

HUMORAL IMMUNITY IN LIBYAN PATIENTS WITH ULCERATIVE COLITIS

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SUMMARY: The aetiology of ulcerative colitis remains uncertain. Most of the clinical manifestations are related to inflammation. Also, the most frequently used drugs in the treatment of ulcerative colitis, sulphasalazine and corticosteroids, have expressed anti-inflammatory properties. In our present study we have investigated serum levels of complements (C3, C4) and immunoglobulins (IgG, IgA, IgM, IgD, IgE) in 29 Libyan patients with ulcerative colitis (age: 17–57 years; sex: 17 males, 12 females) and 29 healthy Libyans (age: 18–59 years; sex: 16 males, 13 females) as controls. It was observed that at active stage of the disease (pretreatment) serum levels of C4, IgA and IgE were significantly elevated, whereas C3 levels were significantly lowered compared to control subjects ($p < 0.00001$). Follow-up studies showed that C3 and C4 levels were brought to normal levels at remission after 2 months of therapy with different drug regimens and at 1 year of maintenance therapy. But serum IgA and IgE levels remained significantly elevated ($p < 0.00001$) even at remission after treatments with different drug regimens, although they were lowered to some extent, but not significantly. The clinical evaluations at 2 months of treatment and at 1 year of maintenance therapy fulfilled the criteria for remission of the disease activity. Our results were therefore taken as indications that alternative pathway activation of the complement system was present which may be responsible for the aggravated inflammation present in ulcerative colitis. It has been proposed that complement activation in our patients may be mediated through IgA and/or IgE-containing immune complexes formed secondary to immune response against initial, but unknown, assaulting agent.

Key Words: Complement, immunoglobulin, inflammation, ulcerative colitis.

INTRODUCTION

The aetiology of ulcerative colitis is unknown although there is an abundance of theories and data implicating genetic predisposition, immunologic alterations, infectious agents and other environmental factors (22). Most of the clinical manifestations are related to inflammation including symptoms of pain, diarrhea, hemorrhage and fever. As yet there is no intervention that prevents the disease nor there is a specific therapy that removes the causative factor, other than perhaps surgical resection. Instead, medical therapy is directed at reducing acute inflammation and treating its manifestations and complications. The dilemma of ulcerative colitis concerning immunology

has not changed in spite of extensive research and many experimental and clinical studies (41). The immunological studies in ulcerative colitis have recently been concentrated largely on mechanism that may be responsible for inflammation relevant to the pathogenesis of the disease initiated for immunological reasons or in response to infection (4). Strickland and Sacher (41) reported that serum levels of immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) were not affected in ulcerative colitis. According to other reports most patients with inflammatory bowel disease (IBD) have elevated levels of immunoglobulins (9, 20, 27, 29, 31, 35) which usually decrease after treatment with sulphasalazine (38). Although ulcerative colitis is considered rare in most of the developing world, there are recent reports that this disorder occurs in Kuwait, Iran, Turkey, India and Saudi Arabia

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(1,18). Literature survey has indicated that no immunological study has been done or reported in Libyans with ulcerative colitis. We have studied and followed-up 29 Libyan patients with ulcerative colitis for serum levels of immunoglobulins and complements before and after treatment with sulphasalazine and/or sulphasalazine plus corticosteroids and the results are reported here.

PATIENTS AND METHODS

Patients

Between 1985–1989, a total of 29 patients (age: 17-57 years, sex: 17 males, 12 females) were diagnosed in the Gastroenterology Clinic, University Teaching Hospital, Benghazi, as having ulcerative colitis. The diagnosis of ulcerative colitis was established by standard clinical, radiological, endoscopic and histopathological methods (2). The main clinical features included diarrhea, general malaise, abdominal pain, rectal bleeding, anorexia, abdominal tenderness, pyrexia, weight loss and frequent bowel movements (3–20 times per day). All the 29 patients were bled and specimens were collected with and without anticoagulant before commencing any treatment. In addition to clinical scoring, the disease activity was ascertained according to standard assessments (7, 10) including complete blood picture, hemoglobin, erythrocyte sedimentation rate, serum albumin, liver function tests, urea and electrolytes, C-reactive protein, urine analysis, blood and stool culture, urine and stool examination for ova, cysts and parasites. At proctosigmoidoscopy (37) one biopsy at least was taken and examined with regard to the activity of the pathologi-

cal process (6). Serum aliquots were kept frozen (-70°C) until immunoglobulins and complement were analyzed. The patients were then given different regimens of treatment as follows: 2–4 gm sulphasalazine daily to 12 patients (Treatment A) and 2–4 gm sulphasalazine daily plus topical corticosteroid daily to 7 patients (Treatment B) for 2 months. To another group of 10 patients 2–4 gm sulphasalazine was given together with 40 mg prednisolone daily orally for the 1st week; prednisolone was reduced to 30 mg daily for the 2nd week and from the 3rd week 20 mg daily for 4 weeks then tailing off the oral dose of steroid to zero till the end of the second month (Treatment C). At 2 months of treatments all these patients were reexamined clinically, reevaluated and had repeated endoscopic examination and blood specimens were collected for immunoglobulin and complement determination. Of the 29 patients, 15 patients with stable remission after 1 year of maintenance therapy (2 gm sulphasalazine daily) reported for follow-up (Table 1). Blood samples were obtained from these patients also for immunoglobulin and complement assays. Twenty nine apparently healthy Libyans (age: 18–59 years; sex: 16 males, 13 females) matched for age, sex and socio-economic background were also included in the study as control subjects (CS).

Estimation of serum immunoglobulins and complements

The serum levels of IgG, IgA, IgM, IgD, C3 and C4 were measured by radial immunodiffusion technique of Mancini *et al.* (28) using immunokits of bioMerieux, France. The serum IgE level was determined by enzyme linked immunosorbent assay (ELISA) technique using the ELISA-Kits of bioMerieux, France.

Table 1: The serum immunoglobulin and complement profiles in control subjects as well as in patients with ulcerative colitis before and after treatments.

Parameters (Mean±SD)	Control subjects (n=29)	Ulcerative colitis patients (UC)* (n=29)	** Different treatment regimens and follow-up								
			Treatment A			Treatment B			Treatment C		
			A1 (n=12)	A2 (n=12)	A3 (n=6)	B1 (n=7)	B2 (n=7)	B3 (n=4)	C1 (n=10)	C2 (n=10)	C3 (n=5)
C3 (mg/dl)	129±28	58±10	57±13	115±19	130±23	59±11	122±19	120±26	66±14	135±23	133±25
C4 (mg/dl)	32±10	69±1	70±21	40±6	37±8	78±15	38±12	42±6	64±16	42±10	35±9
IgG (mg/dl)	1035±148	1218±170	1211±196	1302±201	1246±235	1167±270	1166±225	1020±196	1263±211	1225±168	1288±216
IgA (mg/dl)	235±50	482±93	522±135	445±82	497±67	476±56	427±43	482±90	443±98	422±76	429±26
IgM (mg/dl)	120±25	160±30	165±38	151±30	168±28	157±31	160±35	188±25	156±36	143±31	170±18
IgD (mg/dl)	1.9±0.5	2.4±0.6	2.6±1.0	2.4±0.7	3.2±0.8	1.9±0.7	2.2±0.6	2.2±0.6	2.0±0.7	1.8±0.6	2.3±0.6
IgE (iu/ml)	95±25	512±127	445±253	403±220	489±186	554±230	508±178	508±178	564±170	530±130	560±196

* UC = All patients together (n=29) before commencing any of the treatments, i.e. (A1+B1+C1).

** Treatment A, Treatment B, Treatment C: See Patients and Methods. A1, B1, C1: Before treatment; A2, B2, C2: At 2 months of treatment; A3, B3, C3: At 1 year of maintenance therapy with 2 gm sulphasalazine daily.

Table 2: The results of analysis of variance of the data presented in Table 1.

Groups compared	C3 (mg/dl)			C4 (mg/dl)			IgA (mg/dl)			IgE (iu/ml)		
	F-ratio	d.f.	Significance level	F-ratio	d.f.	Significance level	F-ratio	d.f.	Significance level	F-ratio	d.f.	Significance level
CS, UC (i.e. CS, A1, B1, C1)	134.03	1.56	p<0.00001	81.51	1.56	p<0.00001	111.88	1.56	p<0.00001	83.55	1.56	p<0.00001
CS, A2, B2, C2	1.32	3.54	p=0.278	1.57	3.54	p=0.231	46.64	3.54	p<0.00001	38.01	3.54	p<0.00001
A2, B2, C2	2.19	2.26	p=0.131	0.75	2.26	p=0.483	0.28	2.26	p=761	1.33	2.26	p=0.282
CS, A3, B3, C3	0.15	3.40	p=0.931	1.36	3.40	p=0.265	53.78	3.40	p<0.00001	37.74	3.40	p<0.00001
A3, B3, C3	0.22	2.12	p=0.81	0.85	2.12	p=0.45	1.23	2.12	p=0.33	0.6	2.12	p=0.86

d.f.: Degree of freedom; p<0.05: Significant; p>0.05: Not significant.

Statistical analysis

The statistical significance of the results were evaluated by performing analysis of variance using statgraphics package program in a microcomputer (Model: IBM-XT).

RESULTS

According to the standard criteria for grading the severity of the disease 16 patients (age: 19–43 years; sex: 9 males, 7 females) had mild form of the disease involving rectum only; 10 patients (age: 17–57 years; sex: 6 males and 4 females) had moderate form involving rectum, sigmoideum and left colon and 3 patients (age: 28–39 years; sex: 2 males, 1 female) had pancolitis. The frequency of atopy in our patients was 6.54% i.e., 1 patient had bronchial asthma and 1 patient had hay fever. Blood and stool culture, urine and stool microscopic examinations did not reveal any infective cause (bacterial, fungal or parasitic) in our patients. Barium meal and follow-through examinations were also normal. No small bowel disease was present in our patients. The results of the estimation of serum complements and immunoglobulins and their statistical analyses are shown in Table 1 and Table 2 respectively. It was observed that the 29 patients having active disease had significantly elevated levels of C4, IgA and IgE and reduced level of C3 (p< 0.00001). After treatment for 2 months with various drug regimens, serum C3 and C4 levels were found to be normal, although IgA and IgE levels remained significantly elevated (p<0.00001). The 15 patients in stable remission after 1 year of maintenance therapy still had elevated levels of IgA and IgE (p<0.0001), but C3 and C4 levels were within normal ranges. Serum IgG, IgM and IgD levels were not affected. Regarding treatments, no differences were found among the three regimens of therapy with respect to their effects on serum complement and

immunoglobulin levels. No serious side effects in our patients were observed, although in some studies sulphasalazine induced immunodeficiency were described (24, 39). All our patients at 2 months of treatment and at 1 year of maintenance therapy have satisfied the criteria for remission of the activity of the disease (7,10,37). Some patients with more serious form of ulcerative colitis, not responding to sulphasalazine alone, received oral prednisolone (40 mg daily) together with steroid retention enema and sulphasalazine (2–4 mg daily) and if remission was not obtained these patients were admitted to the hospital and treated for a severe attack (13). This type of patients were, however, not included in our study.

DISCUSSION

The immunology of ulcerative colitis remains a tantalizing and frustrating field (3, 20, 22, 41). The hypothesis was popular that ulcerative colitis was an 'autoimmune disorder' in which antibodies that developed in response to coliform bacterial antigens reacted against epithelial cells (31); against this conception following reasons appeared: the characteristic HLA profile has not emerged (3); auto antibodies against the colon have been demonstrated in other conditions including familial polyposis and pyelonephritis (25). Also, later studies have shown that the association with antibodies to particular bacterial serotypes is not specific (15). It seems probable that there is a variety of gut-associated antigens: bacterial, food-derived, viral etc. Once the gut-associated antigens: bacterial, food-derived, viral etc. Once the gut mucosal defense has been breached secondary immune responses to these antigens seem inevitable and, even if secondary in origin, their expression may be the major contribution to the inflammatory characteristics of ulcerative colitis. One of the striking histological abnormalities

seen in the colonic mucosa with active ulcerative colitis is the considerable increase in plasma cells (34).

An important effector mechanism in the pathogenesis of mucosal inflammation might be the formation of antigen-antibody complexes within the mucosa with subsequent of the complement system (16). Although it seems probable that such mechanisms occur within the mucosa, there is no direct proof that this is so. Nevertheless, there is strong indirect evidence as shown by increased complement metabolism, the presence of complement degradation products in plasma of patients with active disease and by the increased circulation antibody titres to these degradation products (20, 35). Antibodies synthesized within the mucosa against gut-associated antigens might well be a major mechanism not only for causing acute inflammation but also for rendering more inflammation. The complement system consists of a series of proteins which when activated, are the key mediators of the inflammatory response through their capacity to increase vascular permeability, stimulate neutrophil and monocyte chemotaxis, enhance mast cell discharge, induce smooth muscle contraction, and with completion of terminal assembly, produce cell-membrane disruption. The serum level of complement protein, of course, represents the net of synthesis and utilization. Therefore normal levels may be seen despite significant activation. Elevations of serum complements especially C3, C4 and CH50 generally reflect an acute phase reaction. Hodgson *et al.* (17) and Ward and Eastwood (43) have increased C3 turnover *in vivo* in patients with IBD.

Yet the complement aberrations demonstrated in the sera of patients are not very consistent, and may represent little more than non-specific markers of acute inflammation. The C3 conversion products have also been reportedly increased in IBD and in IBD-patients with evidence of circulation immune complexes (CICs) (32, 42).

The elevations of serum C4 component of complement in active disease are usually mild and consistent with an acute phase reaction (25). The serum C4 level was raised significantly in our patients in active phase of the disease compared to normal subjects. However, the post-treatment levels of C4 at 2 months of treatment and at stable remission after 1 year of maintenance therapy were within in the normal range. On the contrary, serum C3 levels in our patients with active disease were significantly lower as compared to normal subjects. These levels were raised significantly after treatments and were observed to be within the normal range at 2-months of treatment and at remission after 1-year of maintenance therapy (Tables 1 and 2). These changes in complement

levels were suggestive that alternative pathway activation of the complement system was present or dominant in our patients during the active phase of the disease which may be responsible for inflammation. Strickland *et al.* (41) showed that immunoglobulin (IgG, IgA, IgM) were not raised, whereas Rubinstein *et al.* (38) observed elevated levels of serum IgA and IgE in most of their patients with IBD. In our study, significantly raised serum levels of IgA and IgE were observed at active stage of the disease. Although these levels were reduced to some extent with the treatment regimens, they remained significantly elevated even at stable remission after 1 year of maintenance therapy (Tables 1 and 2). Rubinstein *et al.* (38) showed increased circulating B-cells in ulcerative colitis and Macdermatt *et al.* (25) explained that the increased IgA synthesis and secretion by peripheral blood monocytes from IBD-patients was due to a systemic immune response by the penetration of injurious agent through initiation of inflammatory process in the gut mucosa. There are evidences of the presence of CICs and increased C3 conversion in CICs-positive patients with ulcerative colitis (8, 21, 32, 36). Pfanfenback *et al.* (36) gathered evidence that IgA-CICs do activate alternative pathway without consuming C4. Also, there is evidence that IgE-CICs can activate complement via alternative pathway (19). The stimulation of normal T-lymphocytes leading to production of T-cytotoxic cells by CICs has also been shown to be dependent on alternative pathway activation of the complement system (40). Hassner and Saxon (14) demonstrated that IgE-isotype specific human T-suppressor cells are generated by IgE-CICs to control excessive IgE-antibody production. If free IgE-antibodies are available in large amounts, then IgE-CICs are unable to stimulate IgE-Fe⁺ T-lymphocytes as the IgE-Fe-receptors are blocked by free IgE. The IgE-antibody production continues due to lack of regulatory IgE-isotype specific T-suppressor cells. Considering the literature cited above, our findings in the present study do not seem to fit with the theories that ulcerative colitis is due to atopic or type I hypersensitivity reactions (5, 36). Secondly, the frequency of atopy in our 29 patients with ulcerative colitis was only 6.54% (1 patient with bronchial asthma and 1 patient with hay fever) similar to many other reports (30, 33, 34, 44). Our observations of significantly higher C4 and lower C3 together with significantly higher IgA and IgE serum levels were, therefore, taken as indications that alternative pathway activation of the complement system and inflammation were present at active stage of the disease. This may be CICs-mediated secondary to immune response against initial assaulting agent or agents. These probable

CICs may be composed of IgA-and/or IgE-class of antibodies and bacterial endotoxins or parasitic or viral antigens or auto antigens (11, 12, 23). Fiocchi *et al.* (12) reported that immune sensitization to intestinal epithelial antigens (auto antigens) in families with chronic inflammatory bowel disease is common; its high frequency among asymptomatic relatives suggested that it may represent a primary phenomenon perhaps predisposing individuals to gut injury. Although the stool and blood culture sensitivity tests, complete blood picture and microscopic examinations of stool and urine ruled out the possibilities of bacterial, fungal or parasitic infections in our patients, the possibility of viral infection could not be ruled out in our patients in the present study. Detailed immunochemical studies on CICs may therefore lead to identification of the antigens involved which may help to uncover the source of the antigen and hence, uncover the aetiology of ulcerative colitis.

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