

A Study of Interleukin-2 Activity in Behçet's Syndrome

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This study was undertaken to investigate immunological mechanisms in Behçet's syndrome, a function considered important in the pathogenesis of the disease. The activity of interleukin-2 (IL-2), which is believed to play a central role in the regulation of both cell-mediated and humoral T cell-dependent immune responses, measured in 46 patients with complete, incomplete or suspected Behçet's syndrome.

The results were as follows:

1. There was no significant difference between the average IL-2 activity of patients and control group.
 2. For each clinical subtype of Behçet's syndrome, IL-2 activity was lower than the control value, but the difference was not statistically significant.
 3. In mucocutaneous and ocular types, the greater the clinical symptoms, the lower the value of IL-2 activity. However, the decrease of IL-2 activity was not statistically significant.
- In conclusion, IL-2 activity of phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes in patients with Behçet's syndrome was not significantly decreased compared to control population. (*Ann Dermatol* 3:(1) 5-11, 1991)

Key Words: Behçet's syndrome, Interleukin-2

Behçet's syndrome, which is characterized by recurrent oral and genital ulcers, skin lesions, and eye involvement mainly iridocyclitis, was first described by Hulusi Behçet in 1937^{1, 2}. Behçet's syndrome is now recognized as a multisystemic disease with mucocutaneous, ocular, intestinal, articular, vascular, urogenital, and neurologic involvement³.

The etiology and pathogenesis of Behçet's syndrome is not yet established⁴. Viral infection¹, immunologic abnormalities³, allergy to streptococcal antigens⁵, and genetic influence⁶ have been suggested in the pathogenesis of this disease.

Interleukin-2 (IL-2), a product of mitogen-or antigen-stimulated T cells⁷, is believed to play a central role in the regulation of both cell-mediated and humoral T cell-dependent immune responses⁸⁻¹³. IL-2 also regulates the clonal expansion and functional maturation of stimulated T cells in a hormonal manner by binding to their specific surface receptors¹¹⁻¹⁵. Thus, deficient IL-2 activity could IL-2 activity has been demonstrated in other autoimmune disorders such as systemic lupus erythematosus¹⁶.

The purpose of this investigation was to evaluate the status of cell-mediated immunity in Behçet's syndrome by measuring IL-2 activity and its relationship to clinical disease activity.

MATERIALS AND METHODS

Materials

The study consisted of 46 patients with complete, incomplete or suspected types of the

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Behcet's syndrome who visited Behcet's Syndrome Specialty Clinic of Severance Hospital, and ten normal, healthy controls with no history of this disease or other immunologic disorders.

Methods

1) Classification of the patients

The patients were classified by the following three methods:

1) Classification by Shimizu *et al*⁵;

- (1) The complete type has all four clinical manifestations, including oral ulcers, genital ulcers, skin and ocular lesions;
- (2) The incomplete type present three clinical manifestations, or ocular lesions together with another lesion;
- (3) The suspected type exhibits two clinical manifestations;
- (4) A possible type has only one clinical manifestation.

The study included the patients with complete, incomplete, and suspected types.

2) Classification by Lehner and Barnes¹⁷:

- (1) The mucocutaneous type involves oral and genital ulcers with or without skin lesions;
- (2) An arthritic type with joint involvement has some or all of the mucocutaneous manifestations;
- (3) A neurologic type with brain involvement demonstrates some or all of the lesions found in the mucocutaneous and arthritic types;
- (4) The ocular type with uveitis shows some or all of the mucocutaneous, arthritic and neurologic manifestations.

3) Classification according to clinical activity:

The clinical symptoms at the beginning of this study included oral ulcer, genital ulcer, skin manifestation such as erythema nodosum-like lesion or erythema multiforme-like lesions, eye involvement, arthritis, thrombophlebitis and neurologic manifestations. One point was given for each clinical symptom, and the sum defined as the number of clinical activities.

2) Purification of peripheral blood lymphocytes

Within two hours of blood sampling in a

heparinized tube, the blood was mixed in the ratio of 1:1 with RPMI 1640 media (M.A. Biproducts, U.S.A.) supplemented with L-glutamine 5000 ug/ml, penicillin 100 unit/ml, streptomycin 100 ug/ml, HEPES buffer 10mM/ml (hereafter referred to as complete media). Lymphocytes were isolated with Ficoll-Hypaque solution (1.077 g/ml density, Pharmacia) and washed two time with complete media.

3) Assay of interleukin-2 (IL-2) activity

Assay of IL-2 activity was performed using a modified method described by Alvarez *et al*¹⁸. Lymphocytes isolated from peripheral blood were cultured at 5×10^6 cells/ml in complete culture medium supplemented with 2% inactivated AB blood type human serum 10 μ g/ml phytohemagglutinin (PHA-P, Commonwealth Serum Lab), and divided into 24 well plastic culture plates (Costar) containing 2 ml each. The cultures were incubated at 37°C, in a 5% CO₂ atmosphere for 24 hours. The cells were then centrifuged for ten minutes at 300xg. and then the supernatants used as IL-2 solution. The quantitative assay of IL-2 was performed by using an analogous microassay described by Gillis *et al*¹⁹ with CTLL2 (cytotoxic lymphoid line 2) which is IL-2 dependent mouse cell line, and by measuring short-term tritiated thymidine (3H-TdR) uptake. We placed 100 μ l of serially diluted supernatants in 96 well round bottomed microtiter plates and added 100 μ l of the complete medium, which was set in 5×10^4 CTLL2 cells/ml supplemented with 5×10^{-5} M/ml 2-Mercaptoethanol. These mixtures were incubated at 37°C in 5% CO₂ atmosphere. CTLL2 cells were used after incubated for three days and washed twice in complete medium. After incubation for 24 hours, 10 μ l of 0.1 mCi/ml 3H-TdR was added to each culture well and the cells were incubated for four hours. Cells were then processed on a cell harvester (Flow Lab) onto glass fiber filters (Cat. No. 78-115-05, Flow Lab) and dried at room temperature. After, placing the dried glass fiber filters containing the cells into 6ml glass vials, and mixing with 5ml of cocktail solution (Toluene, Merck: 0.3% PPO, Merck: 0.01% POPOP, Merck), the activity of the isotype was measuring in cpm of 3H-TdR uptake

on a scintillation beta counter. One unit of IL-2 was expressed as 50% of maximal ^3H -TdR uptake of CTLL2 cells. Purified human IL-2, manufactured by Collaboration, Research Inc., was used as control IL-2.

RESULTS

1. IL-2 activity of peripheral blood lymphocytes from patients with Behçet's syndrome according to classification by Shimizu et al⁵

The mean IL-2 activity of PHA-stimulated peripheral blood lymphocytes from 46 patients with Behçet's syndrome was calculated as 5.7 ± 3.7 units per ml. IL-2 production activity in seven patients with the complete type, 24 patients with the incomplete type, and 15 patients of suspected type were 5.1 ± 3.6 , 5.0 ± 3.1 , 7.1 ± 4.6 units per ml, respectively. IL-2 activity was decreased compared for normal counts, 8.0 ± 5.9 units per ml, but there was no statistical significance (Table 1).

2. IL-2 activity of peripheral blood lymphocytes from patients with Behçet's syndrome according to classification by Lehner and Barnes¹⁷

The mean IL-2 activity of PHA-stimulated peripheral blood lymphocytes from 46 patients with Behçet's syndrome was calculated as 5.7 ± 3.7 units per ml of culture medium. This value expressed was lower than the normal control, 8.0 ± 5.9 units per ml, but the decrease was not statistically significant. IL-2 activity in 26 patients with the mucocutaneous type was 5.8 ± 3.7 units per patients with the neurologic type, 6.6 ± 8.3 units per ml and, in eight patients of ocular type, 5.5 ± 3.4 units per ml. Thus, IL-2 activity was lower than the control value for each type, but there was no statistical significance (Table 2).

3. IL-2 activity tended to be inversely proportionate to the number of clinical activities except for clinical activity 0 and 5. However, there was no statistically significant difference between each group and no statistically significant

Table 1. IL-2 activity of PHA-stimulated peripheral blood lymphocytes from patients according to classification by Shimizu et al^a

Type	Number	IL-2 activity (Unit/ml, Mean \pm S.D.)
Complete	7	5.1 ± 3.6
Incomplete	24	5.0 ± 3.1
Suspected	15	7.1 ± 4.6
Total	46	5.7 ± 3.7
Control	10	8.0 ± 5.9

^aReference 5.

Table 2. IL-2 activity of PHA-stimulated peripheral blood lymphocytes from patients according to classification by Lehner and Barnes^a

Type	Number	IL-2 activity (Unit/ml, Mean \pm S.D.)
Mucocutaneous	26	5.8 ± 3.7
Arthritic	10	5.5 ± 3.9
Neurologic	2	6.6 ± 8.3
Ocular	8	5.5 ± 3.4
Total	46	5.7 ± 3.7
Control	10	8.0 ± 5.9

^aReference 17.

cant decrease than control value (Table 3).

4. IL-2 activity of peripheral blood lymphocytes from patients with Behcet's syndrome according to classification by Shimizu *et al*⁵ and clinical activity.

IL-2 activities were decreased in all clinical activities of complete and incomplete types, and clinical activity 2 of the suspected type, but there was no statistically significant decrease compared to the control value. In the complete type IL-2 activity of clinical activity 3 was lower than that of clinical activity 2, but the difference was not statistically significant (Table 4).

5. IL-2 activity of peripheral blood lymphocytes from patients with Behcet's syndrome according to classification by Lehner and Barnes¹⁷ and clinical activity.

IL-2 activity was lower than in the control values except in clinical activity 2 of the neurologic type. In the mucocutaneous and ocular types, the greater the clinical severity, the lower the value of IL-2 activity. The decrease in IL-2 production was not statistically significant (Table 5).

Table 3. IL-2 activity of PHA-stimulated peripheral blood lymphocytes from patients according to clinical activity

Clinical activity	Number	IL-2 activity (Unit/ml, Mean±S.D.)
0	1	4.1
1	8	7.1±4.9
2	19	6.1±3.8
3	16	5.0±3.2
4	1	0.4
5	1	5.0
Total	46	5.7±3.7
Control	10	8.0±5.9

Table 4. IL-2 activity of PHA-stimulated peripheral blood lymphocytes from patients according to classification by Shimizu *et al*^a and clinical activity

Type	Clinical activity	Number	IL-2 activity (Unit/ml, Mean±S.D.)
Complete	2	2	6.7±8.2
	3	4	4.3±1.1
	ND	1	5.0
Incomplete	1	4	5.3±1.7
	2	8	5.6±2.7
	3	11	5.0±3.6
	ND	1	0.4
Suspected	1	4	8.9±6.7
	2	9	6.5±4.1
	3	1	8.3
	ND	1	4.1
Control		10	8.0±5.9

^aReference 5.

ND: Data not available

Table 5. IL-2 activity of PHA-stimulated peripheral blood lymphocytes from patients according to classification by Lehner and Barnes^a and clinical activity

Type	Clinical activity	Number	IL-2 activity (Unit/ml, Mean±S.D.)
Mucocutaneous	1	7	7.0±5.3
	2	11	5.4±2.7
	3	7	5.4±3.8
	ND	1	4.1
Arthritic	1	1	7.5
	2	4	5.8±4.9
	3	4	6.1±3.2
	ND	1	0.4
Neurologic	2	1	12.5
	3	1	0.7
Ocular	2	3	7.2±5.5
	3	4	4.3±1.1
	ND	1	5.0
Control		10	8.0±5.9

^aReference 17.

ND: Data not available

DISCUSSION

In Behçet's syndrome, changes in the peripheral lymphocyte population are well described. Many studies have been done regarding in the changes of peripheral blood lymphocytes in patients with Behçet's syndrome. These include a decrease in the number of total T lymphocytes^{20, 21}, the decrease of the number of helper T lymphocytes^{21, 25}, and the reduced ratio of helper/suppressor T lymphocyte²³.

Ahmed²⁶ reported decreased response to PHA stimulation and no differentiation into various subpopulations of T cells. Decreased T cell activity and a poor autologous mixed lymphocyte reaction were described by Sakane et al²⁷. Decreased natural killer cell activity has been suggested as an important event in the pathogenesis of Behçet's syndrome^{24, 28, 29}.

IL-2 is a well-known, important lymphokine produced from mitogen-or antigen-stimulated T cells⁷ and is believed to play a role in the regulation of inflammation and immune reaction by inhibiting the induction of growth and differentiation of leukocytes and cellular migration of non-

leukocytes. It appears to play a central role in the regulation of both cell-mediated and humoral T cell dependent immune responses⁸⁻¹³. IL-2 also regulates maturation of stimulated T cell in a hormonal manner by binding to specific receptors on such T cells^{14, 15}. Thus decreased IL-2 activity may influence normal T cell differentiation.

We hypothesized that measurement of IL-2 activity would reflect the cell-mediated immune status of patients with Behçet's syndrome.

In this study, the decrease in mean IL-2 activity from the patients with Behçet's syndrome compared to the control value was not statistically significant. Using the classification schemes by Shimizu et al⁵, and Lehner and Barnes¹⁷ Correlate with clinical activity, IL-2 activity was lower than that of the control value in each type and in each classification. But there was no statistical significance. For the mucocutaneous and ocular types, in the classification by Lehner and Barnes¹⁷ correlate with clinical activity, the greater the clinical activity, the lower the value of IL-2 activity. However, the difference was not statistically significant. Thus, IL-2 activity of PHA-stimulated peripheral blood lymphocytes from the patients with Behçet's syndrome was not

significantly decreased. Our results differed from the data of Lee et al³⁰ who reported a statistically significant decrease of the IL-2 activity according to severity of the disease.

Our data showed some similarity to the results of Sakane et al³¹ who reported no significant decrease of the IL-2 activity in Behcet's syndrome. They suggested that T cells from all patients with Behcet's syndrome could produce normal levels of IL-2 in response to PHA; however, responsiveness to exogenous IL-2 was impaired in the early active phase. They also suggested that this defect was due to a decreased number of cells bearing IL-2 receptors in patients with early active disease, while in patients with chronic active or with inactive disease, there was a decrease in density of IL-2 receptors on T cells bearing these receptors.

Using the scoring method of clinical activity in this study, we tried to evaluate our data objectively. Although there was a slight decrease of IL-2 activity, we felt it may be related with severity and specific clinical types of Behcet's syndrome. Further studies on IL-2 receptors and responsiveness to IL-2 using a scoring method of clinical activity may be useful in defining the role of IL-2 in the pathogenesis of Behcet's syndrome.

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