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BTLA (C272) expression on CD4+ T cells in Behçet patients*

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Aim: To investigate the peripheral blood levels of B and T lymphocyte attenuator (BTLA/CD272) on T cells and its role in Behçet disease (BD), and to define the role of inhibitory receptors on BD.

Materials and methods: Included in this study were 25 patients with BD and 20 healthy control subjects, matched for age and sex.

Results: $CD4^+CD272^+$ ($P < 0.001$) and $CD3^+CD272^+$ ($P < 0.001$) T cell levels were significantly higher in BD patients (active and remission) compared to the healthy controls. However, there was no difference between the active patients and those in remission in terms of $CD4^+CD272^+$ T cells, while $CD3^+CD272^+$ T cell levels were significantly higher in active-phase BD patients compared to patients in the remission phase ($P < 0.05$).

Conclusion: Further functional studies could be implemented to investigate the effectiveness of the present research parameter in the diagnosis or follow-up of certain diseases similar to BD and with obscure etiology, as well as its contribution to immune dysregulation of BD. BTLA can additionally modulate T cell response in BD on different inflammatory areas and it can be upregulated in BD because of T cell activation. The results of the present study indicate that BTLA expression on $CD4^+$ Th (T helper) cells might play a limiting role on the inflammation at peripheral sites or organs in BD.

Key words: Behçet disease, B and T lymphocyte attenuator

Introduction

The immune system is a dynamic process regulated by the balance between different inhibition and activation signals. Knowledge of the inhibitor receptor family is gradually extending at present (1). The recently discovered B and T lymphocyte attenuator (BTLA/CD272) is an inhibitor receptor expressed on T cells, and it negatively regulates the cell activation through phosphatases SHP-1/SHP-2. It has similar structure and function with cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed death 1 (PD-1) (2). Herpes virus entry mediator (HVEM) is a receptor interacting with BTLA and is defined as a member of the superfamily of tumor necrosis factor receptors (3). HVEM is generally expressed on resting T cells, monocytes, immature dendritic cells (DCs), and endothelial cells (4,5). On the other hand, BTLA is expressed on naive $CD4^+$ and $CD8^+$ T cells and then upregulated with T cell activation (6). BTLA is also present in the B cells, macrophages, and DCs of bone marrow origin. T cell receptor (TCR) and HVEM stimulation regulates the surface expression of BTLA and the accumulation in immunologic synapses, and it enables the creation of inhibitor signals, which are effective in the regulation of $CD4^+$ T cell activation (7,8).

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Because BTLA is a negative receptor, T cells give high response rates to TCR-related activation in mice deficient in the full-length form of BTLA (2). Mice deficient in BTLA or its ligand HVEM were reported to be more susceptible to immune and inflammatory diseases and showed severe pathological tissue changes like experimental allergic encephalomyelitis, allergic airway inflammation, and intestinal inflammation. Behçet disease (BD) is a chronic inflammatory disease and is strictly related to T cell stimulation and inflammation. Consequently, the BTLA pathway is considered to have a critical role in immune-inflammatory diseases such as BD (9,10).

Materials and methods

This study was approved by the local ethics committee of Fırat University, Elazığ, Turkey. Patients were diagnosed with BD according to the diagnostic criteria of the International Study Group for BD. Twenty-five BD patients and 20 age-matched healthy control subjects were included in the study. Of the BD patients, 13 were at the active stage of BD and the remaining 12 were in an inactive period. In total, 18 BD patients had ocular complications (9 with active ocular attacks, 2 with inflammatory arthritis, and 7 with uveitis in a remission period) and 5 had mucocutaneous lesions (2 in the active period and 3 in remission).

Blood was collected in EDTA tubes from peripheral venous blood and all of the samples were run within 2 h on the same day. The 3-color-flow-cytometry technique was used to analyze CD4 FITC (IM0448), CD3 PerCP (IM 0479), and CD272 PE (eBioscience 12-5950). Analytic flow cytometry was carried out by counting a total of 10,000 cells, using the Coulter EPICS XL-MCL device (Beckman Coulter, USA). Analyses were made using the Expo-32 analysis program with the same equipment.

Statistical analyses were carried out with SPSS 11.0 for Windows (SPSS Inc., USA). The Mann-Whitney U and Kruskal-Wallis tests were used, and $P < 0.05$ was considered statistically significant.

Results

The number of research groups, age distribution of the control group and BD patients, duration of disease activation criteria, and therapeutic drugs used by the BD patients are given in Tables 1-3, respectively.

BD patients (active and remission period) were observed to have significantly higher $CD4^+CD272^+$ percentages (BD patients: 13.01 ± 1.51%, healthy controls: 2.90 ± 0.814%; $P < 0.001$) and $CD3^+CD272^+$ T cell percentages (BD patients: 23.79 ± 2.27%, healthy controls: 6.27 ± 1.27%; $P < 0.001$) compared to the healthy controls. There was no difference in the $CD4^+CD272^+$ T cell percentages in BD patients in the active and remission phases (active phase: 11.43 ± 2.41%, remission phase: 14.11 ± 1.95%; $P > 0.05$). However, the $CD3^+CD272^+$ values were significantly different in BD patients in the active and remission phases (active phase: 1.06 ± 3.48%, remission phase 27.06 ± 2.74%; $P < 0.05$) (Figures 1-3).

Table 1. The age distribution of the control and BD groups and the duration of disease.

	BD $\bar{x} \pm S\bar{x}$		Control $\bar{x} \pm S\bar{x}$	
Age (years)	37.45	3.25	29.56	2.97
Disease duration (years)	**Active** $\bar{x} \pm S\bar{x}$	**Remission** $\bar{x} \pm S\bar{x}$		
	4.39 ± 1.29	8.13 ± 2.04		

Table 2. Activation criteria in patients with active BD.

	Patients with active BD	
Activation criteria	**n**	**(%)**
Uveitis	9	69.23
Oral/genital ulcers	2	15.38
Arthritis	2	15.38

Table 3. Therapeutic drugs used by BD patients.

Therapeutic drugs	Inactive (n: 13)	Active (n: 12)
Sumatriptan use (%)	30.76	**33.33**
Sulfasalazine use (%)	30.76	**16.66**
Colchicum-Dispert use (%)	61.53	**75.00**
Prednisolone use (%)	**15.38**	**25.00**

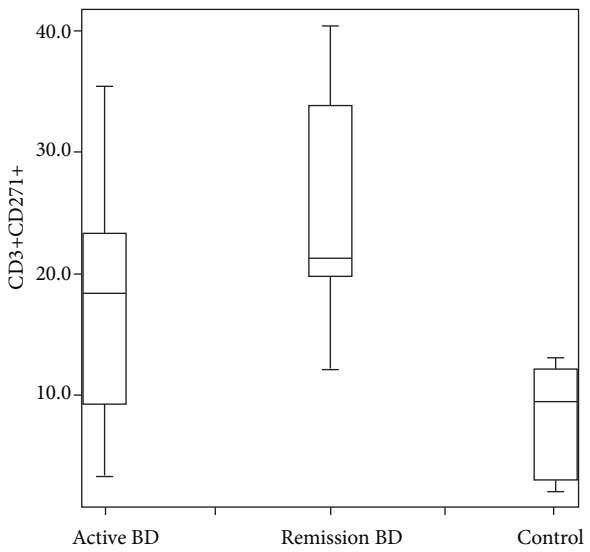

Figure 1. The distribution of BTLA levels in the BD patient and control groups.

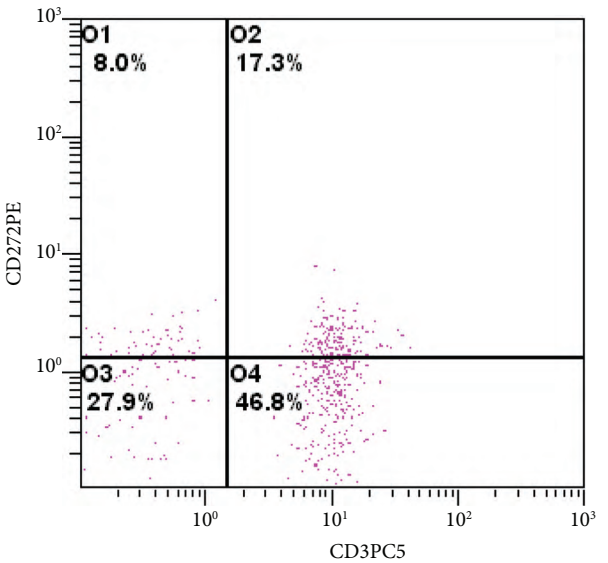

Figure 2. CD272 expression on $CD3^+$ T cells in BD patients.

Discussion

Previous studies demonstrated that inhibitor coreceptors like BTLA are related to the maintenance of homeostasis and tolerance, and, in addition, they inhibit lymphocyte activation (11). In a study reporting that BTLA had an essential role in the maintenance of self-tolerance and thus protected the host from autoimmunity, increases were detected in autoantibodies to nuclear antigens, hypergammaglobulinemia, and the peripheral active T cells of $BTLA^{-/-}$ mice at 12 months of age. In addition, self-activated $CD4^+$ T and natural killer T cells were also increased in $BTLA^{-/-}$ mice. Researchers observed a disease similar to autoimmune hepatitis and inflammatory changes similar to Sjögren syndrome in the salivary glands, lungs, and pancreas. Development of these diseases was attributed to the breakdown in the maintenance of self-tolerance. On the other hand, the same researchers also observed that the susceptibility to experimental autoimmune encephalomyelitis was increased in $BTLA^{-/-}$ mice (2). Accordingly, BTLA was found to be related to the maintenance of tolerance to self-antigens and it played a negative regulatory role in the development of autoimmune diseases (11). Different studies demonstrated the importance of T cells in the pathophysiology of airway inflammation and reported that airway inflammation lasted longer in BTLA-deficient mice (12).

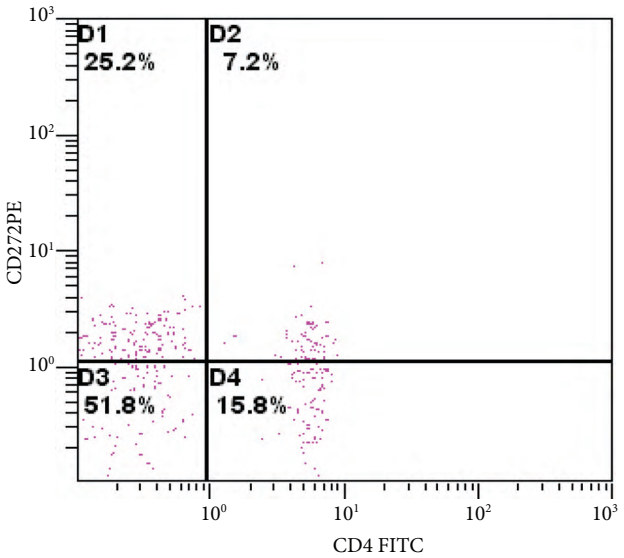

Figure 3. CD272 expression CD4⁺ T cells in BD patients.

Previous studies have indicated that BTLA and PD-1 inhibitor receptors are indispensable parts of acute allergic respiratory tract inflammation (9). In vitro studies demonstrated that BTLA inhibits T cell proliferation (3). Autoimmune encephalomyelitis disorder was even more severe in BTLA-deficient mice. Therefore, BTLA was considered to have dominant inhibitory roles (2).

The inhibitory signals formed by CTLA-4 are highly important in the early phases of T cell response, while inhibitory signals through PD-1 and BTLA pathways, in addition to other receptors, gain importance in the later immune response. Therefore, inhibitor signals through PD-1 and BTLA pathways reduce the inflammatory response rate by dominating over activated signals (13). Immune regulation gains even more importance in this regard. Further studies are required to determine the roles of inhibitor pathway defects as biological reasons for certain inflammatory diseases (14).

Of the inhibitory molecules, CTLA-4 controls the naive T cell activation, while PD-1 is responsible for T cell activation. On the other hand, BTLA is found both on naive and activated T cells and it can control the whole phases of T cell activation, though it is more prevalent on tolerant T cells (15).

BTLA is known to have strong effects on T cell activation in BD that develops uncontrolled inflammatory response on account of a dysfunction in T cell activation; therefore, the expression of BTLA on total T and CD4⁺ T cells was investigated in the present study. The aim was to gain knowledge about the expression of inhibitory receptors in BD based on the BTLA inhibitory receptor. BTLA expression was observed to increase in T cells in the peripheral cycle of BD patients compared to the healthy controls. BTLA binding creates an inhibitory signal to T cells, and thus could play an important role in terms of T cell tolerance. In addition, BTLA could regulate T cell responses in different inflammatory areas in BD. BTLA expression on CD4⁺ Th cells could have a role in the control of inflammation in peripheral sites or organs. Autoimmune events and graft denial increase in BTLA deficiency, which indicates its possible role in autoinflammatory pathogenesis in BD. High levels of BTLA on T cells could limit increased inflammation and autoimmune events. These results indicate that BTLA plays a critical role in the maintenance of self-tolerance and prevention of autoimmune diseases; in addition, increasing BTLA through the signals of agonistic ligands could be helpful in the treatment of autoimmune diseases. Additional detailed and functional studies are necessary for further evaluation of the role of BTLA on BD.

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