Behçet's Syndrome and Autoimmunity

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The aetiology of Behçet's syndrome has long been disputed. Behçet (1937) first suggested a viral hypothesis on the basis of finding inclusion bodies in scrapings from the ulcers. Sezer (1953, 1956) claimed to have isolated a virus from these patients and to have reproduced the disease, and showed a strongly positive complement-fixation reaction in other patients with Behçet's syndrome. Evans *et al.* (1957) also grew a virus, and they showed the presence of neutralizing antibodies in the serum of patients with this disease, but not in control sera. Recently Mortada and Imam (1964) also grew a virus from one patient, but numerous other workers have failed to do so. The literature has been reviewed by Dudgeon (1961) and Dowling (1961).

Oshima *et al.* (1963) and Shimizu *et al.* (1965) have raised the possibility that an autoimmune mechanism may play a part in the pathogenesis of Behçet's syndrome. They showed convincingly in a large series of patients that there is a rise of serum globulin and sialic acid. They have also demonstrated the presence of autoantibodies against oral mucosa by the tanned red cell haemagglutination technique. In an immunofluorescent investigation they claim to have shown significant fluorescence of the cytoplasm of peripheral blood leucocytes and of cells from the oral ulcers. While evidence of an autoimmune reaction does not disprove the viral hypothesis it could be important not only from the aetiological point of view but also in understanding the pattern of the disease and of its response to treatment.

The aim of this investigation was to carry out the haemagglutination, complement-fixation, and Ouchterlony's precipitation test against foetal oral mucosa in 21 patients with Behçet's syndrome, as described by Lehner (1964, 1965) for a series of patients with recurrent oral ulceration. Furthermore, an attempt was made to differentiate the oral ulcers in Behçet's syndrome and to compare them with those of recurrent focal oral ulceration.

Patients and Methods

Oral ulcers in 20 patients with Behçet's syndrome were divided into three groups—(1) aphthous (Mickulicz and Kummel, 1888), (2) periadenitis mucosa necrotica recurrens (Sutton, 1911), and (3) herpetiform (Cook, 1960), as distinguished by clinical (see Figs. 1 and 2), histological, or electron microscopical features (Lehner and Sagebiel, 1966). The ulcer groups were then compared with those in 100 patients with recurrent focal oral ulceration (Table I).

TABLE I.—*Classification of Recurrent Oral Ulcers*

	Aphthous (Mickulicz)	Periadenitis (Sutton)	Herpetiform (Cook)	Herpetiform: Aphthous
Focal oral ulcers (100) . .	84 (84%)	7 (7%)	9 (9%)	1 : 9·3
Behçet's syndrome (20)	9 (45%)	3 (15%)	8 (40%)	1 : 1·1

Clinical and immunological features in 21 patients with Behçet's syndrome are shown in Table II. They were divided into two groups according to whether they had the aphthous (including the "periadenitis") or herpetiform variety of oral ulcers. In one patient the type of oral ulceration could not be determined with any certainty. Twenty sera from patients with focal aphthous ulcers, 10 with focal herpetiform ulcers, and 30 normal randomly collected control sera were used in the immunological tests.

Haemagglutination Test (Boyden, 1951).—The method of preparing the homogenate of oral mucosa was described previously (Lehner, 1964), and the following modifications were applied: (*a*) normal rabbit serum 1/100 in buffered saline *p*H 7.2 was used for serial dilutions of the serum from 1/20 to 1/640 ; (*b*) a new batch of oral mucosa was thawed and then frozen at −20° C. three times before use ; (*c*) antigen was titrated with every new batch of antigen ; the optimum dilution of antigen was usually 1/40 ; (*d*) all sera were decomplemented at 56° C. for half an hour, and they were each absorbed with sheep red cells (volume for volume) ; (*e*) immune absorption tests were carried out with saline extracts of foetal oral mucosa, as well as foetal skin, colon, liver, salivary gland, and striated muscle.

Complement-fixation Test.—A "four drop" test was carried out in perspex trays as modified from Donnelley (1951). The sera were decomplemented and diluted at 1/4 with barbitone buffer *p*H 7.2. Complement was always titrated before the test in the presence of antigen, and the 50% haemolytic dose was recorded. Fixation was allowed for 45 minutes at 37° C., two 50% haemolytic doses of complement being used. Controls set up with every batch of sera tested included known negative and positive sera, complement, buffer, serum, antigen, and cell controls. A positive reading was recorded if less than 50% of the red cells were haemolysed while the corresponding control without antigen showed 100% haemolysis.

Ouchterlony's Precipitation Test.—A micro-Ouchterlony technique was employed after initial failure of the test in 40 cases with the conventional Petri dish method (Ouchterlony, 1964). Two millilitres of 1% Oxoid ion agar prepared with barbitone buffer *p*H 8.6 was poured on a microslide, and circular holes 3 mm. in diameter were cut out. Neat antigen was placed in the central well and the six peripheral wells were filled with neat sera. The slides were incubated in a humid chamber at room temperature for two days ; they were then washed in saline and stained with ponceau S. A number of negative sera were concentrated 10 times by dialysis against Carbowax (Clarry, 1966) and then used as above. Immune absorption tests were performed with tissue homogenates as in the haemagglutination test.

Results

In all but one patient the age of onset of the disease was between 10 and 40 years (Table II). The sex distribution was nearly equal—females 11, males 10—though in other series males have predominated over females by 1.7 : 1 (Dowling, 1961). Three patients showed lesions at all four major sites—the mouth, genitalia, skin, and eyes—eight had three sites and 10 had two sites involved. In addition, five patients showed neurological features, four deep-vein thrombosis, and one arthritis. Oral ulceration was the presenting lesion in 16 out of 21 patients ; two developed skin lesions initially, one genital ulceration, and two iridocyclitis. Twelve patients showed the

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aphthous or periadenitis variety of oral ulceration, and eight showed the herpetiform type (Figs. 1 and 2). Two out of eight patients with herpetiform ulcers were males, as compared with 7 out of 12 with aphthous ulcers. Another difference between the two groups was that while only two patients in the herpetiform group showed eye involvement five in the aphthous group had eye lesions.

TABLE II.—*Clinical and Immunological Features in 21 Patients with Behçet's Syndrome, Classified Into the Aphthous and Herpetiform Groups*

No.	Sex and Age	Age at Onset	Mouth	Genitals	Skin	Eyes	Others	T.R.C.H.	C.F.T.	Precipitation
					I. Aphthous Group					
1	F 40	30	+	+	+	+	Arthritis	320	+	–
2	M 34	14	+	+	+	+	—	640	–	+
3	F 33	30	+	–	+	+	—	80	+	+
4	F 18	6	+	+	+	–	Deep-vein thrombosis and cerebral disorder	80	+	–
5	M 24	22	+	+	–	+	—	160	+	–
6	M 60	26	+	+	+	–	Deep-vein thrombosis and cerebral disorder	40	–	–
7	M 38	23	+	+	+	–	Deep-vein thrombosis	320	–	–
8	F 44	26	+	+	–	–	Cerebral disorder and migraine	320	+	+
9	M 33	32	+	+	–	–	—	40	–	–
10	F 26	14	+	+	–	–	—	160	–	+
11	M 34	28	+	+	–	–	—	320	+	+
12	M 35	15	+	–	–	+	—	160	+	+
					II. Herpetiform Group					
1	F 44	39	+	+	+	+	—	320	+	+
2	M 18	13	+	+	+	–	Deep-vein thrombosis	320	+	+
3	F 37	12	+	+	+	–	Angio-neurotic oedema	160	+	+
4	M 25	25	+	–	+	+		160	+	–
5	F 45	20	+	+	–	–	—	20	–	–
6	F 22	20	+	+	–	–	—	320	+	–
7	F 18	13	+	+	–	–	—	320	+	–
8	F 44	39	+	+	–	–	Urticaria	80	–	+
					Aphthous or Herpetiform					
1	M 30	25	+	–	–	+	—	320	+	+

T.R.C.H. = Tanned red cell haemagglutination.
C.F.T. = Complement Fixation Test.

Type of Oral Ulceration.—The oral ulcers in Behçet's syndrome differed greatly in appearance from patient to patient, and they showed the same clinical variation as the focal lesions. While the oral ulcers in patients in each group of Behçet's syndrome were not distinguishable from patients of the same group with focal oral ulcers, nevertheless there was a significant difference in the incidence of herpetiform ulcers (Table I). The latter appeared to be 8.4 times more common in Behcet's syndrome than in focal oral ulceration.

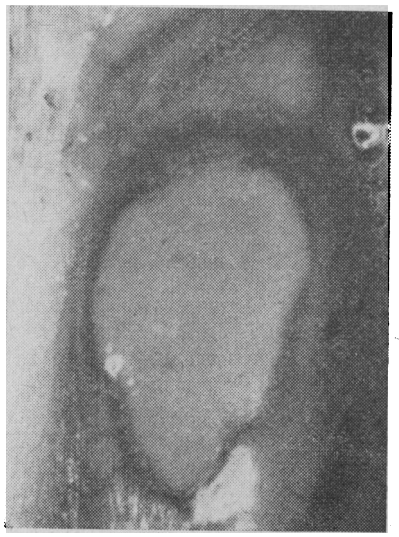

FIG. 1.—Single recurrent aphthous ulcer of the lip.

Haemagglutination Test.—While only 2 (6.6%) of the 30 normal control sera caused haemagglutination at a titre of over 1/40, 18 (85.7%) out of 21 patients with Behçet's syndrome gave a titre of 1/80 to 1/640 (Table II). There was no significant difference between the aphthous and herpetiform groups of patients in Behçet's syndrome—that is, 83.3% and 87.5%. The difference between the corresponding focal oral aphthous and herpetiform ulcers was, however, significant,

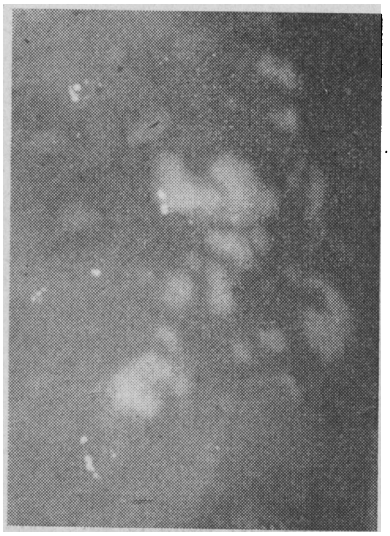

FIG. 2.—A crop of about 25 herpetiform ulcers of the cheek.

70% and 30% respectively. The antibodies were absorbed by foetal oral mucosa, skin, and colon, but not by liver, salivary gland, or striated muscle.

Complement-fixation Test.—Normal control sera gave a positive complement-fixation test in 3 (10%) out of 30 subjects, in contrast to Behçet's syndrome with 14 (66.6%) out of 21 patients. As shown in Table III, the C.F.T. results in the five groups are comparable with those in the haemagglutination test.

TABLE III.—*Combined Haemagglutination, C.F.T., and Precipitation Results in 80 Subjects*

Groups	Total No.	Significant T.R.C.H.	+ C.F.T.	+ Precipitation Test
Healthy controls	30	6·6%	10%	6·6%
Focal oral aphthous ulcers	20	70%	53%	55%
" " herpetiform ulcers	10	30%	20%	30%
Behçet's syndrome with aphthous ulcers ..	12	83·3%	58·3%	50%
Behçet's syndrome with herpetiform ulcers ..	8	87·5%	75%	50%

Precipitation Test.—The micro-Ouchterlony technique showed single lines indicating a precipitating antibody in 6.6% of controls and 50% of patients with Behçet's syndrome. While the results in the various groups were comparable with those of the haemagglutination and C.F.T., a smaller number of patients gave a positive reaction (Table III). This is consistent with the lower sensitivity of the precipitation test. The results of the immune absorption tests were identical with those of the corresponding haemagglutination tests.

Discussion

A commonly expressed view that oral ulceration in Behçet's syndrome is more severe than in focal ulceration was not substantiated. Indeed, the aphthous, periadenitis, and herpetiform types of ulcers were strictly comparable in the focal and Behçet

lesions. The outstanding difference was the relatively higher incidence of herpetiform ulcers in Behçet's syndrome. While the difference between aphthous and periadenitis ulcers is not as yet clear, and appears to be a matter of degree of severity, there are significant clinical, therapeutic, and pathological differences between aphthous and herpetiform ulcers. Herpetiform ulcers resemble clinically herpes simplex ; they usually respond dramatically to tetracycline therapy ; and the epithelial vesicles seen microscopically and intranuclear inclusion bodies observed with the electron microscope are consistent with a viral aetiology (Lehner, 1967a). If a virus were to be grown from this lesion, then it would be tempting to suggest that both recurrent focal oral ulceration and Behçet's syndrome are divisible into two aetiologically distinguishable groups—namely, viral and non-viral. This might explain the serious discrepancies in the reported success and failure in growing viruses from patients with Behçet's syndrome.

It should be noted that, while there is a significant difference in the incidence of antibodies between focal oral aphthous and herpetiform lesions, this is not apparent in the comparable two groups of patients with Behçet's syndrome (Table III). Though this difference seems to reflect well the presumptive double aetiology of focal oral ulceration, the same explanation cannot be applied to the two types of Behçet's syndrome. This suggests that the common denominator in the two groups of Behçet's syndrome may be an autoimmune phenomenon, which would account for the similar clinical syndromes produced by what appear to be two different aetiological agents. Results of the present immune absorption tests suggest that the antibody is not organ-specific for foetal oral mucosa but cross-reacts with foetal skin and colon. It is unlikely that the antibodies develop in response to non-specific mucosal damage, since in other ulcerating mouth lesions significant haemagglutinating levels were not established (Lehner, 1964) ; this is supported by the results with the lymphocyte Evans formation test (Lehner, 1967b).

The relation between focal oral ulceration and Behçet's syndrome is not clear and must at present await further elucidation. Whether patients with the " incomplete Behçet's syndrome "—namely, those showing involvement of two or three of the four major sites—should be classified under Behçet's syndrome has long been disputed. The present immunological tests failed to distinguish the " incomplete " from the " complete " syndrome, except that the latter tended to show higher haemagglutination titres. However, it is significant that patients with eye lesions showed a higher incidence of antibodies ; 100% gave a positive haemagglutination, 87.5% a complement-fixation, and 62.5% a precipitation test, as compared with patients without ocular involvement 77%, 54%, and 46%, respectively. This is of considerable interest, since the greatest diagnostic importance is attached to the ocular lesions, as the risk of blindness outweighs all other considerations.

Virus, mycoplasma, or " L form organisms," and autoantibodies have been implicated aetiologically not only in Behçet's syndrome but also in the related mucocutaneous syndromes and in recurrent oral ulceration. It is of particular interest that Barile *et al.* (1963), Graykowski *et al.*, 1964, and Stanley *et al.* (1964) have isolated " L forms " in a number of patients with recurrent aphthous and periadenitis ulcers, though the herpetiform variety was not differentiated. In Reiter's syndrome also there is evidence for mycoplasma and antibodies to prostatic tissue (Bartholomew and Himes, 1964 ; Grimble, 1964 ; Grimble and Lessof, 1965). Furthermore, Ludlam *et al.* (1964) reported in Stevens–Johnson syndrome the presence of cold agglutinins and complement-fixing antibodies to *Mycoplasma pneumoniae.*

The views expressed in favour of a variety of infectious agents are consistent with the hypothesis that an autoimmune phenomenon may be the common denominator in these lesions. This is especially plausible if it is assumed that an antigenic cross-reactivity may exist between the infective agent and a normal tissue. Indeed, there is convincing evidence for such a relation between beta-haemolytic streptococci and heart tissue in rheumatic fever (Kaplan, 1963), and between *Escherichia coli* O 14 and colonic mucosa in ulcerative colitis (Perlmann *et al.*, 1965). Attempts will now be made to explore any antigenic cross-reactivity between oral epithelium and relevant viruses, mycoplasma, or " L form " organisms.

Summary

Two distinctive types of oral ulcers are recognized in both Behçet's syndrome and focal oral ulceration. The herpetiform ulcers, unlike aphthous ulcers, resemble clinically a known viral lesion, they respond dramatically to tetracycline therapy, and pathologically they show epithelial vesicles and intranuclear inclusion bodies. These differences suggest that Behçet's syndrome may consist of two aetiologically distinguishable groups.

Haemagglutinating, complement-fixing, and precipitating antibodies have been shown to foetal oral mucosa. While autoimmune factors may play a part in the pathogenesis of Behçet's syndrome, the exact nature of this relation needs further investigation.

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