

# Complement inhibitors and immunoglobulins in ulcerative colitis and Crohn's disease

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**SUMMARY** The serum concentrations of the complement inactivators C<sub>1</sub>INH, C3bINA and  $\beta_{1H}$  have been determined in patients with ulcerative colitis and Crohn's disease and their correlation with C3 and properdin factor B examined. The incidence of immunoglobulins (IK) in these patients was investigated. Raised serum concentrations of C<sub>1</sub>INH and C3bINA have been found in patients with active disease, but no significant alteration was found in serum concentration of  $\beta_{1H}$ . An increasing incidence of positive IK titres was found with increased length of disease history. These results suggest continuing complement activation in these diseases.

Serum concentrations of several complement components are known to be altered in ulcerative colitis and Crohn's disease. C3 and properdin factor B are raised and may show a positive correlation with disease activity and the extent of the inflammation.<sup>1,2</sup> Less marked changes have been found for C4<sup>2-4</sup> and no significant change occurs in the serum concentration of C1q.<sup>1</sup> These changes can be interpreted as occurring secondary to acute inflammation. However, direct evidence for increased complement consumption has been presented from metabolic studies of purified C1q and C3.<sup>5,6</sup> Furthermore, an increased incidence of conversion products of C3 and properdin factor B has been found.<sup>7,8</sup>

It is not known whether increased complement activation occurs purely as a result of continuing antigen-antibody reactions or whether there is a defective modulation of the complement cascade. The aim of this study, therefore, was to assay the inhibitors of the complement system and to provide additional evidence of continuing complement activation by measuring the titres of serum immunoglobulins (which are antibodies to activated complement components).

## Methods

### PATIENTS

Blood was obtained from 45 patients with ulcerative colitis, 41 patients with Crohn's disease, and 32

normal healthy control subjects. Sex and age distribution in each group was similar. Sera from all the controls were collected during the winter period (November to February) and from 85% of patients with ulcerative colitis and 88% with Crohn's disease.

Ulcerative colitis and Crohn's disease were diagnosed on standard clinical, radiological, and histological criteria. Disease activity was graded according to the criteria of Truelove and Witts<sup>9</sup> for ulcerative colitis and de Dombal *et al.*<sup>10</sup> for Crohn's disease.

### ESTIMATION OF COMPLEMENT COMPONENTS AND INHIBITORS

Sera were separated within two hours of collection. They were stored in aliquots at -20°C until use, when they were rapidly thawed in a water bath at 37°C. C3 and properdin factor B were estimated by single radial immunodiffusion using Diffugen plates (Searle) and Partigen plates (Hoechst) respectively. C1 esterase inhibitor (C<sub>1</sub>INH) and C3b inactivator (C3bINA or KAF) were determined by rocket immunoelectrophoresis into barbital buffered agarose (0.075M, pH 8.6) containing specific antisera (Hoechst & Flow Laboratories).  $\beta_{1H}$  determinations were performed by single radial immunodiffusion into barbital buffer agarose containing monospecific antiserum to  $\beta_{1H}$ .<sup>11</sup> All concentrations, except C3bINA, were determined in absolute terms (mg/l) by reference to serial dilutions of known standards (Protein Standard Serum and Plasma, Hoechst). C3bINA concentrations were determined in reference to a pool of sera from 25 normal control subjects (%NP).

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## IMMUNOCONGLUTININS

Immunoconglutinins were detected by the method of Lachmann and Hobart.<sup>12</sup> All sera under test were first de complemented by heat inactivation at 56°C for 30 minutes and then absorbed with half their volume of sheep erythrocytes. Serial dilutions were then made and 0.05 ml of each placed in plastic agglutination trays. To each well was added 0.05 ml of a 1% suspension of EAC43 antrypol (human) reagent and the cells allowed to settle at 4°C. EA cells were used as a negative control and rabbit antiserum to human C3 and C4 as a positive reaction. A positive immunoconglutinin titre was taken as greater than 1:4 under these conditions.

## STATISTICS

All data except that for the immunoconglutinins (1K) were subjected to analysis of variance. 1K data were analysed using the McNemar test for significance of change.

## Results

The serum concentrations of  $\bar{C}1\text{INH}$ ,  $\text{C3bINA}$ , and  $\beta_{1\text{H}}$  are shown in Figs. 1-3. Tables 1 and 2 give the mean concentrations for these inhibitors and for C3 and properdin factor B. The Tables also give the mean values of patients in remission and for those

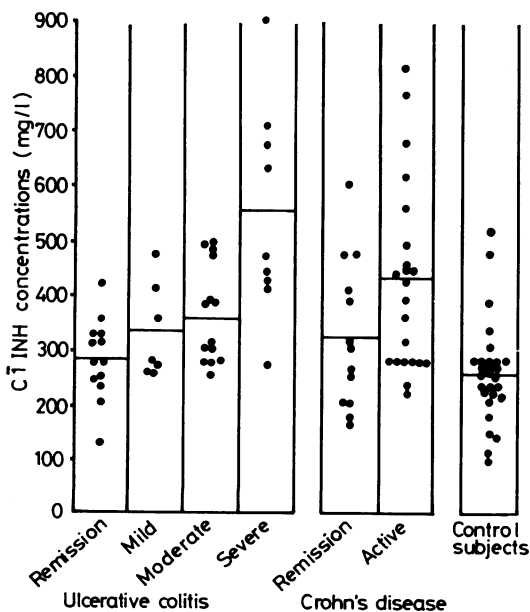


Fig. 1 Serum concentrations of  $\bar{C}1\text{INH}$  in control subjects and in patients with inflammatory bowel disease.

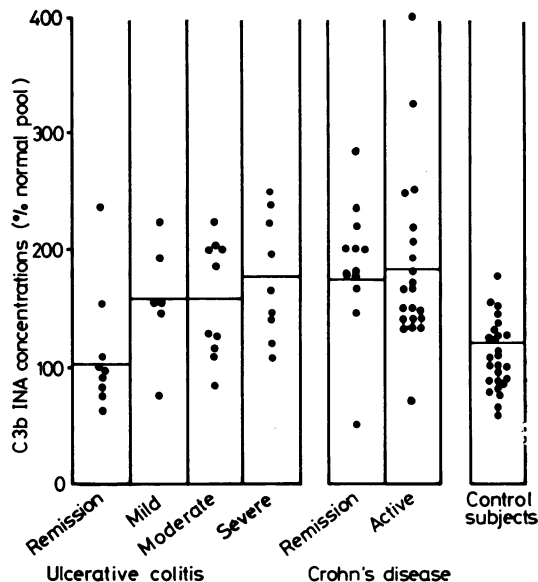


Fig. 2 Serum concentrations of  $\text{C3bINA}$  in control subjects and in patients with inflammatory bowel disease.

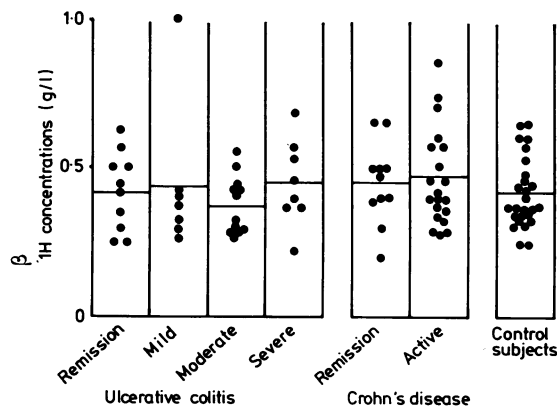


Fig. 3 Serum concentrations of  $\beta_{1\text{H}}$  in control subjects and in patients with inflammatory bowel disease.

with active disease. Table 3 contains the immunoconglutinin data for these patients in relation to disease activity and Table 4 the same data in relation to disease history.

 $\bar{C}1\text{INH}$ 

Serum  $\bar{C}1\text{INH}$  concentrations were raised both in the group of patients with ulcerative colitis and those with Crohn's disease ( $P < 0.01$ ). The mean values for patients with active disease was significantly higher than for those in remission ( $P < 0.01$ ).

Table 1 Serum concentrations\* of complement components and inhibitors in patients with ulcerative colitis

Group	Patients (no.)	C3 (g/l)	Factor B (mg/l)	C <sub>1</sub> INH (mg/l)	C3bINA		β <sub>1</sub> H	
					(no.)	(%NP)	(no.)	(mg/l)
All patients	42	1.45 ± 0.08	241 ± 15	373 ± 24	35	147 ± 9	37	414 ± 23
Remission	13	1.07 ± 0.10	180 ± 13	286 ± 20	10	102 ± 18	10	419 ± 41
Mild	7	1.63 ± 0.22	234 ± 47	332 ± 32	6	158 ± 20		420 ± 10
Moderate	13	1.36 ± 0.14	260 ± 23	358 ± 24	10	158 ± 16	12	370 ± 27
Severe	9	1.98 ± 0.13	309 ± 28	553 ± 20		176 ± 17	8	450 ± 50
Control subjects	28	1.16 ± 0.06	168 ± 10	261 ± 18	26	108 ± 6	26	417 ± 23
Significance		P < 0.01	P < 0.01	P < 0.01		P < 0.01		NS

\*Mean ± SEM

Table 2 Serum concentrations\* of complement components and inhibitors in patients with Crohn's disease

Group	Patients (no.)	C3 (g/l)	Factor B (mg/l)	C <sub>1</sub> INH (mg/l)	C3bINA		β <sub>1</sub> H	
					(no.)	(%NP)	(no.)	(mg/l)
All patients	35	1.43 ± 0.12	286 ± 17	390 ± 29		178 ± 12	32	460 ± 28
Remission	14	1.56 ± 0.24	253 ± 22	326 ± 35		173 ± 17	12	448 ± 37
Active	21	1.35 ± 0.12	308 ± 22	432 ± 38		182 ± 16	20	467 ± 36
Control subjects	28	1.16 ± 0.06	168 ± 10	261 ± 18	26	108 ± 6	26	417 ± 23
Significance		NS	P < 0.01	P < 0.01		P < 0.01		NS

\*Mean ± SEM.

Table 3 Immunoconglutinin titres

	0	1/4	1/8	1/16	1/32	1/64	1/128	1/256
Ulcerative colitis n = 45	29	3	5	2	3	1	1	1
Crohn's disease n = 41	29	3	0	2	1	3	3	1
Controls n = 32	24	4	1	0	2	0	1	0

Table 4 Immunoconglutinins in inflammatory bowel disease and control subjects

Group	n	% IK Positive (titre > 1:4)
Ulcerative colitis	45	38
Active	28	43
Remission	17	29
Crohn's disease	41	29
Active	27	37
Remission	14	14
Controls	32	25

From Fig. 1 it will be seen that, for patients with ulcerative colitis, the mean values increased with increasing severity of the disease.

#### C3bINA

The mean serum levels for C3bINA were increased in all patients with inflammatory bowel disease (P < 0.01). In patients with ulcerative colitis those with active disease had significantly higher serum

concentrations and the mean values correlated with disease activity, whereas those in remission tended towards normal values. No such distinction could be made between active and inactive disease in patients with Crohn's disease.

#### β<sub>1</sub>H

All except two values were within the normal range for this protein. There was no significant difference between the patients with inflammatory bowel disease and the control subjects.

#### CORRELATIONS BETWEEN SERUM CONCENTRATIONS

There was no general correlation with any two serum components and inhibitors measured over the entire group of subjects. However, C3bINA did correlate with C3 in Crohn's disease (r = 0.418, P < 0.01) and with factor B in ulcerative colitis (r = 0.527, P < 0.01), and control subjects (r = 0.169, P < 0.01).

#### IMMUNOCONGLUTININS

The titres obtained in each group are shown in Table 3. In both ulcerative colitis and Crohn's disease there was a greater incidence of positive 1K titres (> 1:4) in active disease than in the control subjects (P < 0.01). The incidence in remission, however, was within the normal range (Table 4). When patients were examined for positive 1K titres in relationship to disease history, it was found that both groups showed a similar pattern and are therefore grouped together in Table 5. From this

**Table 5** Disease history and immunoconglutinin titre in inflammatory bowel disease

Patients	1st attack	5 years	> 5 years
No.	18	43	25
% 1K positive (Controls 25% 1K positive)	17	35	44

Table it can be seen that the incidence of positive 1K titres increases significantly with length of history. Seventeen per cent of patients during their first attack had a positive 1K titre and this rose to 44% in patients who had had their disease longer than five years. This increase of 1K titres in patients with long-standing disease was independent of disease activity.

### Discussion

Immune complexes have been found in the sera of patients with ulcerative colitis and Crohn's disease by a variety of methods.<sup>8 13-15</sup> Earlier workers, using an indirect test, have reported the presence of endotoxin in these patients.<sup>16</sup> Complement may, therefore, be activated by both classical and alternative pathways. Many workers have found alterations in the serum concentration of classical pathway components<sup>13,14</sup> and in factor B<sup>2</sup>. We have previously shown that there is increased catabolism of C1<sub>q</sub>,<sup>5</sup> a classical pathway component, and of C3,<sup>6</sup> which is common to both pathways in patients whose serum concentrations of these components were raised. What was not apparent from these earlier studies was whether increased activation was due to antigen-antibody reactions and/or endotoxin, or whether it resulted from a failure to modulate the system.

The present study has shown that the concentrations of C1INH and C3bINA, modulators of the classical and alternative pathways respectively, rise during a relapse of ulcerative colitis and return to normal levels as the disease goes into remission. A similar pattern was seen for C1INH in patients with Crohn's disease but no such change occurred for C3bINA. The concentrations of  $\beta_{1H}$ , another modulator of the alternative pathway, were within the normal range in both diseases and showed no change with disease activity. This is in agreement with the finding of Charlesworth *et al.*<sup>11</sup> who found that  $\beta_{1H}$  did not behave as an acute phase reactant when compared with C-reactive protein.

However, functional activity of these complement modulators cannot be deduced from serum concentrations. Similarly, serum concentrations provide no information of the catabolism of these proteins. Studies of functional activity and catabolism are

required to elucidate the effectiveness of the control of the complement system. A functional abnormality in the alternative pathway in inflammatory bowel disease has been suggested by the diminished activation by cobra venom factor (CoVF).<sup>4</sup> Serum properdin and properdin convertase activity are also diminished.<sup>4</sup> These changes are not specific for inflammatory bowel disease, as diminished activation by CoVF has been reported in other situations<sup>17,18</sup> and similar changes in properdin have been found in systemic lupus erythematosus,<sup>19</sup> membranoproliferative glomerulonephritis,<sup>20</sup> and in association with endotoxin.<sup>21</sup>

Initial observations by Pepys and co-workers<sup>22</sup> showed a 41% incidence of positive 1K titres in patients with inflammatory bowel disease, an incidence similar to that reported in the present study. It has now been shown that the height of the titre correlates positively with the duration of the disease which strongly suggests that there is continuing complement activation in these patients. There is some controversy, however, over the presence of C3 conversion products in these patients. Teisberg and Gjone<sup>7</sup> have shown C3 activation products in the sera of many of these patients, but they are rarely found if plasma is used.<sup>22</sup> C3 has been observed as deposits in the intestinal wall by some<sup>23,24</sup> but not by others.<sup>25</sup>

The results of this study, therefore, provide additional evidence compatible with the view that complement activation is a factor in the pathogenesis of mucosal inflammation in ulcerative colitis and Crohn's disease. There is no quantitative deficiency in the modulators and inhibitors of the complement system and more sophisticated studies are now required to assess their functional activity.

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