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Complement activation in chronic liver disease

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SUMMARY

Patients with HBsAg positive chronic active liver disease (CALD) and primary biliary cirrhosis (PBC) exhibit increased C3d concentrations and changes in the serum concentrations of the complement components consistent with activation of the classical and alternative pathways. In these patients the concentrations of the regulatory proteins, C3b inactivator (C3bINA) and βIH globulin, are normal. Patients with HBsAg negative CALD and alcohol induced liver disease (ALD) exhibit no evidence of an increased level of complement system activation. In these patients diminished serum concentrations of complement components appear to be related to diminished hepatic synthetic function. C4 synthesis may be specifically reduced in autoimmune chronic active liver disease.

INTRODUCTION

Increased catabolism of C3 has been demonstrated in primary biliary cirrhosis (PBC) (Potter, Elias & Jones, 1976) and HBsAg positive chronic active liver disease (HBsAg positive CALD) (Thomas *et al.*, 1979), and normal catabolism in HBs antigen negative CALD and alcohol induced liver disease (ALD) (Thomas *et al.*, 1979; Thomas, Potter & Sherlock, 1977). Increased C1q catabolism in these patients (Potter *et al.*, 1980) suggests that this increased catabolism of C3 is initiated by activation of the classical pathway.

Cleavage products of C3 have been demonstrated in the plasma of patients with PBC (Teisberg & Gjone, 1973). During C3 cleavage, C3b, a biologically active fragment is formed. C3b triggers the alternative pathway feedback loop resulting in further C3 cleavage (Lachman & Muller-Eberhard, 1968). The enzymatic degradation of C3b is an important step in controlling alternative pathway turnover, and is mediated by at least two serum proteins, C3b inactivator (C3bINA) (Ruddy & Austen, 1969; Whaley & Ruddy, 1976a) and its cofactor, βIH globulin (Weiler *et al.*, 1976); Mancini, Carbonara & Heremans, 1965). Together these proteins produce at least one proteolytic cleavage in the alpha chain of C3b, the resultant molecule C3bi lacking C3 and C5 convertase activity (Pangburn, Schreiber & Muller-Eberhard, 1977). C3bi is degraded to C3c and C3d by trypsin like proteases (Abramson *et al.*, 1971). When either of these regulatory proteins is absent, C3b accumulates causing uncontrolled activation of the alternative pathway, with depletion of the alternative pathway complement components and C3 (Abramson *et al.*, 1971; Thompson & Lachman, 1977; Whaley & Thompson, 1978).

In this study we have examined the plasma of patients with a variety of liver diseases for evidence of increased C3 catabolism, and attempted to define the relationship between increased C3

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catabolism to classical and alternative pathway activation, and the serum concentrations of the control proteins—C3b inactivator and βIH globulin.

METHODS

Blood was allowed to clot in a glass tube for 2 hr before the serum was separated. EDTA-plasma was separated immediately after venepuncture. Both were stored at −70°C in aliquots.

Radial immunodiffusion, or rocket immunoelectrophoresis in agarose, was used to estimate concentrations of the various complement components. Commercial antisera were available for C1q, C4, C3, Factor B (Hoescht), and C3b inactivator (Kent Labs). βIH globulin (Whaley & Ruddy, 1976b) and properdin (Fearon, Austen & Ruddy, 1974) were purified and used to immunize rabbits. These antisera were incorporated in agarose plates (1·5% agarose in isotonic veronal buffered saline containing 0·01 M EDTA, pH 7·5). Diffusion was allowed to proceed for 48 hr at room temperature. Properdin plates were then washed in saline for 24 hr at room temperature, and stained with 1% tannic acid solution. Results were expressed as a percentage of a plasma pool from five control subjects or against standard solutions of each component.

The ^{125}I-C1q binding capacity of EDTA plasma was measured by a standard technique (Zubler, Lange & Lambert, 1976).

C3d was measured by rocket immunoelectrophoresis using a rabbit anti-C3d serum (Netherland Red Cross). In this method, native C3 was precipitated with 12% polyethylene glycol and C3d was then assayed in the supernatant (Perrin, Lambert & Meischer, 1975).

Serum immunoglobulins and liver function tests were determined by standard techniques.

Analysis of data. Analysis was undertaken with Dunnett's test, for making a comparison between several experimental groups and a single control group i.e. several comparisons which are not independent of each other (Dunnett, 1964).

RESULTS

C3 turnover

Reduced levels of C3 were present in some patients from each group, but only in the HBsAg positive CALD group was the mean C3 level significantly reduced (Table 1). Plasma C3d levels were significantly elevated in the HBsAg positive CALD and the PBC groups (Table 1), whereas they were normal in the HBsAg negative CALD and ALD groups. Increased serum levels of C1q binding

Table 1. Concentrations (mean ± s.e.m.) of complement components, C1q binding and C3d levels in patients with chronic liver disease

Variable (mg/l)	Controls	HBsAg +ve CALD	HBsAg −ve CALD	ALD	PBC
C1q	129±4	137±13	132±5	135±5	137±6
C4	320±30	200±50	160±20‡	280±50	290±50
C3	1020±50	690±100*	820±40	1030±40	1050±60
Factor B	190±20	170±30	160±20	230±30	280±30‡
Properdin	32±2	34±4	33±3	28±3	33±3
C3b ina‡	57±4	46±4	36±4*	48±4	64±5
βIH	245±12	298±22	215±12	310±15°	340±30‡
C1q Binding§	7±1	19±7	12±1*	13±2	13±1*
C3d	23±6	53±12*	13±6	13±5	53±11*

* $P<0·05$; † $P<0·01$; ‡ $P<0·001$; § % std = % laboratory standard.

activity were detected in all the groups studied but were only significantly elevated in the HBsAg negative and the PBC groups (Table 1).

Classical pathway

C1q levels did not differ significantly from normal in any of the four groups of patients studied (Table 1). Although individual patients in each group had low C4 levels, only in the HBsAg negative CALD group was the mean C4 levels significantly lower than the mean of the control group (Table 1).

Alternative pathway

Although occasional patients in all the patient groups had reduced levels of factor B (Table 1), the mean levels of this protein were not reduced. Indeed, in the PBC group the mean factor B level was higher than the controls.

The mean levels of properdin in the patient groups were not significantly different from the controls (Table 1).

Control proteins

C3bINA levels were significantly reduced in the HBsAg negative CALD group only (Table 1). The βIH levels were significantly elevated in the ALD and PBC groups (Table 1).

Correlation coefficients

Table 3 shows the correlation coefficients between the various complement components of the classical and alternative pathways with serum albumin concentration and the degree of prolongation of the prothrombin time (seconds).

Table 2. Summary of the results of mean values of % C1q binding and concentrations of the classical and alternative complement components in chronic liver disease

	HBsAg positive CALD ($n=11$)	HBsAg negative CALD ($n=15$)	ALD ($n=26$)	PBC ($n=19$)
C3	↓*	n	n	n
C3d	↑*	n	n	↑*
% C1q binding	↑	↑*	↑	↑
C1q	n	n	n	n
C4	n	↓↓↓***	n	n
Properdin	n	n	n	n
Factor B	n	n	n	↑↑↑‡
β1H	n	n	↑↑↑‡	↑↑↑†
C3bINA	n	↓↓†	n	n

* $P<0{\cdot}05$; † $P<0{\cdot}01$; ‡ $P<0{\cdot}001$; n = number of patients.

DISCUSSION

The serum concentrations of complement proteins will be affected by changes in either synthesis or catabolism. In this study the serum concentrations of components of the classical and alternate pathways of complement activation and the concentrations of the regulatory proteins, β1H globulin and C3b inactivator, have been related to serum concentrations of C3d, a catabolic product of C3. In previous studies, the fractional catabolic rate of C3 has been shown to be

Table 3. Significant positive correlations ($P<0.05$) between complement components, % C1q binding, albumin and prothrombin time (seconds prolonged) in different types of chronic liver disease

Variable	HBsAg positive CALD	HBsAg negative CALD	ALD	PBC
C3d		Factor B*	C4	
C3	C1q, C4, Factor B	Prot. time*	Prot. time*, C4, Factor B, C3bINA, β1H	Properdin, factor B, β1H, C3bINA
%C1q binding	Albumin*	Properdin*	Prot. time, albumin*	
C1q	Factor B, C3, β1H	Albumin*		Albumin*, C3INA*
C4	C3	Prot. time*	C3d, factor B, Properdin C3bINA, C3, β1H, albumin, prot. time*	Factor B
Properdin	β1H	C1q binding*	β1H, C4	C3, β1H
Factor B	C1q, C3	C3d*, β1H, albumin	β1H, C4, C3, albumin, C3bINA, prot. time*	C4, C3, β1H
β1H	C1q, Properdin	Prot. time*, Factor B	Factor B, Properdin, C4, albumin, C3, C3bINA	Factor B, Properdin, C3, albumin, C3bINA
C3bINA			C4, factor B, β1H, C3	C3, C1q*, β1H

* inverse correlation; Prot. Time = Prothrombin time.

increased in HBsAg positive CALD (Thomas *et al.*, 1979) and in primary biliary cirrhosis (Potter *et al.*, 1976) and normal in auto-antibody positive CALD and alcoholic liver disease (Thomas *et al.*, 1979, 1977). In this study, C3d concentrations were increased in the same patient groups as have previously been shown to have increased fractional catabolic rates of C3. It seems probable therefore that C3d concentrations reflect the rate of catabolism of C3. In comparing the serum concentrations of the various complement components and regulatory proteins to C3d concentrations, we have attempted to relate the concentrations of these proteins to complement consumption.

In HBsAg positive CALD, serum concentrations of C4 and C3 are decreased. In previous studies the fractional catabolic rate of C3 has been shown to be increased, whereas the synthesis rate of this protein is normal (Thomas *et al.*, 1979). It seems probable, therefore, that the diminished C3 and probably the diminished C4 concentrations as well, in this study are a reflection of increased consumption of these proteins by activation of the classical pathway. The diminished concentrations of factor B, and the positive correlation between factor B and the components of the classical pathway, suggest that the activation of the classical pathway also results in increased activity of the alternative pathway. The concentrations of the regulatory proteins which modulate the activity of the alternative pathway, are within the normal range. Thus, in HBsAg positive CALD, it is probable that an increased rate of C3b formation due to activation of the classical pathway results in turnover of the alternative pathway.

In HBsAg negative CALD, C3d concentrations were normal. These data suggest that significant activation of the complement system does not occur in this disease, and this is consistent with the observation that the fractional catabolic rate of C3 is normal (Thomas *et al.*, 1979). C3 and C4 concentrations were diminished in these patients and in view of the evidence that consumption of at least C3 is normal, we must conclude that the diminished concentrations of both proteins are probably related to impaired hepatic synthesis. The serum concentrations of properdin and factor B were normal. Thus, in this disease, there is no evidence of activation of either the classical or the alternative pathway. The concentrations of the regulatory proteins were difficult to interpret. β1H globulin concentrations were within normal limits, whereas C3b inactivator concentrations were significantly reduced. These patients had a significant impairment of the coagulation system signifying a reduced capacity of the liver to synthesize protein, and it seems probable that the low levels of C3b inactivator and also C4 and C3 may be related to this impaired hepatic synthetic capacity. Metabolic studies of these regulatory proteins are needed to examine this possibility further.

In previous studies of lupoid CALD patients, increased C1q binding (Thomas *et al.*, 1978) was demonstrated in the presence of normal C1q and C3 catabolism (Thomas *et al.*, 1979; Potter *et al.*, 1980), and in this study increased C1q binding has been demonstrated in the presence of normal C3d levels. This suggests that either the C1q binding moiety in these sera is not an immune complex, and does not result in the generation of C3 convertase activity, or that there is a primary or acquired defect in the early part of the classical pathway of complement in these patients. Against this background it is difficult to explain why these patients exhibit a highly significant reduction in serum C4 concentrations in the presence of an insignificant reduction in C3 levels. It is possible that C4 synthesis is specifically reduced in this disease.

In patients with ALD, C3d concentrations were normal and previous studies have shown normal fractional catabolic rates of C3 in this condition (Thomas *et al.*, 1977). Serum concentrations of the components of the classical (C4 and C3) and of the alternative pathway (properdin and factor B) were all within the normal range. Thus, in this disease, there is no evidence of significant activation of either the classical or alternative pathways of complement. The concentrations of β1H globulin were significantly increased but C3b inactivator concentrations were normal. It seems probable that increased synthesis accounts for the raised levels of β1H globulin. The positive correlations between the serum concentrations of the majority of the components of the classical and alternative pathways suggests that, in this group of patients, serum concentrations are principally influenced by changes in the synthesis rates of these components.

In PBC, C3d concentrations were markedly elevated. C3 concentrations are normal in these patients reflecting an increase in synthesis of this protein to compensate for increased catabolism. That the increased activation of C3 is dependent on activation of the classical pathway in this

disease is implied by the observation that the catabolic rate of C1q is also increased in these patients (Potter *et al.*, 1980). C4 concentrations however, are normal. Although serum concentrations of the components of the alternative pathway were within the normal range, other authors have demonstrated conversion products of factor B in the serum of these patients (Wands *et al.*, 1978), and it seems probable that there is significant activation of the alternative pathway. The normal serum concentrations of these complement components suggest that this increased activation is accompanied by increased synthesis of properdin and factor B. β1H globulin concentrations were significantly increased and C3b inactivator concentrations were normal in these patients. It seems probable therefore, that the activation of C3 by the classical pathway is accompanied by activation of the alternative pathway which is adequately controlled by the C3b regulatory proteins. The role of immune complexes is unclear. C1q binding is highest in the patients with the most severe disease (Epstein *et al.*, 1979), raising the possibility that many immune complexes accumulate because hepatic phagocytic function is impaired in these patients. Indeed, a specific defect of kupffer cell C3b receptors has recently been reported (Jaffe *et al.*, 1978). However, it remains possible that the immune complexes present in the early phase of the disease are responsible for the increased level of complement activation and may have pathogenetic significance (Thomas *et al.*, 1977).

These studies provide further evidence of an increased level of complement activation in HBsAg positive CALD and PBC. Activation of C3 resulting in the formation of C3b, will result in the activation of the alternative pathway. This will result in an amplification of the degree of C3 conversion. This process is regulated by the proteins C3b inactivator and β1H globulin (Lachman & Muller-Eberhard, 1968; Ruddy & Austen, 1969; Weiler *et al.*, 1976; Whaley & Ruddy, 1976b; Whaley, Schur & Ruddy, 1976) and at least one additional trypsin like enzyme (Lachman & Muller-Eberhard, 1968; Pangburn *et al.*, 1977). Complete absence of either C3b inactivator or β1H permits the uncontrolled activation of the alternative pathway (Abramson *et al.*, 1971; Thompson & Lachman, 1977; Whaley & Thompson, 1978). In this study, in patients with evidence of activation of the classical pathway, the serum concentrations of the components of the alternative pathway were relatively normal. This suggests that the degree of recruitment of the alternative pathway is not excessive, presumably because the rate of C3b production does not outstrip the capacity of C3b inactivator and β1H to degrade it.

The changes in serum concentrations of the components of the classical and alternative pathways and of the regulatory proteins seen in autoimmune CALD, are probably related to primary or secondary changes in the hepatic rate of synthesis of these proteins, since there is no evidence either from metabolic studies (Thomas *et al.*, 1979) or from studies of the activation products of C3, for increased activation of C3 in these patients. In alcohol induced disease, alcohol itself has been shown to have an inhibiting effect on the secretion of proteins by hepatocytes (Lieber, 1980) and this may contribute to the changes in the serum concentrations of complement components and the strong correlation of these changes amongst the various components.

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