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Immune complexes in Behçet's syndrome and recurrent oral ulceration

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Summary

Seventeen patients with Behçet's syndrome, 11 with recurrent oral ulceration, and eight controls were studied in an investigation of the part possibly played by immune complexes in the transition from focal oral ulceration to the multifocal syndrome. Changes in the distribution of C3 within the first peak of Sephadex G200 fractionated plasma were found in nine of the 17 patients with Behçet's syndrome (55%), three of the 11 patients with recurrent oral ulcers, and none of eight controls. These findings provide indirect evidence that immune complexes are found in the plasma of these patients. Immune complexes were more common in patients with the neuro-ocular type of Behçet's syndrome than in those with the mucocutaneous type, and in those with herpetiform ulcers than in those with major or minor aphthous ulcers. Immune complexes were also associated with active disease. These findings support the hypothesis that the formation of immune complexes is an important step in the pathogenesis of Behçet's syndrome.

Introduction

Behçet's syndrome is a disease that affects many tissues; the most common manifestations are orogenital ulceration, skin lesions, uveitis, arthralgia, neurological lesions, and vascular thrombosis. The cause of the syndrome is unknown but most of the evidence suggests some abnormality in the immune mechanism. Complement components have recently been

investigated and although C3 and C4 concentrations are normal, high levels of total haemolytic complement and C9 have been found in patients with Behçet's syndrome.¹⁻³ There is also some evidence that complement consumption by the classical pathway may occur in Behçet's syndrome since low levels of C2, C4, and C3 have been found before an attack of uveitis.¹ These observations raise the possibility that the complement system might be implicated in the pathogenesis of Behçet's syndrome and recurrent oral ulceration and that immune complexes might play a part in the transition from focal oral ulceration to the multifocal Behçet's syndrome.

Patients and methods

The series consisted of 17 patients with Behçet's syndrome, 11 with recurrent oral ulceration, and eight controls. Patients with Behçet's syndrome were further classified according to the type of tissues that were affected⁴: four patients had the mucocutaneous type of the syndrome, with orogenital and skin manifestations; four had the arthritic type, with joint involvement and some or all of the mucocutaneous lesions; and nine had the neuro-ocular type with neurological or ocular manifestations, or both, in addition to some or all of the lesions found in patients with the mucocutaneous or arthritic types. The mouth ulcers found in these patients and the 11 with recurrent ulceration were classified into minor aphthous ulcers, major aphthous ulcers, and herpetiform ulcers.⁵

A specimen of blood was collected from each subject into edetic acid (final concentration 0.01 mol/l). All the samples were coded and the code was broken after completion of the assay. The plasma was separated and either used immediately or stored in small aliquots at -70°C. Plasma was fractionated on Sephadex G200, and the concentration of C3 in the first peak fractions (20-26) was determined by a sensitive haemagglutination inhibition assay. Details of these methods have been published elsewhere.⁶

Results

The elution profile of control plasma fractionated on Sephadex G200 is shown in Fig 1a. Only a small quantity of C3 was found in the first peak and it was confined to the last three fractions (fractions 24-26). Most of the C3 was found under the second peak and the position corresponded to its molecular weight of 185 000. The elution profile of plasma obtained from a patient with Behçet's syndrome showed an

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increase in the amount of C3 in the first peak, and C3 was found in all the fractions of the first peak (fig 1b). This sample was taken when the patient had active oral ulceration and papular skin lesions. Two months later, during a remission with no active clinical manifestations, the distribution of C3 was similar to that found in the control sample (fig 1c).

Distribution of C3 in plasma of control subject (a) and patient with Behçet's syndrome during relapse (b) and remission (c). Values are expressed as optical density at 280 nm and reciprocal inhibition titre. Shaded columns indicate amount of C3 under first peak.

Disease activity was assessed by the erythrocyte sedimentation rate (ESR) or the presence of active disease at any one of the affected sites. We could find no correlation between the level of the ESR and the presence of an abnormal C3 distribution within the first peak, but an abnormal C3 distribution was found in 12 patients with active disease and no patients without active disease. Of the remaining 16 patients 11 showed active disease (of which four were during the healing stage of ulceration) and five had no disease activity.

The amount and distribution of C3 in all the patients and controls are shown in the table. C3 in the controls was confined to the last four fractions (23-26) of the first peak. No control had detectable C3 at or before the void volume (fraction 22). Nine out of 17 patients with Behçet's syndrome and three out of 11 with recurrent oral ulceration had C3 distributed in the void volume fractions. The difference in the prevalence of C3 in the void volume fractions between those with Behçet's syndrome and those with recurrent ulceration was not significant, but the difference in prevalence between those with Behçet's syndrome and controls was significant at the 5% level ($\chi^2 = 6.617$). Further analysis according to the type of Behçet's syndrome showed the lowest frequency of C3 in the void volume fractions among patients with the mucocutaneous type (one out of four patients) and the highest among those with the neuro-ocular type (six out of nine). Two of the four patients with the arthritic type had C3 in the void volume fractions. Classification of patients with Behçet's syndrome and recurrent oral ulceration according to the type of mouth lesion showed that C3 was found most often in those with herpetiform ulcers (in all three patients) and less often in those with major (four out of 11) and minor (five out of 14) aphthae. The finding of abnormal amounts of C3 in all the patients with herpetiform ulcers (two with Behçet's syndrome and one with recurrent oral ulceration) is noteworthy and will have to be examined in a larger series.

Distribution and concentration (reciprocal inhibiting titre) of C3 in first peak of plasma samples fractionated on Sephadex G200

Case No	Fractions in protein peak 1						
	20	21	22	23	24	25	26
Patients with Behçet's syndrome							
1	1	2	4	4	2	8	16
2	2	1	2	4	4	2	2
3		1	1	4	2	4	16
4						2	8
5					2	4	4
6					4	8	8
7		1	4	8	16	8	16
8			1	2	4	4	16
9					4	4	8
10					4	32	32
11		1	2	4	4	8	16
12	2	1				8	16
13				2	8	16	32
14				2	4	16	16
15	1	1	1	1	2	2	2
16	2	1	4	2	4	8	16
17					2	2	4
Patients with recurrent oral ulcers							
18				1	1	2	4
19					4	4	8
20				1	1	4	8
21	4	1			4	8	8
22					4	8	8
23					4	8	16
24					4	8	32
25					1	16	16
26					1	8	16
27		1	2	4	8	16	32
28		1	2	16	4	2	16
Controls							
29				1	2	4	16
30				1	1	8	32
31					1	4	16
32					2	2	8
33				2	2	8	8
34					1	4	16
35					2	8	32
36					4	2	4

Figures shown represent the concentration of C3 in each of the first peak fractions.

Discussion

Activation of the complement system by immune complexes changes the molecular weight of a proportion of the C3 present in the serum. Some of the C3 is bound to the immune complex but some seems to be bound to other complement proteins or other serum proteins. The increase in the molecular weight is reflected in an increased quantity and changed distribution of C3 in the first peak, obtained by fractionating plasma on Sephadex G200. For convenience we refer to this as macromolecular C3.

Plasma from nine of the 17 patients with Behçet's syndrome showed changes in the distribution of C3 within the first peak. A clinical division of Behçet's syndrome into three progressively more severe types suggested that there was an increasing frequency and concentration of C3 in the first peak. Only one out of four patients with the mucocutaneous type had macromolecular C3, while two of the four with the arthritic type and six of the nine with the neuro-ocular type had macromolecular C3. These results are consistent with the fact that more precipitating, complement-fixing, and haemagglutinating antibodies to homogenates of fetal oral mucosa are seen in the neuro-ocular variety of Behçet's syndrome than in the mucocutaneous type.⁷

As Behçet's syndrome is commonly preceded by recurrent oral ulceration, an attractive hypothesis is that the transition from focal oral ulceration to the multifocal Behçet's syndrome might be mediated by immune complexes. But three of the 11 patients with recurrent oral ulceration also showed putative immune complexes and this finding either invalidates the hypothesis or suggests that these patients might be particularly prone to develop extraoral manifestations. Longitudinal studies have now been started to find out whether these patients are at special risk. Attempts to associate the presence of immune complexes with disease activity showed that although all patients with immune complexes had active disease, several patients with active disease did not have the immune complexes. It might be

relevant that disease activity was clearly correlated with the concentration and distribution of C3 in one patient (fig 1); whether this finding applies also to other patients with Behçet's syndrome requires confirmation in a large prospective study.

Our findings also suggest that complement activation occurs in Behçet's syndrome. This may be started by circulating complexes of antigen and antibody, although extravascular complement activation, through the interaction of complement-fixing antibodies and tissue antigens, might also produce similar changes. Preliminary evidence in favour of complement activation by immune complexes is provided by finding increased quantities of IgG in association with macromolecular C3. This concept is further supported by the demonstration of low levels of C4, C2, and C3 before an attack of uveitis in Behçet's syndrome; by the association between other types of uveitis and immune complexes⁶; and by our observations that macromolecular C3 is seen most often in patients with the neuro-ocular type of the syndrome. The assay we have used, however, provides no information on the amount of immune complexes circulating in the plasma or the way in which they activate the complement system.

It is difficult at present to reconcile the normal levels of C3 and C4 and the raised levels of C9 in Behçet's syndrome with our findings that immune complexes also occur in this disease. Normal levels of C3 and C4, however, are found even though

their catabolism has been increased. While it is possible that increased levels of C9 may reflect a temporary imbalance between the synthesis and breakdown of this protein after complement activation, it is more likely that the raised levels of C9 reflect an acute-phase response to immunologically induced tissue injury.³ If the presence of immune complexes is confirmed by different techniques the relation of these complexes to cell-mediated immune responses will have to be investigated before one understands the immunopathological mechanisms operating in Behçet's syndrome.

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Vaccination scar with soft-tissue atrophy restored by local insulin treatment

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Summary

A woman who had a deep scar on her left arm as a result of an influenza vaccination was treated with insulin. Monocomponent porcine insulin was injected into each quadrant of the pit. After 82 days' treatment her arm appeared normal and has remained so over seven months. Using insulin's ability to promote fat and protein synthesis is a simple and effective way of treating atrophied scars.

Introduction

Lipoatrophy caused by insulin treatment in diabetics has been successfully treated by injecting alternative, preferably purer, forms of insulin into the atrophied areas. This technique was first reported by Collens¹ in 1949 and several subsequent reports have established its usefulness in diabetics.

I can find no reference to the use of this technique in non-diabetics, who may accept ugly atrophied scars as irremediable, except by complex and not altogether successful plastic surgery. This case report should serve as a reminder that a simple and complete remedy does exist.

Top: front and side views of left arm showing vaccination scar before treatment. Bottom: front and side views of same arm on 21 May, after treatment had stopped.

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