The Evaluation of T Lymphocytes and Subsets, B Lymphocytes, and Natural Killer (NK) Cells in Behçet's Disease

BEHÇET HASTALARINDA T LENFOSİT, B LENFOSİT VE NATURAL KİLLER (NK) HÜCRELERİNİN DEĞERLENDİRİLMESİ

Sevgi BAHADIR*, Rebiay APAYDIN**, Gülseren ÇİMŞİT***, Yavuz TEKELİOĞLU****, Perihan PARLAT*****, Fahrunnisa BOZOK*****, Nilgün BİLEN**, Köksal ALPAY******

* Doç.Dr., Karadeniz Teknik Üniversitesi Tıp Fakültesi Dermatoloji AD, TRABZON
** Yrd.Doç.Dr., Kocaeli Üniversitesi Tıp Fakültesi Dermatoloji AD, KOCAELİ
*** Yrd.Doç.Dr., Karadeniz Teknik Üniversitesi Tıp Fakültesi Dermatoloji AD,
**** Uzm.Dr., Karadeniz Teknik Üniversitesi Tıp Fakültesi Histoloji - Embriyoloji AD,
***** Dr., Karadeniz Teknik Üniversitesi Tıp Fakültesi Dermatoloji AD,
****** Prof.Dr., Karadeniz Teknik Üniversitesi Tıp Fakültesi Dermatoloji AD, TRABZON

Summary

The percentages of total T, B, natural killer (NK) cells, and T cell subsets in the peripheral blood obtained from 37 patients with active Behçet's Disease (BD), 14 patients with inactive BD, and 20 healthy control subjects were analyzed by using flow cytometry in this study. The percentages of total T lymphocytes were lower in patients with active and inactive stages than control subjects ($p<0.001$ and $p<0.001$, respectively). The decrease in CD4+ cells in patients with active stage was statistically significant when compared with patients with inactive stage and control subjects ($p<0.001$ and $p<0.001$, respectively). The percentages of CD8+ cells were higher in patients with active and inactive stages than control subjects ($p<0.001$ and $p<0.001$, respectively). The CD4+/CD8+ ratio was lower in patients with active stage than patients with inactive stage and control subjects ($p<0.001$ and $p<0.001$, respectively). The percentage of B lymphocytes was decreased in patients with active stage compared with control subjects ($p<0.001$) while increased in patients with inactive stage compared with control subjects ($p<0.001$). In contrast, the percentage of NK cells increased in patients with active stage compared with control subjects ($p<0.001$) while decreased in the patients with inactive stage compared with control subjects ($p<0.001$).

Our results support the idea that immunological reactions have an important role in the pathogenesis of BD.

Key Words: Behçet's disease, CD4+ cells, CD8+ cells, B cells, NK cells

T Klin J Dermatol 2000, 10:7-12

Özet

Çalışmamız, Behçet Hastalığının patogenezinde immünolojik reaksiyonların rolünü değerlendirmek amacıyla, 37'si aktif, 14'ü inaktif, toplam 51 Behçet Hastası ile 20 sağlıklı bireyde yapıldı. Total T lenfosit yüzdesi aktif ve inaktif hasta grubunda, kontrol grubuna göre anlamlı derecede düşük bulundu (sırasıyla $p<0.001$, $p<0.001$). Aktif hasta grubunda T helper (Th) hücrelerinin azalması, inaktif hasta ve kontrol gruplarına göre anlamlı idi (sırasıyla $p<0.001$, $p<0.001$). T supresor (Ts) hücre yüzdesi ise, hem aktif hem de inaktif hasta grubunda, kontrol grubuna göre yüksek bulundu (sırasıyla $p<0.001$, $p<0.001$). T helper/T supresor (Th/Ts) oranı aktif hasta grubunda, inaktif ve kontrol grubuna göre anlamlı oranda düşük bulundu (sırasıyla $p<0.001$, $p<0.001$). B lenfosit yüzdesi aktif hasta grubunda kontrol grubuna göre düşük bulunurken ($p<0.001$), inaktif hasta grubunda kontrol grubuna göre yüksek idi ($p<0.001$). Natural Killer (NK) hücreleri ise aktif hasta grubunda kontrol grubuna göre yüksek bulunurken ($p<0.001$), inaktif hasta grubunda kontrollere göre azalmıştı ($p<0.001$).

Sonuç olarak bu bulguların Behçet Hastalığının patogenezinde immünitenin rolünü desteklediği görüşüne varıldı.

Anahtar Kelimeler: Behçet hastalığı, T Lenfosit, B Lenfosit, Natural killer hücreler

T Klin Dermatoloji 2000, 10:7-12

Geliş Tarihi: 05.05.1999

Yazışma Adresi: Dr.Sevgi BAHADIR
Karadeniz Teknik Üniversitesi Tıp Fakültesi
Dermatoloji AD, TRABZON

Behçet's disease (BD) is a chronic, inflammatory, and multisystemic condition of unknown etiology, clinically characterized by recurrent oral and genital ulcerations and skin eruptions, inflamma-

tory ocular involvement, and in some cases, by arthritis, vasculitis, inflammatory bowel disease, and neurologic manifestations (1-5). It is mainly observed in Turkey, other Mediterranean areas, and Japan (3). Viral, genetic, and environmental factors have been implicated in the pathogenesis of the disease. Several immunological abnormalities have been described such as presence of circulating immune complexes, raised serum levels of C9 and acute phase proteins, autoantibodies to oral mucosa (4), decreased chemotaxis, an imbalance in subsets of peripheral lymphocytes (2-4), lack of peripheral blood NK cell activation (4), and excessive B cell stimulation (5).

In order to understand the relationship between the immunological reactions in active and inactive stages of BD, we analyzed the percentages of T, B, NK cells and T cell subsets and the ratio of helper/inducer T (Th) cells to suppressor/cytotoxic T (Ts) cells in the peripheral blood obtained from the patients with active and inactive stages of BD using flow cytometry. The results were compared with healthy control subjects.

Material and Methods

Patients

This study included 51 patients suffering from BD, 21 males and 30 females (mean age 34.9±8.5 years). The diagnosis was based on the criteria of International Study Group. Thirty-seven patients (15 males and 22 females, mean age 36.1±9.1 years) had active clinical manifestations at the time of the study. 32 cases were under the treatment with colchicine while 5 recently diagnosed cases have not received any treatment (Table 1). The inactive group consisted of 14 patients (6 males and 8 females, mean age 34.2±9.3 year) who have not shown any clinical sign for at least 1 month. Nine cases were under the treatment with colchicine while the treatment had been stopped in 5 cases. Twenty healthy persons (11 males and 9 females, mean age 29.8±7.6 year) with similar age and sex distribution were selected as control group.

Methods

Analysis of cells with monoclonal antibodies

Automated whole blood lysis method was used for preparation of cells. In brief, whole blood samples drawn from patients and control subjects were taken into 5 ml EDTA Vacutainer tubes. After staying on the mixer at least for half an hour, blood cells were counted on a Coulter Counter. Then, with appropriate concentration, 100 µl of each blood was pipetted into 12X75 mm plastic tubes. Titered amounts of conjugated isotypic control CD3, CD4, CD8, CD19, CD56 (Immunotech-Coulter, Miami, FL) were pipetted onto appropriate tubes and incubated for 20 minutes at room temperature in dark. For lysing and fixing procedures, automated Multi-Q prep system with Immunprep kit (Coulter diagnostic, Miami, FL) was used. Samples were analysed within 2 hours at room temperature.

Flow cytometry

Epics Elit ESP (Coulter, Miami, FL) was used for flow cytometric analyses. Daily quality control and calibration was performed with flow check and flow set fluorospheres. Laser alignment was controlled with flow check (Coulter, Miami, FL) and fluoroscence calibration was done with flow set fluorospheres. Each protocol consisted SS/FS (side scatter/forward scatter), LFL1/LFL2 dot plot graphics, and LFL1 and LFL2 histograms. IgG1-FITC/IgG1-PE isotypic controls were analysed as the negative control and appropriate markers were placed. 5000 cells in lymphocyte gate was counted for each sample. Results were reported after list mode data analysis was completed.

Statistical Analysis

The statistical significance of results was assessed by Mann Whitney U test.

Results

T lymphocytes and T cell subsets

The percentages of total T lymphocytes and T cell subsets in the peripheral blood obtained from patients and control subjects are outlined on Table 2. While there was no significant difference between the active and inactive stages ($p>0.05$), the percentages of total blood T lymphocytes in patients with both active and inactive BD were low compared with control subjects ($p<0.001$ and $p<0.001$, respectively).

Table 1. Clinical characteristic of patients with active Behçet's disease (1)

No	Age	Sex	Recurrent oral ulceration	Recurrent genital ulceration	Eye lesions	Skin lesions	Positive pathergy test	Treatment
1	36	F	+	+	Ø	E.nodosum	+	Colchicine
2	55	M	+	+	Ø	E.nodosum	Ø	Colchicine
3	25	F	+	+	Ø	Pustular	Ø	Colchicine
4	43	M	+	Ø	Ø	Pustular	+	Colchicine
5	43	F	+	+	Ø	Pustular	+	Colchicine
6	64	M	+	+	Ø	Ø	+	Colchicine
7	29	M	+	+	Ø	E.nodosum	Ø	Colchicine
8	46	M	+	Ø	Ø	Pustular	+	Colchicine
9	42	M	+	+	Ø	Pustular	+	Colchicine
10	23	F	+	Ø	Ø	Pustular	+	No
11	33	F	+	+	Ø	Ø	+	Colchicine
12	33	M	+	+	+	Ø	Ø	Colchicine
13	38	F	+	+	Ø	E.nodosum	Ø	Colchicine
14	34	F	+	Ø	+	Ø	+	Colchicine
15	33	M	+	Ø	+	Pustular	Ø	Colchicine
16	28	F	+	+	Ø	Ø	+	Colchicine
17	33	M	+	+	Ø	Ø	+	Colchicine
18	41	F	+	+	+	Ø	Ø	No
19	27	M	+	+	Ø	Pustular	+	Colchicine
20	27	M	+	Ø	+	E.nodosum	Ø	Colchicine
21	33	M	+	+	Ø	Ø	+	Colchicine
22	25	F	+	+	Ø	Pustular	+	Colchicine
23	41	F	+	+	Ø	Ø	+	Colchicine
24	47	F	+	Ø	+	E.nodosum	+	Colchicine
25	49	F	+	+	Ø	Ø	+	Colchicine
26	31	F	+	+	Ø	Pustular	Ø	Colchicine
27	34	M	+	+	Ø	E.nodosum	Ø	Colchicine
28	38	M	+	+	Ø	Ø	Ø	Colchicine
29	39	F	+	Ø	+	E.nodosum	Ø	Colchicine
30	24	F	+	+	Ø	Ø	+	Colchicine
31	36	F	+	+	+	Pustular	Ø	No
32	40	F	+	+	Ø	Ø	+	Colchicine
33	40	F	+	+	Ø	E.nodosum	Ø	Colchicine
34	21	F	+	+	+	Pustular	Ø	Colchicine
35	27	F	+	Ø	+	Pustular	Ø	No
36	32	M	+	+	+	Ø	Ø	Colchicine
37	40	F	+	+	+	Ø	Ø	No

A significant decrease in the CD4+ cells was found in patients with active stage compared with patients with inactive stage and control subjects ($p<0.001$ and $p<0.001$, respectively). There was not a significant change between patients with inactive stage and control subjects ($p>0.05$).

The percentages of CD8+ cells were similar in patients with active and inactive stages ($p>0.05$), but the values of both groups were statistically higher than control group ($p<0.001$ and $p<0.001$, respectively).

The CD4+/CD8+ ratio decreased significantly in patients with active stage compared with patients in inactive stage and controls ($p<0.001$ and $p<0.001$, respectively). Although the CD4+/CD8+ ratio increased slightly in inactive stage, it was statistically lower than control subjects ($p<0.001$).

B lymphocytes

The percentages of B lymphocytes are shown on Table 2. The decrease in the percentage of B cells in patients with active stage was statistically

Table 2. Comparison of the results of active, inactive stages of Behçet's disease and control groups

Cells	Active stage (mean±SD)	% Inactive stage (mean±SD)	Controls (mean±SD)
CD4+	23.0 ± 2.1*,**	39.8 ± 1.8	40.3 ± 1.2
CD8+	30.4 ± 2.9 **	29.1 ± 1.8***	23.3 ± 1.2
CD4+/CD8+	0.7 ± 0.1*,**	1.3 ± 0.1***	1.6 ± 0.1
CD3+	81.4 ± 1.5**	80.5 ± 1.8***	83.2 ± 1.7
CD19+	7.3 ± 0.9*,**	11.6 ± 1.2***	8.4 ± 0.6
CD56+	8.1 ± 0.7*,**	5.3 ± 0.7***	6.4 ± 0.7

* : different from inactive stage, $p < 0.001$
** : different from control group, $p < 0.001$
*** : different from control group, $p < 0.001$

significant compared with both patients with inactive stage and control subjects ($p<0.001$ and $p<0.001$, respectively). In inactive stage, the percentage of B lymphocytes was higher than control group ($p<0.001$).

NK cells

The percentage of total NK cells in patients with active and inactive stage and control group are presented in Table 2. The percentage of NK cells was higher in active stage compared with inactive stage and control group ($p<0.001$ and $p<0.001$, respectively). In the inactive stage, it was lower than the mean value in control group ($p<0.001$).

Discussion

Currently, it is considered that the inflammation induced by immune mechanisms plays an important role in the pathogenesis of BD.

The immune system is a complex, interrelated system of cells, antibodies, lymphokines, and mediators. T cells seem to play a central role in directing the immune response (6). Although the CD4+/CD8+ ratio was found to be reduced in almost all previous studies on circulating T lymphocytes and the T cell subsets, there was uncertainity about changes in T cell subsets. A reduction in the percentages of total T lymphocytes and Th cells and a concomitant significant increase in the percentage of Ts cells have been suggested by Lim et al. (7) Similar results were reported by Kahan et al. (8) and Hamzaoui et al. (4) On the other hand, Victorino et al. reported that the population of circulating Ts cells were unaltered whereas Th cells were reduced compared with normal individuals (2). In contrast, Valesini et al. reported that Th cells were decreased, but the decrease in Th cells was not found to be statistically significant and Ts cells were increased in the patients with BD, when compared with normal subjects (3).

Tokgöz et al. reported that there was an increase in total T lymphocytes in patients with active BD, and in Ts cells in patients with both active and inactive BD, but there were no important differences in Th cells related to activity (9). On the other hand Gürer et al. found that there was a reduction in total T lymphocytes, and increase in Ts cell in patients with both active and inactive BD. They suggested that there was a suppresion in cell-mediated immunity (10). In our study, the CD4+/CD8+ ratio was lower in patients with active BD than control subjects, in accordance with previous studies (4,7,8). This result was related to both reduction of CD4+ cells and increase of CD8+ cells. In patients with inactive BD, the CD4+/CD8+ ratio was higher than active patients, that seemed to be related to the increase in CD4+ cells.

Although the percentages of circulating B and T lymphocytes in BD were evaluated in many studies, different results were reported. While in some studies, the percentages of B and T cells in the peripheral blood were found normal (11), in some other studies, abnormally decreased B cells and proportionally increased T cells (9) or increased B cells and decreased T cells were found (12). In our investigation, we found that the percentage of circulating total B lymphocytes was decreased in patients with active BD compared with both patients

with inactive BD and control group, whereas in inactive stage, it was significantly higher than control group.

While one subset of Th cells helps B cells to proliferate and differentiate and thereby augments antibody production, the other subset induces suppressor cells. In contrast, Ts cells inhibit the function of Th cells via a feedback mechanism and thereby suppress both the humoral and cell-mediated immune responses (6). For this reason, decreases in percentage of B lymphocytes in active stage seems to be related to decreased CD4+ cells and increased CD8+ cells. Sakane et al. showed that suppressor T cell activity in patients with preactive disease impaired both T and B cell functions and they suggested that the failure of suppressor T cell activity could be largely attributable to either the dysfunction or the reduction in CD4+ cells that provide suppressor function (13). Suzuki et al. showed polyclonal B cell activation in patients with BD (5). Fortune et al. demonstrated that there was no significant difference in percentage of B lymphocytes between patients with BD and controls, but percentage of B cells with membrane-bound Ig A was significantly increased (14).

We could not evaluate the function of cells, however, to our knowledge, our findings of the reduction of both T and B cells were previously reported only by Haim et al (15).

NK cells are also important members of immune system. It has been demostrated that in patients with active BD while the number of NK cells were increased (4,12,16), the activity of these cells were decreased (4,12,17), whereas in patients with inactive BD, there were either an increase in NK cell activity (4,17) or an insignificant difference compared with normal subjects (12). The NK cells are activated by the cytokine IFN-gamma and inhibited by the cytokine PGE2 (17). It was demonstrated that the serum level of IFN was reduced in active stage of BD and increased in inactive stage (18). Therefore, it has been suggested that the reduced activity of NK cells may be due to low serum titer of IFN- gamma. In addition, the decrease in NK cell function may be related to the presence of immature forms of NK cells in the peripheral blood or the high titer of PGE2. In our study, while percentage of NK cells in patients with active BD was increased compared with patients with inactive BD and control subjects, it was decreased in patients with inactive BD compared with control subjects.

This study provides evidences regarding the changes in humoral and cell-mediated immunity in BD, but the question from where these changes start can not be answered.

REFERENCES

1. Jorizzo JL, Abernethy JL, White WI, Mangelsdorf HC, Zouboulis CC, Sarıca R, et al. Mucocutaneous criteria for the diagnosis of Behçet's disease: an analysis of clinicopathologic data from multiple international centers. J Am Acad Dermatol 1995; 32:968-76.
2. Victorino RMM, Ryan P, Hughes GRV, Hodgson HJF. Cell-mediated immune functions and immunoregulatory cells in Behçet's syndrome. Clin Exp Immunol 1982; 48:121-8.
3. Valesini G, Pivetti-Pezzi P, Mastrandrea F, Moncada A, Cuomo M, Natali PG. Evaluation of T cell subsets in Behçet's syndrome using anti-T cell monoclonal antibodies. Clin Exp Immunol 1985; 60:55-60.
4. Hamzaoui K, Ayed K, Hamza M, Touraine JL. Natural killer cells in Behçet's disease. Clin Exp Immunol 1988; 71:126-31.
5. Suzuki N, Sakane T, Ueda Y, Tsunematsu T. Abnormal B cell function in patients with Behçet's disease. Arth Rheum 1986; 29:212-9.
6. Dahl MV. T cells and cell-mediated immunity. In: Clinical immunodermatology, second ed. Chicago: Year Book Medical Publishers. 1987:35-51.
7. Lim SD, Haw CR, Kim NI, Fusaro RM. Abnormalities of T cell subsets in Behçet's syndrome. Arch Dermatol 1983; 119:307-10.
8. Kahan A, Hamzaoui K, Ayed K. Abnormalities of T lymphocyte subsets in Behçet's disease demonstrated with anti-CD 45RA and anti-CD 29 monoclonal antibodies. J Rheumatol 1992; 19:742-6.
9. Tokgöz G, Düzgün N, Özoran K, Gürler A, Tutkak H. Peripheral blood lymphocyte population in patients with Behçet's disease and recurrent oral ulceration. VIII. Eular symposium "Vasculites". Clin Rheum 1994; 13:385-6.
10. Gürer MA, Baydar İ, Önder M, Bozkurt M, Şaşmaz R. Behçet Hastalığında hücresel immünite değişiklikleri. XII. Ulusal Dermatoloji Kongre kitabı. 27-30 Eylül İstanbul 1988; 65-75.
11. Carroll ET, Miller M, South MA, Abdou NL. Behçet's disease: possible role of secretory component deficiency, synovial inclusions, and fibrinolytic abnormality in the various manifestations of the disease. J Lab Clin Med 1978; 91:409-22.
12. Kaneko F, Takahashi Y, Muramatsu R, Adachi K, Miura Y, Nakane A, Minagawa T. Natural killer cell numbers and function in peripheral lymphoid cells in Behçet's disease. Br J Dermatol 1985; 113:313-8.

13.Sakane T, Kotani H, Takada S, Tsunematsu T. Functional aberration of T cell subsets in patients with Behçet's disease. Arth Rheum 1982; 25:1343-51.

14.Fortune F, Walker J, Lehner T. The expression of γδ T cell receptor and the prevalence of primed, activated and Ig A-bound T cells in Behçet's syndrome. Clin Exp Immunol 1990; 82:326-32.

15.Haim S, Mekori T, Sobel JD, Robinson E. Aspects of lymphcyte function in Behçet's disease. Dermatologica 1976; 153:34-7(Abst).

16.Suzuki Y, Hashi K, Matsuda T, Mizushima Y. Increased peripheral blood gamma delta + T cells and natural killer cells in Behçet's disease. J Rheumatol 1992; 19:588-92.

17.Önder M, Bozkurt M, Gürer MA, Gülekon A, Sezgin P, İmir T. Natural cellular cytotoxicity in Behçet's disease. J Dermatol 1994; 21:239-43.

18.Fujii N, Minagawa T, Nakane A, Kato F, Ohno S. Spontaneous production of gamma-interferon in cultures of T lymphocytes obtained from patients with Behçet's disease. J Immunol 1983; 130:1683-86.