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The levels of serum chemokines in patients with Behçet's disease

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Aim: As chemokines are involved in the regulation of immune responses and inflammatory reactions, this study aimed to investigate the serum levels of some chemokines to determine whether they play roles in the pathogenesis of Behçet's disease (BD).

Materials and methods: The study comprised 64 cases, allocated to active or inactive patient groups. The levels of serum macrophage inflammatory protein-1α (MIP-1α), epithelial neutrophil-activating protein-78 (ENA-78), and neutrophil-activating peptide-2 (NAP-2) were measured.

Results: The serum levels of MIP-1α and ENA-78 were statistically higher in BD patients than controls (P = 0.032 and P = 0.023, respectively). However, NAP-2 levels did not differ significantly between patients and controls (P > 0.05). A significantly higher mean serum level of ENA-78 was found in patients with active disease compared to patients with inactive disease and controls (P = 0.029 and P = 0.003, respectively). However, mean serum MIP-1α and NAP-2 levels did not differ significantly between active and inactive patients (P > 0.05).

Conclusion: In this study, there was a significant elevation of MIP-1α and ENA-78 supporting the role of neutrophil hyperactivity in BD. However, because chemokines insert their effects in the microenvironment and serum concentrations reflect a developing inflammatory process indirectly, we suggest that more comprehensive studies are needed to reveal the role of chemokines in the pathogenesis of the disease.

Key words: Behçet's disease, pathogenesis, macrophage inflammatory protein-1α, epithelial neutrophil-activating protein-78, neutrophil-activating peptide-2

Introduction

Behçet's disease (BD) is a chronic inflammatory disease of unknown etiology, accompanied by vasculitis in various organs, and active leukocytes play a pivotal role in its pathogenesis. Prominent neutrophil infiltration is present in typical BD lesions (1,2). Immunological mechanisms occupy an important place in the pathogenesis of BD. Therefore, studies investigating the role of immune mechanisms in the pathogenesis of the disease have been increasing recently (3-6).

Chemokines are chemotactic factors for leukocytes. They mediate the migration and activation of leukocytes at sites of inflammation. They also have important roles in hematopoiesis, angiogenesis, tumor growth, and various autoimmune diseases, including rheumatoid arthritis, systemic sclerosis, and BD (6). The CXC chemokines predominantly activate neutrophils, while the CC chemokines generally activate monocytes, lymphocytes, basophils, and eosinophils. Macrophage inflammatory protein-1α (MIP-1α) (CCL3) is a member of the CC chemokine

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subfamily. This molecule plays an important role in inflammation. MIP-1α activates inflammatory cells and acts as a regulator in growth and differentiation of various cells. It also exerts a chemotactic effect for monocytes and T cells (7,8). Epithelial neutrophil-activating protein-78 (ENA-78) (CXCL5) is a member of the CXC chemokine family. Its structural features and biological activities are similar to those of interleukin (IL)-8 (9-11). Neutrophil-activating peptide-2 (NAP-2) (CXCL7) also belongs to the CXC chemokine family and is responsible for the chemotaxis and activation of neutrophils. It was reported that Th1 activity increases in BD, particularly in active BD (7,12). As chemokines are involved in the regulation of immune responses and inflammatory reactions, we investigated whether they may play a role in the pathogenesis of BD.

Materials and methods

Sixty-four Turkish patients with BD and 24 matched healthy controls were enrolled in this study. Patients were diagnosed according to the International Study Group (ISG) diagnostic criteria for BD at the Dermatology Clinic of the Firat University School of Medicine (13). Patients selected had received no antiinflammatory therapy in the last month, taking into consideration that it can suppress chemokines response. However, blood samples were obtained in pretreatment period from patients with systemic involvement who needed immunosuppressive therapy. The patients were grouped as active or inactive. At the time of the study, the patients who met at least one criterion of the ISG for BD were considered to be in the active stage of the disease. Patients who had been free of lesions for the previous 30 days or more were accepted as having inactive BD. Necessary permission concerning the study was obtained from the local ethics committee. Each patient was informed in detail about the study and those who agreed to be included were asked to sign an informed consent form.

Preparation of serum samples

Venous blood samples were taken from the antecubital vein for biochemical assays. These samples were centrifuged at 4000 rpm for 5 min with a Hettich Universal 32 centrifuge (Germany). The sera were separated and kept at –80 °C until the day of analysis.

Chemokine investigation

A human MIP-1α ELISA kit from BioSource International Inc. (USA) was used to determine MIP-1α. A RayBio Human NAP-2 ELISA kit from RayBiotech Inc. (USA) was used to determine NAP-2. A human ENA-78 Quantikine® ELISA kit from R&D Systems Inc. (USA) was used to determine ENA-78. Analyses were performed in immunology laboratories with a Triturus Grifols (Spain) fully automatic ELISA analyzer by using controls and calibrations in accordance with the manufacturer's recommended study protocols. For each chemokine, all sera were tested in the same plate following the same study procedure. Eight wells per plate were used for calibrators. The results were read on a spectrophotometer at 450 nm. The results of the study were calculated based on their optic densities by drawing curves from one point to another.

Statistical analysis

SPSS 11.0 was used for data analysis (SPSS Inc., USA). All values are given as mean ± standard deviation. The chi-square test was used for sex distribution among the groups, while the Mann-Whitney U test was used for comparing the data between groups. Additionally, the Kruskal-Wallis test was used for triple comparison of the data from active and inactive patients and controls. The Mann-Whitney U test was used for paired comparison of groups in statistically significant values. $P < 0.05$ was accepted as indicating statistical significance.

Results

A total of 64 BD patients (27 males, 37 females), including 46 active patients and 24 healthy controls (10 males, 14 females), were enrolled in the study. The mean age of the BD group was 36.81 ± 11.65 years (age range: 17- 66 years) and that of the control group was 33.88 ± 9.51 years (age range: 25-28 years). There were no statistically significant differences in age and sex distribution between the groups ($P > 0.05$). The distribution of present symptoms and findings together with the demographic features of the patients included in the study are listed in Tables 1 and 2.

Table 1. The demographic features of the patients included in the study.

	Patient group	Control group	P
Number of cases (n)	64	24	
Number of active patients (n)	46		
Age (years)	36.81 ± 11.65	33.88 ± 9.51	>0.05
Sex (M/F)	27/37	10/14	>0.05
Duration of the disease (years)			
Active patients	5.06 ± 6.31		
Inactive patients	6.63 ± 7.68		

Table 2. Distribution of the symptoms and findings in Behçet's disease.

Symptoms and findings	Number of cases (n)
OA	30
GU	12
EI	6
Arthralgia	28
Arthritis	16
AL	16
EN	11
Thrombophlebitis	4
GSI	1
NI	5
PTP	8

OA: Oral aphthous ulcer, GU: genital ulcer, EI: eye involvement, AL: acneform lesions, EN: erythema nodosum, GSI: gastrointestinal system involvement, NI: neurological involvement, PTP: pathergy test positivity.

Significantly higher mean serum levels of MIP-1α and ENA-78 were found in the patient group compared to the controls (P = 0.032 and P = 0.023, respectively). NAP-2 levels did not differ significantly between patients and controls (P > 0.05). The mean serum levels of the 3 chemokines studied are listed in Table 3. A significantly higher mean serum level of ENA-78 was found in patients with active disease compared to patients with inactive disease and controls (P = 0.029 and P = 0.003, respectively). However, mean serum MIP-1α and NAP-2 levels did not differ significantly between active and inactive patients (P > 0.05).

Discussion

The etiopathogenesis of BD has not yet been clarified, but it might involve immune dysfunction (6). Chemokines enable the firm adhesion of leukocytes to endothelial cells, migration to interstitial tissue, and direct leukocyte activation (9). Additionally, chemokines play a role in attracting cell groups to inflammation sites at different times. Neutrophils are suggested to have a primary role in BD lesions, such as in the pathergy phenomenon and acne-like lesions (6). MIP-1α is bound to the CCR5 receptor expressed by Th1 and also to the CCR1 receptor expressed by

Table 3. Mean sera levels of the 3 chemokines studied.

	Patient group (n = 64)		Control group
	Active patient group (n = 46)	Inactive patient group (n = 18)	(n = 24)
MIP-1α (pg/mL)	96.10 ± 15.21[a]	98.32 ± 18.33	88.92 ± 5.32
	95.23 ± 13.93		
ENA-78 (pg/mL)	1274.80 ± 686.45[b]	1023.41 ± 726.79	926.12 ± 558.33
	1373.17 ± 651.90[c,d]		
NAP-2 (pg/mL)	470.13 ± 47.85	469.81 ± 47.13	492.05 ± 54.12
	470.26 ± 48.65		

[a]: vs. control group, P = 0.032.
[b]: vs. control group, P = 0.023.
[c]: vs. control group, P = 0.003.
[d]: vs. inactive group, P = 0.029.

both Th1 and Th2, which indicates that MIP-1α particularly stimulates Th1 activity. It was reported that Th1 activity increases in BD, particularly in active patients (14). Similar to our results, Ozer et al. reported higher MIP-1α and MCP-1 levels in active BD patients than controls (12). We think that these chemokines have an important role in leukocyte activation and migration, and in addition to this role, they have an effect on Th1 polarization, which is cited in the BD pathogenesis. All of these increase Th1 activities and inflammatory cell accumulation in the inflammatory area. MIP-1 was found to be increased in the serum samples in other studies (12,15). Saruhan-Direskeneli et al. and Miyagishi et al., in 2 different studies, determined high MIP-1α levels in the cerebrospinal fluid in BD patients with neurological involvement (14,16).

In our patients, serum MIP-1α levels were significantly higher than in the controls, similar to the previously mentioned studies. However, there was no significant difference in MIP-1α levels between active and inactive patients. Unfortunately, our patients were not grouped by neurological or other organ involvement, and this patient group was too limited to interpret these results according to diagnostic criteria or to shed light upon the specific etiopathogenesis of BD.

ENA-78 is a potent neutrophil activator with similar biological properties as demonstrated for IL-8, NAP-2, and growth-related oncogenes-α (17). IL-8 mRNA expression was more prominent in patients with active BD than in patients with inactive disease (18). In a study conducted by Sugiyama et al., ENA-78 and interferon gamma-inducible protein (IP)-10 levels in the bronchoalveolar lavage (BAL) fluid and sera of 41 patients in various phases of pulmonary sarcoidosis were measured. They reported that IP-10 was increased in both serum and BAL fluid in the healthy control group in comparison to BD patients, and ENA-78 was increased only in stage 3 sarcoidosis. They suggested that the increase in ENA-78 in stage 3, where fibrosis occurred, might be due to its fibrogenetic function (19). Olszyna et al. researched whether chemokines and ENA-78 played a role in infections other than chronic diseases such as sarcoidosis. They detected that chemokines (including ENA-78) were significantly increased in urine samples in urosepsis patients, but not in plasma samples. As a result, they concluded that CXC chemokines were produced primarily from the urinary channel in patients with urosepsis, and thus neutrophils gathered in the urinary site (20). Z'Graggen et al. demonstrated that ENA-78 mRNA gene expression increased in intestinal epithelial cells of patients with Crohn's disease and ulcerative colitis (21). Those data indicate that ENA-78 might play a pivotal role in patients with BD. We could not find other previous studies investigating serum levels of ENA-78 in patients with BD for comparison with our

results. In our study, we detected significantly higher ENA-78 serum levels in BD, particularly in the active patient group, when compared to the control group. This can be attributed to neutrophils gathering at inflammatory sites and exacerbations of Behçet's vasculitis and inflammation in different sites of the body. For more specific results, different body fluids should be investigated in different BD involvements.

In our study, we aimed to investigate NAP-2, which belongs to the CXC chemokine family and is responsible for neutrophil chemotaxis and activation, and particularly its role in the pathogenesis of BD. Since NAP-2 is primarily a thrombocyte-derived chemokine, most studies are focused on the diseases in which thrombocytes play a role in the pathogenesis (22). NAP-2 was investigated in the pathogenesis of coronary artery disease. The authors found both high plasma NAP-2 level and increased CXCR2 receptors in monocytes with stable and particularly unstable angina in comparison to the control group. Thrombocyte-derived NAP-2 especially enhanced the expression of chemokines and adhesion molecules in endothelial cells (23). According to previous studies, NAP-2 is formed by platelet basic protein and connective tissue-activating peptide III released by active thrombocytes, and it leads to neutrophil gathering in thrombocyte clumps (24). According to other results, NAP-2 can be an effector chemokine, especially for thrombus formation; however, thrombocytes are not the primary effector cells in BD and the definitive pathogenetic mechanism of thrombotic tendency of BD is not fully elucidated. None of our patients had thrombotic tendency and we were unable to determine any increase in NAP-2 serum levels.

In conclusion, in the current study, in which the levels of some chemokines that are known to play a role in neutrophil activity were investigated, the significant elevation of MIP-1α and ENA-78 supports the role of neutrophil hyperactivity in this disease. However, chemokines insert their effects in the microenvironment and serum concentrations reflect a developing inflammatory process indirectly. Thus, for clearer results, tissue levels of these chemokines should be determined, as well as the relation of disease activation and the correlation of therapy response. We suggest that more comprehensive studies are needed in order to reveal the role of chemokines.

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