Clin. exp. Immunol. (1976) 25, 36–39.

Acute phase proteins and C9 in patients with Behcet's syndrome and aphthous ulcers

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(*Received* 17 *February* 1976)

SUMMARY

Estimation of the concentration of C9, C-reactive protein (CRP) and α1-antitrypsin in forty sera from patients with Behcet's syndrome and recurrent oral ulcers showed significantly increased amounts of C9 and CRP in Behcet's syndrome. The concentration of C9 was also significantly raised in recurrent oral ulceration, though to a lesser extent than in Behcet's syndrome. The assay C9 and CRP might be useful in the differential diagnosis of Behcet's syndrome, especially from recurrent oral ulcers. It is suggested that during epithelial inflammation in recurrent oral ulcers some of the acute phase proteins are increased and in some patients these may modulate the immunological mechanism in such a way as to induce a transition from focal oral ulceration to the multifocal Behcet's syndrome.

INTRODUCTION

The current views about the aetiology of Behcet's syndrome (BS) and recurrent oral ulceration (ROU) are that they are autoimmune conditions (Oshima *et al.*, 1963; Lehner, 1964, 1967a, b; Dolby, 1969; Rogers, Mitchell, Sams & Shorter, 1974) or microbial infections (Dudgeon, 1961; Sallay *et al.*, 1973). The two views can be reconciled in terms of antigenic cross-reactivities, or autoimmune effects subsequent to microbial initiation of the lesion. Complement components have received less attention, though recently normal concentrations of C3 and C4 (Kawachi-Takahashi *et al.*, 1974) but high levels of total haemolytic complement titre and concentrations of C9 were found in patients with BS (Shimada *et al.*, 1974; Kawachi-Takahashi *et al.*, 1974).

The interpretation of these findings is not clear but one possibility is that C9 belongs to the group of plasma proteins known as acute phase proteins (APP) which increase in concentration in the acute phase of a variety of inflammatory reactions (Koj, 1975). The evidence for inclusion of C9 among the APP is that the amount of C9 increases with turpentine-induced inflammation (Kawachi-Takahashi *et al.*, 1974). Furthermore, the concentration of C9 can be correlated with the erythrocyte sedimentation rate and the concentration of α1-antitrypsin (an established APP) in some diseases (Kawachi-Takahashi *et al.*, 1975). The aims of this investigation were to estimate the levels of serum C9 and two APP, C-reactive protein (CRP) and α1-antitrypsin (α1-AT), in BS and extend this to ROU, as there are no adequate laboratory criteria to differentiate between them.

MATERIALS AND METHODS

The series consisted of a group of twenty patients with BS, twenty patients with ROU and fourteen matched control subjects. The oral ulcers were classified (Table 1) into: minor aphthous ulcers (MiAU), major aphthous ulcers (MjAU) and herpetiform ulcers (HU) (Lehner, 1969). Patients with BS were further classified according to the site of involvement; five patients had oro-genital lesions, seven had these mucous and additional cutaneous lesions, and the rest had muco-cutaneous lesions with uveitis (three), arthritis (three) or neurological manifestations (two). A specimen of venous blood was taken from each subject. The levels of C9 were measured by the single radial diffusion technique using rabbit monospecific immune serum (Adinolfi & Beck, 1975). The levels of C9 were expressed as a percentage of the amount of this protein present in a pool of normal adult serum. The serum concentration of CRP and α1-AT were measured by the single radial immunodiffusion technique (Behringwerke, AG, Marburg-Lahn, Germany), and were expressed in mg/100 ml.

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RESULTS

The individual levels and the mean (±s.e.) of the assays given in Fig. 1 showed no differences in α1-AT between BS (333·9±28·5), ROU (358±27·6) and controls (334·4±38·6). However, significantly in-

TABLE 1. Classification of the oral ulcers

	Minor aphthous ulcers	Major aphthous ulcers	Herpetiform ulcers
Recurrent oral ulceration	11	4	5
Behcet's syndrome	7	11	2
Total	18	15	7

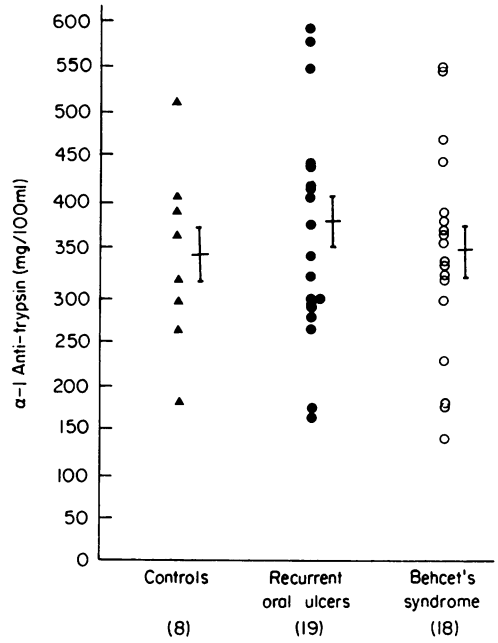

FIG. 1. Serum concentrations of α1-antitrypsin.

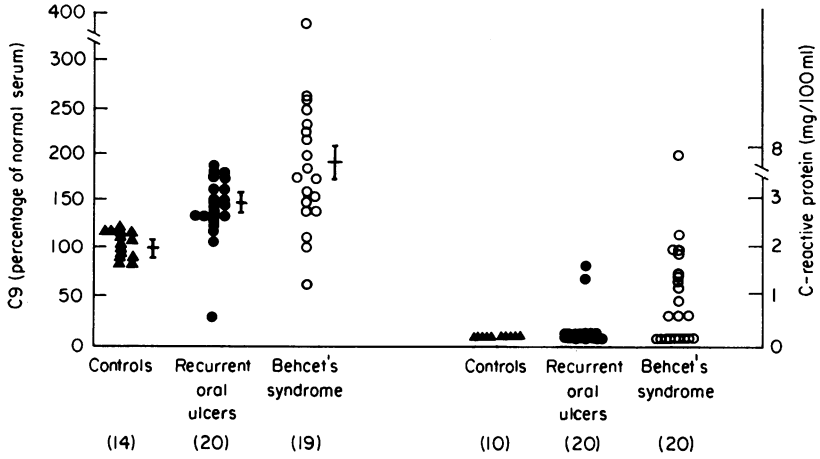

FIG. 2. Serum concentrations of C9 and C-reactive protein.

creased amounts of C9 (Fig. 2) were found in BS as compared with controls ($t = 4{\cdot}58$, d.f. 31, $P<0{\cdot}001$) or with ROU patients ($t= 2{\cdot}59$, d.f. 37, $P<0{\cdot}02$). This was not specific to BS as the levels of C9 in ROU were also significantly raised when compared with those in controls ($t = 6{\cdot}399$, d.f. 32, $P<0{\cdot}001$). A difference was not detected among patients with different sites of involvement of BS. A further analysis of all the patients into the three types of ROU (Table 1) suggested that a larger proportion of patients with MjAU (8/15) had increased concentrations of C9 (>175%) than MiAU (5/18) or HU (1/7).

The concentrations of CRP were low (Fig. 2) and of the positive sera all but one showed a level less than 3 mg/100 ml. However, very significant differences were observed when the number of sera with positive CRP among the BS patients (13/20) was compared with those in ROU (2/20; $\chi^2 = 8{\cdot}64$, $P<0{\cdot}005$) or controls ($\chi^2 = 10{\cdot}0$, $P<0{\cdot}005$). Analysis into different sites of involvement in BS or the three types of ROU revealed no differences.

DISCUSSION

The results show that of the three acute phase proteins assayed C9 and CRP were significantly increased in patients with BS. This is consistent with the increased levels of C9 reported by Kawachi-Takahashi *et al.* (1974), but a correlation between C9 and α1-AT was not found in BS or ROU, unlike that reported by Kawachi-Takahashi *et al.* (1975) in Herpes zoster or pustulosis. However, CRP was detected predominantly in BS ($P<0{\cdot}005$), although the levels were rather low. The level of C9 in ROU was also significantly increased when compared with the controls, but there was a further rise in C9 in BS. There is no reliable laboratory test for the diagnosis of BS and particularly for differentiating it from ROU. These data suggest that measurement of the serum concentrations of C9 and CRP may be used in the differential diagnosis of BS; a level of C9 greater than 175% of that found in normal serum and CRP detectable by immunodiffusion suggests the diagnosis of BS. It is possible that the few patients with ROU and a level of C9 greater than 175% and detectable CRP are the ones that may develop BS at a later stage, as the extraoral manifestations may develop after an interval of 1–21 years. It should, however, be emphasized that in practise a very small proportion (probably less than 1%) of patients with ROU develop into BS.

The increased concentration of C9 is not specific to BS, as this is also found in a variety of microbial infections and skin diseases with autoimmune manifestations (Kawachi-Takahashi *et al.*, 1975). An overlap in the levels of C9 between patients with BS and ROU is consistent with previous findings of autoantibodies and the proliferative response of lymphocytes to oral mucosa in both BS and ROU, though these are more common and of greater magnitude in BS (Lehner, 1964, 1967a, b). CRP is also found in a variety of inflammatory diseases and one of its functions might be to stimulate phagocytosis (Hokama, Coleman & Riley, 1962; Kindmark, 1971).

It might be expected that other APP than those tested might also be increased, as APP increase non-specifically after various types of inflammation or injury. However, an increase in one APP may not necessarily be associated with an increase in another APP; indeed in systemic lupus erythematosus α1-AT and C9 levels are normal but haptoglobin, orosomucoid and caeruloplasmin levels are increased (Koj, 1975; Kawachi-Takahashi *et al.*, 1975). Furthermore, repeated injury may alter the level of APP, and the concentration of some APP, such as fibrinogen may be decreased and others such as complement and α2-AP globulin may be increased (Weimer & Humelbaugh, 1967; Darcy, 1966). A lack of relation between some of the APP was also found in BS, in that normal levels of α1-AT were associated with increased levels of CRP and C9. In ROU and BS there is repeated breakdown of epithelium and other tissues, and this might have a differential effect on the concentrations of the various APP.

Accurate studies in animals suggest that the APP concentration is correlated with the severity of injury (Mouray, 1966; Darcy, 1970; Wycoff, 1970). Although this is difficult to assess clinically it is of interest that a larger proportion of patients with MjAU (8/15) yielded higher concentrations of C9 than patients with MiAU (5/18). It is relevant that MjAU in contrast to MiAU not only show greater severity of ulceration, but also higher ESR values and decreased serum iron concentrations which might be an indirect index of loss of epithelium (Challacombe, Barkhan & Lehner, 1976).

An alternative view to be considered is that C9 might play a part in lysis of the affected cells in BS and ROU. There is as yet no evidence for such a complement-dependent cytotoxic mechanism. However, other complement components, namely C3, C4 and C2 were markedly reduced before an attack of uveitis, suggesting complement consumption by the classical pathway (Shimada *et al.*, 1974). We have been unable to demonstrate tissue-bound C3, except in 5/24 oral biopsies of early aphthous lesions in which C3 was found in the cytoplasm of epithelial cells (Lehner, 1969). Immune complexes were not detected in the sera of four patients with BS (unpublished results).

A more significant relationship between CRP and the complement system can be envisaged as CRP can activate complement and sensitize for complement-dependent haemolysis (Kaplan & Volanakis, 1974; Osmand *et al.*, 1975). CRP can also affect some T-cell functions in that it binds selectively to human T lymphocytes and inhibits their ability to form spontaneous rosettes and the mixed lymphocyte reaction (Mortensen, Osmand & Gewurz, 1975). It is therefore postulated that during epithelial inflammation in ROU APP are formed; some of the APP such as CRP may modulate the immunological mechanism by their capacity to bind to T lymphocytes, to promote phagocytosis and to activate complement. Under some, as yet undefined, conditions the effects of APP on immunological responses might induce the transition from focal oral ulceration to the multifocal Behcet's syndrome.

We thank Dr H. J. Müller-Eberhard for his help in the preparation of anti-C9 and Miss Susan Beck for her skilful technical assistance.

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