largely in accordance with earlier reports.^{5 24-26} The level of complement proteins reflects the net synthesis and utilisation. Normal concentrations may therefore be seen in spite of significant activation of the complement system. Raised complement concentrations generally reflect an acute phase reaction, whereas depression reflects utilisation or decreased production. In patients with alcoholic liver disease all mechanisms may operate simultaneously so obscuring each other. This may explain why we are unable to demonstrate any uniform abnormality of either the classical or alternative activation pathway of the complement system.

In conclusion, CIC is found in about half of the patients with alcoholic liver disease. No relation between CIC and complement concentrations on one hand and complement concentrations and liver function on the other could be established. Therefore, determination of CIC does not seem to have diagnostic or pathogenetic value in patients with alcoholic liver disease. The increased prevalence of CIC in patients with alcoholic liver disease may be due to an inhibitory effect of alcohol on clearance of CIC or an increased immunological reactivity, or a combination of these effects.

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