IMMUNOLOGICAL STUDIES ON BEHÇET’S SYNDROME

BY

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Summarized results of the clinical and laboratory findings in 85 cases of Behçet’s syndrome seen at our clinic between September, 1957, and April, 1961, have already been reported (Oshima, Shimizu, Yokohari, Matsumoto, Kano, Kagami, and Nagaya, 1963). Investigation revealed a high incidence of abnormalities, not only in the mouth, eyes, and genital organs, but also in the intestine, cerebrospinal fluid, and plasma proteins. A homologous antibody against human oral mucous membrane tested by the tanned-cell haemagglutination technique proved positive in seventeen of forty cases of Behçet’s syndrome and the titre changed along with the clinical course of the disease, though it proved negative in eighteen healthy controls. These data suggest that the disease must be systemic. Moreover, recurrence of the multiple manifestations characterizes the clinical picture of Behçet’s syndrome (Mavioğlu, 1958; Strachan and Wigzell, 1963; Shimizu, Kagami, Matsumoto, and Matsumura, 1963). No causative micro-organism was found in spite of fever, general malaise, and the progressive course of the disease.

These findings suggest that an autoimmune mechanism may be concerned in the pathogenesis and development of these characteristic recurrent clinical features.

The present investigation deals mainly with immunological studies on our cases of Behçet’s syndrome.

Material and Methods

132 subjects seen at our clinic between September, 1957, and March, 1964, comprised 85 males and 47 females (sex ratio 1:8:1:0), in whom diagnosis of Behçet’s syndrome was established by the recurrent appearance of mucocutaneous-ocular symptoms and other manifestations, and the previously-mentioned laboratory findings.

The following methods were used:

1) Immediate intradermal reaction with heat-aggregated human gamma globulin (H.y.G.).—Heat-aggregated H.y.G. was prepared by the method of Ishizaka and Ishizaka (1959). A 2 per cent. solution of pooled H.y.G. was heated for 20 min. at 63°C. The aggregated H.y.G. was precipitated from this preparation with 0·62 M sodium sulphate, dissolved, and dialysed against borate buffered saline. Intradermal injection of this preparation in volume of 0·1 ml. containing 5·0 μg. N. was given into the forearm. After 20 min. the diameters of the erythema and the wheal were measured. Control sites were injected with 0·1 ml. physiological saline and 0·1 ml. saline containing 50 μg. N. of untreated H.y.G.

Because of skin hyperirritability in Behçet’s syndrome, several kinds of allergen extracts for bronchial asthma were also tested.

2) Haemagglutination test against heat-aggregated H.y.G.—Tanned sheep red cell coated with heat-aggregated H.y.G. was used according to the method of Boyden (1951). Sera tested were obtained from patients with Behçet’s syndrome, systemic lupus erythematosus (SLE, rheumatoid arthritis (RA), other connective tissue diseases, other miscellaneous diseases, and healthy controls.

3) Immunofluorescent staining.—Using fluorescein isothiocyanate (F.I.) conjugated rabbit anti-human-gamma-globulin serum (absorbed with acetone dried liver powder), the smears of oral aphthous ulcers and peripheral blood from the patients were stained. Sera from cases of Behçet’s syndrome, SLE, RA, other connective tissue diseases, and normal healthy controls were studied by the indirect method (Beutner, 1961). In this method, the smears were fixed with acetone for 10 min., after 30 min. incubation with sera at room temperature, stained with F.I.-conjugated antisera for 30 min., and washed three times with buffered saline. The cells were examined through a microscope equipped with a dark field condenser: UV I (2 mm.) B’ 12 mm.) as ultraviolet exciting filters; UV II (2 mm.) and Y (2 mm.) as barrier filters (Perma Ray Indus. Co. Japan).

Results

1) The immediate intradermal reaction with several kinds of allergen extract for bronchial asthma, such as house dust, ragweed, fungi, etc., proved negative in the cases of Behçet’s syndrome tested, while it was strongly positive with heat-aggregated H.y.G. in most cases, as shown in the Table (opposite). Moreover, it was mostly negative with
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TABLE
IMMEDIATE INTRADERMAL REACTION IN 10 CASES OF BEHÇET'S SYNDROME
Diameter of Wheal (mm.)/Diameter of Erythema (mm.)

<table>
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<th>33</th>
<th>27</th>
<th>37</th>
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<td>0/5</td>
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<td>6/6</td>
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untreated H.γ.G., even in the amount of 50·0 μg. N. With heat-aggregated H.γ.G. erythema and wheal formation began within 3 to 5 min. after injection, reaching the maximum at 15 to 25 min. and decreasing in intensity thereafter.

However, in the patients with Behçet's syndrome, a sterile pustule formation with surrounding redness often appeared 24 to 72 hrs after the injection, and this was never seen in patients with other diseases. Between the patients with Behçet's syndrome and those with other diseases there was a significant difference ($P < 0.001$) in the intensity of this immediate intradermal reaction with heat-aggregated H.γ.G. (Fig. 1).
(2) The haemagglutination test against heat-aggregated H.γ.G. was positive in twenty out of thirty cases of Behçet's syndrome, in twelve out of thirteen cases of RA, and in ten of eleven cases of other collagen diseases, while it was mostly negative in cases of other controls (Fig. 2). A titre of anti-heat-aggregated H.γ.G. antibody was likely to be slightly low in Behçet's syndrome than in RA and other connective tissue disorders.

(3) Immunofluorescent studies revealed that polymorphnuclear leucocytes in peripheral blood and the cells of smears of oral aphthous ulcers from the patients with Behçet's syndrome were stained with F.I.-labelled anti-globulin serum, the fluorescence being localized in the cytoplasm of the cells. In the leucocytes of blood smears from normal subjects, this cytoplasmic fluorescence was absent or much weaker. By the indirect method, the fluorescence was observed in leucocyte cytoplasm of normal blood smears incubated with sera of Behçet's syndrome, which was distributed in the form of very small granules, while the cell nuclei remained unstained (Fig. 3, and Fig. 4, opposite). The fluorescent staining was inhibited by incubation with unconjugated antisera.

Fig. 3.—Indirect immunofluorescent staining of leucocytes in peripheral blood incubated with serum from Behret's syndrome, showing a cytoplasmic fluorescence. Stained with anti-H.γ.G. rabbit anti-serum conjugated with F.I. × 400.

Fig. 2.—Haemagglutination test with aggregated human γ-globulin.
D.M. = Dermatomyositis
S.D. = Scleroderma
P.N. = Periarteritis nodosa
R.A. = Rheumatoid Arthritis
S.L.E. = Systemic Lupus Erythematosus

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The fluorescent staining of the peripheral blood leucocytes of Behçet’s syndrome was similar to that of RA, dermatomyositis, and polyarteritis nodosa (Fig. 5), and a nucleic fluorescence seen frequently in SLE was never seen in Behçet’s syndrome, showing a lack of antinuclear factors in the serum.

These findings showed that Behçet’s syndrome responded similarly to RA and other collagen diseases in the above immunological tests.

(4) As the serum mucoprotein level was raised in Behçet’s syndrome (Oshima and others, 1963), serum sialic acid, a component of mucoprotein, was determined, using the thiobarbituric acid method of Warren (1959).

Fig. 6 (overleaf) shows that the serum sialic acid level in 43 cases of Behçet’s syndrome (97 mg./100 ml. in average) is as high as in RA (98 mg./100 ml. in eleven cases) and in SLE (97 mg./100 ml. in ten cases), and is much higher than in chronic recurrent aphthosis without other manifestations (76 mg./100 ml. in ten cases), bronchial asthma (75 mg./100 ml. in 27 cases), and other miscellaneous diseases (74 mg./100 ml.). Normal range is 60 ± 12 mg./100 ml.

The serum sialic acid level reflects very sensitively the relapsing course of the disease; Fig. 7 (overleaf) shows the changes recorded in ten cases of Behçet’s syndrome.

Discussion

Many papers on Behçet’s syndrome published in the past 30 years deal with the striking manifestations which were first described by Behçet (1937). Our previous studies of 85 cases reported the symptoms of its systemic features and discussed the possibility that an autoimmune mechanism plays a
role in its pathogenesis (Oshima and others, 1963). We have now studied the immunological aspects of the disease, although the primary causative factor has not yet been confirmed.

Sezer (1953, 1956) claimed to have isolated a virus from pathological material and to have reproduced similar changes in experimental animals. Then Evans, Pallis, and Spillane (1957) reported complement-fixation and virus-neutralization tests. The question of virus infection remains obscure, as many workers have repeatedly failed to isolate a virus. Oshima and others (1963) demonstrated circulating antibody against oral mucous membrane in sera from the patients.

In Behçet's syndrome the hyperirritability of the skin against many kinds of stimuli has been noted and considered nonspecific. Jadassohn (1958), Jadassohn, Franceschetti, and Golay (1957), and Jadassohn, Franceschetti, and Hunziken (1961)
demonstrated a tuberculin-type skin reaction after the injection of autogenous ulcer extract tissue, which they designated "Behçetin-reaction".

Our studies of immediate intradermal reaction showed that the skin of patients makes a strongly positive response to heat-aggregated H.y.G. despite a mainly negative reaction against several kinds of allergen extracts for bronchial asthmas and untreated H.y.G.

The intensity of this skin reaction appeared much more strongly in the Behçet’s patients than in the control subjects.

The haemagglutination test with heat-aggregated H.y.G. was also positive in twenty of thirty cases of Behçet’s syndrome (67 per cent.), in twelve of thirteen cases of rheumatoid arthritis and ten of eleven cases of other collagen diseases, but it was mostly negative in cases of other controls.

The earlier immunofluorescent studies of Behçet’s syndrome, described by Shimizu and others (1963), showed a cytoplasmic fluorescence in the leucocytes in peripheral blood and in the cells of smears from aphthous ulcers.

Similar findings were reported by Hartl (1963), who demonstrated a cytoplasmic fluorescence in a group of systemic diseases such as dermatomyositis, cold agglutinin syndrome, and Stevens-Johnson’s syndrome, which belong to the category of mucocutaneous-ocular syndromes, and observed a similarity between the skin- and leucocyte-antigen. Our studies show that such a cytoplasmic fluorescence is distributed in the form of very small granules, and a similar finding was noted in cases of rheumatoid arthritis, polyarteritis nodosa, and dermatomyositis.

Hess and Ziff (1961) described a positive fluorescent test, staining the leucocytes in peripheral blood with F.I.-labelled H.y.G. in about 90 per cent. of rheumatoid arthritis. Recently Cormane (1964) and Kalsbeek and Cormane (1964) demonstrated with an immunofluorescent technique a “bound” globulin factor in the skin of the patients with discoid lupus erythematosus and systemic lupus erythematosus.

Even though the positive reaction in the immediate intradermal test and haemagglutination test with aggregated H.y.G. and the demonstration of cytoplasmic fluorescence by an immunofluorescent staining can not yet sufficiently prove that autoimmunization is a primary causative factor, these findings strongly suggest that there must be a common or related autoimmune mechanism in pathogenesis of Behçet’s syndrome and other collagen diseases.

The elevation of the serum sialic acid level in Behçet’s syndrome and other collagen diseases suggests that there may be similar systemic connective tissue changes in these diseases. This rise in serum sialic acid, especially in acute phases of Behçet’s syndrome, was thought to be important for differential diagnosis between chronic recurrent aphthous ulcer and scanty symptoms of Behçet’s syndrome.

**Summary**

In ten to thirty of 132 cases of Behçet’s syndrome seen in our clinic, immuno-chemical studies were carried out, using an immediate intradermal reaction, a haemagglutination test with heat-aggregated human gamma globulin, and an immunofluorescent staining technique. A high incidence of strong positives in the former reactions and the demonstration of a cytoplasmic fluorescence in the leucocytes of peripheral blood and cells of aphthous ulcers of the cases tested suggests that there may be a related autoimmune mechanism in Behçet’s syndrome and in collagen diseases. The rise in the serum sialic acid level and changes with the clinical course of the disease also suggest that the systemic connective tissue damage is similar to that found in the collagen diseases.

**REFERENCES**


Etudes immunologiques du syndrome de Behçet

**RéSUMÉ**

Chez dix à trente, sur 132 cas de syndrome de Behçet vus dans notre clinique, on a procédé à des études immuno-chimiques en utilisant la réaction intradermique immédiate, la réaction d'hémagglutination avec la globuline gamma humaine agrégée à la chaleur et la méthode de coloration immuno-fluorescente. Une grande fréquence de fortes réactions positives et la mise en évidence d'une fluorescence cytoplasmique dans les leucocytes du sang périphérique et dans les cellules des ulcères aphteux des cas examinés indiquent la possible existence d'un rapport entre le mécanisme auto-immun dans le syndrome de Behçet et celui dans les maladies du collagène. Une augmentation du taux sérique de l'acide sialique et des alterations de l'évolution clinique de la maladie indiquent aussi que la lésion du tissu conjonctif est similaire à celle trouvée dans les maladies du collagène.

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Estudios inmunológicos del síndrome de Behçet

**SUMARIO**

En diez a treinta de 132 casos de síndrome de Behçet, vistos en nuestra clínica, se realizaron estudios inmuno-químicos empleando la reacción intradérmica inmediata, la reacción de hemaglutinación con la globulina gamma humana agregada por el calor y el procedimiento de coloración inmunofluorescente. La gran incidencia de fuertes reacciones positivas y la demostración de una fluorescencia citoplásica en los leucocitos de la sangre periférica y en las células de las úlceras aftosas de los casos examinados sugiere la existencia de una relación entre el mecanismo auto-inmune en el síndrome de Behçet y el observado en las enfermedades del colágeno. Un aumento de las cifras séricas del ácido sialico y las alteraciones de la evolución clínica de la enfermedad sugieren también que la lesión sistémica del tejido conjuntivo es similar a la encontrada en las enfermedades del colágeno.