Acute phase proteins, C9, factor B, and lysozyme in recurrent oral ulceration and Behçet's syndrome

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SUMMARY The concentrations and sequential changes of some acute phase proteins, factor B, and lysozyme have been assayed in recurrent oral ulceration and Behçet's syndrome. C9 was elevated in both groups of patients and was the most sensitive index of disease activity; however, it failed to discriminate between the three types of recurrent oral ulcers and four types of Behçet's syndrome. The level of α 1 acid glycoprotein and lysozyme were significantly increased predominantly in the ocular type, whereas factor B was significantly increased especially in the neurological type of Behçet's syndrome. It is suggested that the changes in the concentrations of some plasma proteins may help our understanding of tissue involvement in Behçet's syndrome, as well as in the selection of therapeutic agents in this disease.

An earlier investigation of some acute phase proteins has shown that C-reactive protein (CRP) but not α 1 antitrypsin, is significantly increased in Behçet's syndrome.¹ Serum C9 concentration is also significantly increased in patients with Behçet's syndrome,¹⁻³ and in those with recurrent oral ulcers.¹ The functional significance of these findings is not clear, and two views have been particularly considered. Firstly, that in Behçet's syndrome and recurrent oral ulcers there is repeated damage of epithelial and other tissues, and this might induce a non-specific increase in acute phase proteins. An alternative view ascribes more specific functions to these proteins; CRP may activate complement, enhance phagocytosis, and depress some T cell functions.⁴⁻⁶ C9 might play a part in lysis of the affected cells in Behçet's syndrome and recurrent oral ulcers.

In this investigation we have measured the concentrations of CRP, C9, and α 1 acid glycoprotein (α 1AG; orosomucoid) among the acute phase proteins. We have also assayed factor B, as this is involved in the alternative complement pathway activation,⁷ and lysozyme, as this may play a part in antimicrobial activity of the mucosal surfaces,⁸ and serum lysozyme levels may be an index of the turnover of neutrophils.⁹

Material and methods

PATIENTS

A group of 60 patients with Behçet's syndrome, 85 with recurrent oral ulcers, and 34 matched controls were investigated. Patients with Behçet's syndrome were subdivided according to their tissue involvement into four types:¹⁰ (a) mucocutaneous type with oral and genital and with or without skin manifestations (9 patients); (b) arthritic type with joint involvement and two or more of the mucocutaneous lesions (14 patients); (c) neurological type with brain manifestations and some or all lesions found in the mucocutaneous and arthritic types (10 patients); and (d) ocular type with uveitis in addition to some or all lesions found in the mucocutaneous, arthritic, and neurological types (17 patients). Patients with recurrent oral ulcers were subdivided into minor aphthous ulcers (MiAU; 51 patients), major aphthous ulcers (MjAU; 14 patients), and herpetiform ulcers (HU; 20 patients).¹¹

Samples of 10 ml of blood were taken from each patient, and serial samples of blood were collected from some patients at one- to six-monthly intervals for up to three years. The protein concentrations were related to a clinical index of activity during remissions and exacerbations of the disease. Each ulcer was given an index of 1, and any cutaneous, arthritic, or ocular manifestation of disease was given an index of 1; healing or clearing lesions were

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Table 1 Concentration of C9, C-reactive protein (CRP), lysozyme (LZM), α 1 acid glycoprotein (α 1AGP), factor B, and the ESR in controls, minor aphthous ulcers (MiAU), major aphthous ulcers (MjAU), and herpetiform ulcers (HU)

	Controls			MiAU		MjAU		HU	
	n	Mean $\pm$ SD	Mean + 2 SD	n	Mean $\pm$ SD	n	Mean $\pm$ SD	n	Mean $\pm$ SD
C9*	34	93.9 $\pm$ 19.96	133.8	51	121.4 $\pm$ 34.8	14	153.9 $\pm$ 89.4	20	173.0 $\pm$ 62.6
CRP (mg/100 ml)	29	0.07 $\pm$ 0.25	0.57	51	0.1 $\pm$ 0.4	11	0.25 $\pm$ 0.6	20	0.64 $\pm$ 0.8
LZM*	26	88.1 $\pm$ 18.4	124.9	41	94.7 $\pm$ 20.6	12	90.2 $\pm$ 22.9	14	113.0 $\pm$ 43.7
α 1AGP (mg/100 ml)	33	75.0 $\pm$ 20.6	116.0	26	81.2 $\pm$ 38.9	10	88.1 $\pm$ 32.9	16	78.4 $\pm$ 28.9
Factor B (mg/100 ml)	24	13.4 $\pm$ 3.7	20.8	26	13.2 $\pm$ 6.5	11	19.1 $\pm$ 5.8	13	15.9 $\pm$ 6.4
ESR (mm/h)				32	6.1 $\pm$ 5.6	14	10.3 $\pm$ 7.3	17	14.2 $\pm$ 10.8

*% of normal serum.

given an index of 0.5, and absence of any clinical features was denoted by 0. The individual values were added up to give a clinical index of disease activity.

ASSAY OF CRP, C9, α 1AG, LYSOZYME, FACTOR B, AND ESR

The levels of C9, factor B, CRP, and α 1AG were estimated by the radial diffusion technique using commercial antisera (Behringwerke, AG, Marburg-Lahn, Germany). Details of the methods have been published elsewhere.¹ The levels of factor B, CRP, and α 1AG are expressed in mg/100 ml. Those of C9 are expressed as a percentage of the concentration present in a pool of normal sera containing 23 mg/100 ml of C9. Lysozyme was measured by the lysoplate method, as described by Osserman and Lawlor¹² using a pool of normal serum as standard. The ESR was assessed by the method of Westergren and expressed in mm in 1 hour.

Results

ESR AND CONCENTRATIONS OF PROTEINS

A significant increase in the ESR was considered to be a level greater than 15 mm in 1 hour. As the ESR was not assessed in controls, those with MiAU were considered as a baseline, and the Fisher's exact

test was used for comparison with the other groups. A significant increase in the ESR was found in all the groups except the neurological type of Behçet's syndrome (Tables 1 and 2). The highest frequency of raised ESR was found in the arthritic type of Behçet's syndrome.

The mean ($\pm$ SD) of the concentration of each protein in the controls, three types of recurrent oral ulcers, and four types of Behçet's syndrome are given in Tables 1 and 2. The individual levels of C9 and factor B are shown in Figures 1 and 2. It can be seen that there was a common pattern of increase in concentration from MiAU to MjAU, sometimes in HU and then the mucocutaneous, arthritic, neurological, and ocular types of Behçet's syndrome. These results were plotted in a histogram (Fig. 3) to facilitate comparison between the different proteins in the various clinical groups. A positive value was ascribed to one greater than the mean + 2 SD of the controls, and the percentage of positive values was determined for each group. Significant differences were established by Fisher's exact test for all five proteins between patients with the various types of Behçet's syndrome and controls; the highest p value was reached with C9 ($p = 4.9 \times 10^{-11}$) and the lowest value for lysozyme ($p = 0.03$). Only C9 showed a significantly increased value in recurrent oral ulcers ($p = 0.0001$).

Table 2 Concentration of C9, C-reactive protein (CRP), lysozyme (LZM), α 1 acid glycoprotein (α 1AGP), factor B, and the ESR in controls, mucocutaneous (M-C), arthritic, neurological, and ocular types of Behçet's syndrome

	Controls			Mucocutaneous		Arthritic		Neurological		Ocular	
	n	Mean $\pm$ SD	Mean + 2SD	n	Mean $\pm$ SD	n	Mean $\pm$ SD	n	Mean $\pm$ SD	n	Mean $\pm$ SD
C9*	34	93.9 $\pm$ 19.96	133.8	19	167.5 $\pm$ 75.4	14	237.0 $\pm$ 131.0	10	167.0 $\pm$ 84.0	17	197.0 $\pm$ 67.4
CRP (mg/100 ml)	29	0.07 $\pm$ 0.25	0.57	19	0.66 $\pm$ 0.86	13	6.0 $\pm$ 16.3	8	4.5 $\pm$ 12.3	17	5.6 $\pm$ 15.6
LZM*	26	88.1 $\pm$ 18.4	124.9	15	95.7 $\pm$ 25.8	14	111.6 $\pm$ 29.4	9	101.6 $\pm$ 16.6	14	120.0 $\pm$ 51.0
α 1AGP (mg/100 ml)	33	75.0 $\pm$ 20.6	116.0	14	78.1 $\pm$ 30.4	12	123.0 $\pm$ 56.0	8	101.4 $\pm$ 32.0	15	117.4 $\pm$ 55.7
Factor B (mg/100 ml)	24	13.4 $\pm$ 3.7	20.8	12	19.0 $\pm$ 5.8	12	22.6 $\pm$ 6.8	10	29.3 $\pm$ 13.8	16	23.1 $\pm$ 8.6
ESR (mm/h)				16	19.0 $\pm$ 13.9	13	31.2 $\pm$ 19.2	9	28.7 $\pm$ 45.8	16	25.1 $\pm$ 28.9

*% of normal serum.

Fig. 1 Concentration of C9 in the three types of recurrent oral ulcers and the four types of Behçet's syndrome; shaded area indicates mean + 2 SD.

Key to abbreviations in Figs 1, 2, and 3

MiAU Minor aphthous ulcers
MjAU Major aphthous ulcers
HU Herpetiform ulcers
M-C Mucocutaneous
A Arthritic
N Neurological
Oc Ocular
C Controls
ROU Recurrent oral ulcers
BS Behçet's syndrome

Analysis of the individual groups of patients revealed that C9 was increased significantly in all groups, though with great differences in the *P* values (Fig. 3). CRP was also increased significantly in all groups except in MiAU, MjAU, and the neurological type of Behçet's syndrome. In contrast, both α 1AG and lysozyme showed very significantly increased values, predominantly in the ocular type of Behçet's syndrome. Although factor B was significantly increased in all types of Behçet's syndrome the prevalence was greatly increased only in the neurological type. There was no significant difference between MiAU and MjAU with any of the proteins.

CORRELATION BETWEEN DIFFERENT PROTEINS AND OTHER INDICES

The relationship between different proteins in each group of patients and the controls was analysed by calculating the correlation coefficient between the proteins (Table 3). The controls and patients with

Fig. 2 Concentration of factor B in the three types of recurrent oral ulcers and the four types of Behçet's syndrome; shaded area indicates mean + 2 SD.

MjAU, HU, and the mucocutaneous type of Behçet's syndrome showed no significant correlations except that between C9 and α 1AG in MjAU. However, significant correlations were found between C9, factor B, and α 1AG in MiAU. The arthritic type of Behçet's syndrome showed significant correlations between C9 and ESR, factor B, α 1AG, and CRP. However, in the neurological and ocular types, factor B was correlated with ESR, C9, CRP, and, in addition, α 1AG in the neurological, and lysozyme in the ocular, types only. C9 was also correlated with ESR and α 1AG in the neurological type of Behçet's syndrome (Table 3). The lysozyme concentration was also examined in relation to the combined neutrophil and monocyte count, and a significant negative correlation was found between them ($r = 0.4342$, $DF\ 24$, $P < 0.05$).

SEQUENTIAL ANALYSIS

Examination of serial samples of sera and the ESR during remissions and exacerbations of the disease over a period of up to three years revealed quantitative changes in the concentrations of the proteins tested and the ESR. A significant decrease in the clinical index in Behçet's syndrome from $2.5 (\pm 1)$ to $0.6 (\pm 0.8)$; $t = 6.9391$, $P < 0.0001$ was not associated with a significant decrease in the ESR in Behçet's syndrome (Fig. 4; $t = 2.0409$, $DF = 10$). Similarly, an increase in the clinical index was not associated with a significant increase in the ESR. Significant changes in the numbers of ulcers in recur-

Fig. 3 Percentage of significant serum proteins in the three types of recurrent oral ulcers and four types of Behçet's syndrome.

rent oral ulcers were hardly associated with any change in the ESR (not shown).

A decrease or increase in the number of ulcers in recurrent oral ulcers was associated with a corresponding decrease or increase in the concentrations of C9 in 16/19 (84%) patients (Fig. 5). A decrease in the number of ulcers ($t = 4.8925$, $DF 10$, $P < 0.001$) from $3.4 (\pm 1.8)$ to $0.6 (\pm 0.92)$ was associated with

a significant decrease in the concentration of C9 ($t = 3.1934$, $DF 10$, $P < 0.01$). An increase in ulcers from $0.3 (\pm 0.46)$ to $2.56 (\pm 1.05)$ was not associated with a significant increase in the concentration of C9 ($t = 1.6239$). A decrease in the clinical index ($t = 8.174$, $DF 15$, $P < 0.0001$) in Behçet's syndrome (Fig. 6) was also correlated with a very significant decrease in the concentration of C9 ($t = 3.720$, $DF 15$,

Table 3 Correlations between concentrations of C9 or factor B and ESR, $\alpha 1$ acid glycoprotein ($\alpha 1AGP$), C-reactive protein (CRP), and lysozyme (LZM); P values of correlation coefficients are given

Group	C9 versus:					Factor B versus:				
	ESR	Factor B	$\alpha 1AGP$	CRP	LZM	ESR	C9	$\alpha 1AGP$	CRP	LZM
Controls	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
MiAU	NS	<0.05	<0.01	NS	NS	NS	<0.05	<0.01	NS	NS
MjAU	NS	NS	<0.05	NS	NS	NS	NS	NS	NS	NS
HU	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mucocutaneous	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Arthritic	<0.01	<0.01	<0.01	<0.01	NS	NS	<0.01	NS	NS	NS
Neurological	<0.001	<0.05	<0.05	NS	NS	<0.002	<0.05	<0.01	<0.05	NS
Ocular	NS	<0.05	NS	NS	NS	<0.05	<0.05	NS	<0.02	<0.05

NS not significant.

Fig. 4 Changes in ESR during changes in the clinical index of patients with Behçet's syndrome.

$P < 0.005$) but not with those of CRP, lysozyme, factor B, or $\alpha 1AG$ (the latter two are not shown). An increase in the clinical index was not associated with a significant increase in any of the proteins, though the concentrations of lysozyme increased in 6/9 and that of C9 in 7/10 patients.

The effect of prednisolone and azathioprine on the clinical index and the concentration of C9 was examined in eight patients; these received between 15 and 40 mg of prednisolone and 200 mg of azathioprine daily for a period of six months to two years (Table 4). They were clearly divisible into two groups. Four patients whose C9 levels before

Fig. 5 Sequential assay of serum C9 concentrations in patients with recurrent oral ulcers: $\blacktriangle$ minor aphthous ulcer; $\triangle$ major aphthous ulcer; $\bullet$ herpetiform ulcer.

Fig. 6 Sequential assay of serum C9, lysozyme, and C-reactive protein (CRP) in patients with Behçet's syndrome: $\blacktriangle$ mucocutaneous; $\triangle$ arthritic; $\blacksquare$ neurological; $\bullet$ ocular.

treatment were greater than 200% showed a considerable clinical improvement with a fall in the clinical index and a fall in the level of C9. In contrast

Table 4 Effect of prednisolone and azathioprine on the clinical index and C9 levels

Patient	C9 (% normal serum)		Clinical index	
	Before*	After*	Before	After
1	430	136	3	0.5
2	400	140	3	0
3	352	122	2.5	0.5
4	240	96	2	0.5
5	198	162	3	3
6	124	158	2	3
7	124	146	2	2
8	118	166	2	1

*Before or after treatment started with prednisolone and azathioprine.

the other group of four patients whose C9 levels before treatment were less than 200% showed little clinical change, and the C9 levels increased in three of the four patients after treatment with prednisolone and azathioprine. The changes in the concentrations of C9 were therefore not related to these drugs but to the clinical changes. Furthermore, patients with a high level of C9 (> 200%) responded readily to the treatment, as compared with those whose initial C9 levels were below 200%.

Discussion

The changes in the concentrations of some proteins in sera from patients with recurrent oral ulcers and Behçet's syndrome may be significant in the pathogenesis of the spectrum of diseased tissues. They may also help in the diagnosis and prognosis of these disease states. It is thought unlikely that most of the proteins under examination increased as a result of tissue damage, as there were significant differences in concentrations between different groups of patients. C9 and CRP were increased indiscriminately in all or most of the groups of patients studied, as would be expected with acute phase proteins. In contrast to C9 and CRP, α 1 antitrypsin was not increased¹ and α 1AG was increased predominantly in the ocular type of Behçet's syndrome (Fig. 3).

The most significant increases in concentration were found with C9, which was also the most sensitive index of disease activity. An increase or decrease in the number of ulcers was associated with a corresponding change in concentration of C9 in 84% of patients with recurrent oral ulcers. A change in the clinical index of Behçet's syndrome was also correlated with a corresponding change in C9 in 77% of the patients. Perhaps a surprising finding was that C9, factor B, and α 1AG were significantly correlated in MIAU, to a limited extent in MjAU, and not at all in HU. C9 and factor B were correlated in the arthritic, neurological, and ocular, but not in

the mucocutaneous, types of Behçet's syndrome. However, as C9 increased across the whole spectrum of Behçet's syndrome and in all types of recurrent oral ulcers, it is not a good discriminator between the different types of Behçet's syndrome or recurrent oral ulcers. This is consistent with both views that C9 is involved in cellular damage or as an acute phase protein modulating the immune response to damaged tissue.

Although the concentration of factor B was significantly increased in all types of Behçet's syndrome, a very significant prevalence was found only in the neurological type (Fig. 2). Factor B was significantly correlated with C9 and CRP in the neurological and ocular types of Behçet's syndrome (Table 3), and this raises the possibility that the alternative complement pathway might be involved. This hypothesis is consistent with the presence of normal serum concentrations of C4 in recurrent oral ulcers and Behçet's syndrome¹³ and with C3, in the absence of immunoglobulin deposits, in the walls of blood vessels from these patients.¹⁴ However, factor B concentration was not significantly correlated with the clinical index.

The increased levels of CRP in all but the neurological type of Behçet's syndrome might also be related to activation of complement.^{4,5} However, CRP may have a paradoxical effect in increasing complement-dependent reactions and decreasing cell-mediated immune responses by inhibiting T cell function.⁶

The concentration of lysozyme was significantly increased and correlated with factor B only in the ocular type of Behçet's syndrome (Fig. 3 and Table 3) and seems to be a good discriminator for this type of disease. These findings suggest the possibility that activation of the alternative complement pathway might be involved in tissue damage and induce monocytes and neutrophils to release lysozyme. There is experimental evidence in rabbits that serum lysozyme is derived mostly from neutrophil destruction.⁹ Indeed, a significant negative correlation was found between the concentration of lysozyme and the number of these leucocytes in Behçet's syndrome. These results suggest that some patients with Behçet's syndrome may have an increased destruction of neutrophils and monocytes and that the greater the number of leucocytes destroyed, the greater the amount of lysozyme released into the circulation. Membrane fragments with complement holes have been found by electron microscopy in the circulation of some patients with Behçet's syndrome,¹⁵ and it has been suggested that these fragments might be the remnants of damaged neutrophils. Furthermore, a relationship between the lysozyme concentration and increased disease

activity has been seen in 5/8 patients, and this will be further pursued by frequent serial examinations, as the half-life of lysozyme is only 2 to 3 hours.¹⁶

The concentration of α 1AG was very significantly increased in the ocular type of Behçet's syndrome ($P < 0.001$). This glycoprotein contains about 41% carbohydrate, and it has been proposed that it may coat damaged tissue and thereby decrease their potential antigenicity.¹⁷ Whether increased formation of α 1AG might be protective in the ocular type of Behçet's syndrome is not clear at the present time but should be considered in long-term studies of the prognosis of uveitis in Behçet's syndrome.

It is of considerable interest that in Behçet's syndrome, in which a sequence of tissue damage of increasing severity and morbidity may occur, a variety of plasma proteins show differential increases in concentrations. These studies may help to define an association between tissue damage and some plasma proteins. There is also evidence that a satisfactory response to treatment with steroids and azathioprine may be correlated with an initial very high level of C9, which falls with an improvement in the disease activity. However, if the initial C9 level is not significantly increased then treatment with steroids and azathioprine does not lead to a significant change in the disease activity. These findings may be helpful in predicting whether administration of steroids and azathioprine might be effective and hence in the selection of drugs in this intractable disease.

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