Significance of serum complement levels in patients with gastrointestinal disease

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SUMMARY Levels of the serum complement components, C₃ and C₄, in patients with Crohn's disease, ulcerative colitis, and miscellaneous gastrointestinal disorders were compared with those of normal blood donors. Significant increases of both components were found in all three patient groups, the highest being in patients with Crohn's disease. Generally, levels of C₃ and C₄ were lower in patients with inactive rather than active Crohn's disease and ulcerative colitis.

These results provide some evidence in support of an immunological basis for inflammatory bowel disease. However, in view of the frequent elevation of C₃ and C₄ in other gastrointestinal diseases, it is equally possible that the complement components are behaving as acute phase proteins.

In patients with Crohn's disease (CD) and ulcerative colitis (UC) there is evidence of complement activation by both classical and alternative pathways. Thus in active UC elevation of total serum haemolytic complement activity has been reported (Thayer and Spiro, 1963; Fogel *et al.*, 1967) and the C₃ component has been demonstrated in the rectal mucosa (Ballard and Shiner, 1974). Further, the finding by Doe *et al.* (1973) of an increased incidence of precipitin reactions with C1q (a fragment of C₁ from the classical pathway) in both diseases could indicate the presence of complement-binding immune complexes. Raised serum levels of C₃, C₄, and a component of the alternative pathway, factor B, have recently been described (Ward and Eastwood, 1975; Feinstein *et al.*, 1976; Hodgson *et al.*, 1977). Taken together, these findings have been accepted as evidence in favour of an immunological basis for the two diseases, but there has been conflicting evidence, particularly with regard to complement changes in UC (Teisberg and Gjone, 1975; Ward and Eastwood, 1975). We have therefore prospectively compared serum levels of C₃ and C₄ in patients with CD and UC and in a miscellaneous group of other gastrointestinal patients.

Material and methods

Serum C₃ and C₄ levels were measured in three serial groups: 39 patients with CD, 35 with UC, and 70 other patients investigated in a gastrointestinal unit (gastrointestinal controls, Table 1) and also in a group of 100 normal blood donors. The diagnosis of CD and UC was based on accepted clinical, radiological, and pathological criteria; disease activity was determined using the criteria of Truelove and Witts (1955) for UC and of De Dombal *et al.* (1974) for CD, taken in conjunction with the results of serum levels of albumin and orosomucoid.

Serum albumin was determined by a standard autoanalytical technique (Northam and Widdowson, 1967) and serum orosomucoid by a M-Partigen Immunodiffusion plate (Behringwerke AG, West Germany); serum C₃ and C₄ levels were measured by the modified single diffusion technique of Mancini *et al.* (1965) using monospecific antisera.

Statistical analysis was performed by calculating fiducial limits based on Student's *t* distribution, as described by Bliss (1967) for samples with unequal variances.

Results

The mean (± 1 SD) levels of serum C₃ and C₄ in the four groups of subjects, together with the *P* value for the difference between these means, are shown in Table 2, and the results for individual patients are presented in Figures 1 and 2.

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Table 1 Patient diagnosis in gastrointestinal controls; the numbers in each diagnostic category with an elevated serum C₃ and/or C₄ level are also shown

Diagnosis	Number of patients	Individuals with elevated levels*	
		C ₃	C ₄
Celiac disease	15	1	2
Irritable bowel syndrome	14	4	7
Iron and/or folate deficiency	9	4	4
Peptic ulceration/post-gastrectomy diarrhoea	4	2	1
Pernicious anaemia	3	2	2
Chronic pancreatitis	3		1
Whipple's disease	2		1
Hypogammaglobulinaemia and malabsorption	2		1
Alcoholic cirrhosis	2		
Ileocaecal tuberculosis	2		1
Thyrototoxic diarrhoea	2		
Malabsorption due to intestinal resection	2		1
α-Heavy chain disease	1		1
Pneumatosis cystoides	1	1	
Osler-Weber-Rendu syndrome	1		
Nutritional vitamin D deficiency	1	1	
Lactose intolerance	1		
Tropical sprue	1		
Small intestinal diverticula	1		
Diverticulitis	1		1
Eosinophilic gastroenteritis	1	1	1
Primary intestinal lymphangiectasia	1	1	1

*Elevated level = value > normal control mean + 2 SD

Comparison between C₃ and C₄ levels in each of the three patient groups showed significant differences, the CD group having the highest mean values. In addition, the mean C₃ and C₄ values in these three patient groups, which included the gastrointestinal control group, were significantly higher than in the normal controls. Of 10 CD patients with a C₃ level within the normal control range (mean ± 2 SD), only four had active disease. The complement levels in two of these four patients were measured at a time when they were extremely ill with CD, and both have subsequently died of their disease. Complement levels in patients with inactive disease tended to be lower than in those with active disease; 10 out of 11 (C₃) and nine out of 11 (C₄) patients with inactive CD had levels below the mean of the CD group, while seven out of 13 (C₃)

and nine out of 13 (C₄) with inactive UC had levels below the mean of their group. Concurrent elevation of both C₃ and C₄ in individual patients occurred in about 50% of cases. The diagnoses of the gastrointestinal control patients with elevated C₃ and/or C₄ levels are listed in Table 1.

There was no correlation between serum orosomucoid levels and levels of C₃ or C₄ in either CD or UC, in serial samples taken from individual patients.

Discussion

Our results clearly indicate an altered complement metabolism with respect to C₃ and C₄ complement components in CD and UC. Only 16% of patients with active disease had complement values within the normal control range, and, in addition, levels of

Table 2 Mean levels of serum C₃ and C₄ (± 1 SD) in the three patient groups and in the normal controls, together with the significance of the difference between means. Complement expressed as mg/100 ml

Complement component	Patient group	Mean ± 1 SD	Significance of difference between means		
			CD	UC	Gastrointestinal control
C ₃	CD	199 ± 56	—	—	—
"	UC	174 ± 43	P < 0.05	—	—
"	Gastrointestinal control	146 ± 40	P < 0.001	P < 0.01	—
	Normal control	120 ± 21	P < 0.001	P < 0.001	P < 0.001
C ₄	CD	56 ± 20	—	—	—
"	UC	48 ± 13	P < 0.05	—	—
"	Gastrointestinal control	42 ± 17	P < 0.001	P < 0.05	—
"	Normal control	30 ± 7.5	P < 0.001	P < 0.001	P < 0.001

Fig. 1 C_3 levels in individual patients. Brackets indicate mean $\pm$ SD for that group. White circles indicate inactive disease.

both components were lower in quiescent CD and UC.

These findings are in keeping with the results reported by Thayer and Spiro (1963) for total haemolytic complement in UC, elevated values being found in active disease. Subsequently, Feinstein *et al.* (1976) and Hodgson *et al.* (1977) found the serum factor B level to be raised in both conditions, particularly in CD, and remission of the inflammatory bowel disease was associated with a fall in this level. Hodgson *et al.* (1977) also reported significantly elevated levels of serum C₃ in CD and an elevation of serum C1q in both CD and UC. In contrast, Teisberg and Gjone (1975) and Ward and Eastwood (1975) were unable to demonstrate significant elevation of C₃ or C₄ in inflammatory bowel disease (except for C₄ in CD in the latter study).

In spite of these varying results there is additional evidence of altered complement activity in inflammatory bowel disease, namely (i) C₃ conversion as shown by the presence of C₃ breakdown products in blood (Teisberg and Gjone, 1975), (ii) the presence

Fig. 2 C_4 levels in individual patients. Brackets indicate mean $\pm$ SD for that group. White circles indicate inactive disease.

of low titres of immunoglobulins reflecting continuing complement activation (Pepys *et al.*, 1977), and (iii) an increased fractional catabolic rate as well as synthetic rate of the C₃ component (Hodgson *et al.*, 1977).

With respect to mechanisms, it has been postulated that the site of complement activation is within the gut mucosa itself, initiated by antigen-antibody reactions. However, it is equally possible that the high titres of C₃ and C₄ demonstrated in this study represent acute phase reactants. C₃, C₄, C₅, C₆, and factor B are known to be 'acute phase' proteins of acute inflammation (Schutte *et al.*, 1974; Lachmann, 1975). Both C₃ and factor B originate in the liver, as do other acute phase proteins; these include orosomucoid, which is elevated in active CD (Cooke *et al.*, 1958). Nevertheless, in our patients with active CD comparison of serial C₃ or C₄ levels with orosomucoid levels showed no correlation.

Additional evidence that C₃ and C₄ behave as acute phase reactants is suggested by the elevated values found in patients with gastrointestinal disease other than inflammatory bowel disease, and in hospital patients without immunological disorders in the report from Hodgson *et al.* (1977). It is difficult to explain the cause of raised complement levels in seven out of 14 patients who, after intensive

investigation, including jejunal biopsy and barium studies, were classified as having an irritable bowel syndrome, and this finding is perhaps worthy of further study.

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