Acta Med Scand 1984; 215: 375–8

Polymorphism of Complement C3 in Chronic Inflammatory Bowel Disease

Predominance of the $C3^F$ Gene in Crohn's Disease

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ABSTRACT. Elmgreen J, Sørensen H, Berkowicz A. (Department of Medical Gastroenterology, Herlev University Hospital, Herlev, and the Blood Bank, Department of Clinical Immunology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.) Polymorphism of complement C3 in chronic inflammatory bowel disease. Acta Med Scand 1984; 215: 375–8.

Polymorphism of the third component of complement (C3), occupying a key position in cascade reactions, was investigated in 125 consecutive outpatients, 53 with Crohn's disease and 72 with ulcerative colitis. A sample of 1378 randomly selected healthy volunteers of Danish origin served as controls. Occurrence of the F and FS phenotype of C3 (C3F and C3FS) was increased in the group of Crohn's disease patients ($\chi^2=2.80$, $p<0.05$, one-tailed test) and in a subgroup of Crohn patients with the gastrointestinal disease process confined to ileum ($\chi^2=6.91$, $p<0.01$). C3 phenotype distribution was unaffected in ulcerative colitis. Only S and F alleles of C3 ($C3^S$ and $C3^F$) were recognized and $C3^F$ frequencies were 0.33 in Crohn patients with small bowel disease, 0.23 in all Crohn patients, 0.18 in ulcerative colitis patients and 0.17 in healthy volunteers. The results are compatible with a positive association of the $C3^F$ gene and Crohn's disease located in the small bowel. *Key words: C3 polymorphism; colitis, ulcerative; complement component C3; Crohn's disease.*

The third component of complement (C3) occupies a key position in complement cascade reactions involving both classical and alternative pathway (1). Complement may be important for perpetuation of chronic inflammation. During activation, split products with potent chemotactic, opsonic, and anaphylatoxic properties are released from the centrally placed complement proteins as C3 and C5 and activation of terminal components may lead to cytolysis (1). In chronic inflammatory bowel disease, particularly Crohn's disease, hypercatabolism of C3, suggesting involvement of complement cascade reactions, has been revealed by unrelated immunologic techniques (2, 3, 4).

The aim of this report was to study polymorphism of C3 in chronic inflammatory bowel disease. Two common alleles, F and S, of C3 ($C3^F$ and $C3^S$) have been identified by the electrophoretic mobilities of the corresponding phenotypes of C3: F, FS, and S (C3F, C3FS and C3S) (5, 6). Concentrations of the two gene products in serum from heterozygotes are approximately equal (5) but their function in immune adherence reactions is different (7).

STUDY POPULATION

Typing of C3 was performed in 125 patients with well established chronic inflammatory bowel disease, 53 with Crohn's disease and 72 with ulcerative colitis. The patients were consecutively

Abbreviations: C3 = complement 3, C5 = complement 5, $C3^F$ = F allele of C3, $C3^S$ = S allele of C3, C3F = F phenotype of C3, C3FS = FS phenotype of C3, C3S = S phenotype of C3.

admitted to our regional Outpatient Clinic for a routine clinical assessment. Diagnostic criteria have been published previously (8). None suffered from complicating conditions such as hypertension, arteriosclerotic vascular disease or rheumatoid arthritis, which are associated with changes of C3 phenotype distribution (9–12).

At the time of the study the median duration of Crohn's disease was 4 years (range 0–11). Clinical parameters had been recorded prospectively during this period on a standard form. The course of disease was classified as severe if at least one of the following three criteria was fulfilled during the observation period: 1) two or more intestinal resections, 2) systemic treatment with steroids for at least 3 months, 3) total elimination of working capacity for at least one month due to inflammatory bowel disease. Assessment of disease localization was evaluated by at least one pair of simultaneous X-ray examinations of small bowel and colon, performed during an active stage of the disease.

The control sample consisted of 1378 healthy persons of Danish origin. Of these, 750 had participated in parternity investigations in the Institute of Forensic Medicine, Copenhagen (10) and an additional 628 had been investigated in a population study in a Copenhagen suburb, Glostrup. C3 distribution did not differ between the two populations. Rare alleles, found in 10 of the healthy volunteers, were not included in the control material. Informed consent was obtained from all participants.

METHODS

Plasma for C3 typing was drawn in EDTA (10 mM) and kept at −70°C until analysis by horizontal high voltage electrophoresis according to Teisberg (13).

Statistics

χ^2-test corrected for continuity was applied for comparing larger samples (number of observations ≥60) and the fourfold table test (14) for smaller samples. Unless otherwise stated, p-values for two-tailed test are given.

RESULTS

Distribution of C3 phenotypes among Crohn's disease patients, patients with ulcerative colitis, and normal controls is shown in Table I. An increased frequency of the $C3^F$ gene was revealed in Crohn's disease ($\chi^2=2.80$, $p<0.05$, one-tailed test) but not in ulcerative colitis ($\chi^2=1.40$, $p>0.20$) compared to the controls.

Table II shows the subgroups of Crohn's disease patients divided according to clinical parameters recorded during the course of the disease. Patients with small bowel disease had an increased frequency of the $C3^F$ gene compared to controls ($\chi^2=6.91$, $p<0.01$). The subgroup with small bowel and/or colon affection had a $C3^F$ frequency similar to that of the controls (Table II). $C3^F$ frequencies did not correlate with the presence of arthritis episodes or severity of disease as assessed during the median observation period of 4 years (Table II). There was, however, a trend towards increased $C3^F$ frequency in the subgroup

Table I. *Distribution of C3 phenotypes in patients and normal controls*

	Phenotypes							
	F		FS		S		Total	$C3^F$
	n	%	*n*	%	*n*	%	(*n*)	frequency
Crohn's disease	1	2	22	42	30	57	53	0.23
Ulcerative colitis	0	0	26	36	46	64	72	0.18
Normal controls	43	3	391	28	944	69	1378	0.17

Patients with Crohn's disease vs. normal controls $\chi^2=2.80$, $p<0.05$, one-tailed test.

with arthritis which did not reach statistical significance compared to controls ($\chi^2=2.08$, $p>0.10$).

DISCUSSION

The present paper demonstrates a significantly increased frequency of the $C3^F$ gene in consecutive, well established cases of Crohn's disease. A subgroup of patients with small bowel disease accounted for the predominance of $C3^F$ genes. Occurrence of C3 phenotypes did not relate to the severity of the diseases or arthritis episodes as assessed during a median observation period of 4 years. Patients with ulcerative colitis did not differ from healthy volunteers in respect to $C3^F$ gene frequency.

Increased $C3^F$ frequency in Crohn's disease is not specific. A positive association between other chronic inflammatory conditions such as rheumatoid arthritis (11, 12) and multiple sclerosis (15) and the $C3^F$ gene has previously been reported. The $C3^F$ frequencies in these groups of patients are similar to those seen in our patients with Crohn's disease.

Experimental evidence concerning the function of different C3 phenotypes in sparse. Total haemolytic capacity of sera is unrelated to the C3 phenotype (5). However, cytolysis may not be the critical function of C3. The C3F protein exhibits increased capacity for binding onto receptors of mononuclear cells compared to the C3S protein (7), suggesting that the structural features responsible for C3 polymophism affect the functionally active site of the molecule. Investigations of opsonization, release of anaphylatoxins and immune adherence by different C3 phenotypes in normal controls and patients with well defined chronic inflammatory conditions are in progress.

ACKNOWLEDGEMENTS

The study was supported by the Danish League against Rheumatism and by Johann and Hanne Weiman née Seedorf's Foundation.

Table II. *C3 phenotype distribution in relation to clinical parameters in patients with Crohn's disease and healthy volunteers*

	C3 phenotype			
	F+FS	S	Total	$C3^F$ frequency
Healthy volunteers	434	944	1 378	0.17
Crohn's disease patients				
Disease localization				
Small bowel	10	5	15	0.33
Small bowel and/or colon	13	25	38	0.18
Arthritis				
Present	11	12	23	0.26
Absent	12	18	30	0.20
Course of the disease				
Severe	10	14	24	0.23
Mild	13	16	29	0.22

Crohn patients with small bowel disease vs. healthy volunteers $\chi^2=6.91$, $p<0.01$. Changes in C3 phenotype distribution between corresponding subgroups of Crohn patients did not reach statistical significance ($p>0.10$).

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Received June 27, 1983.

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