

1-1-2012

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

NAFİYE FULYA İLHAN

NESRİN DEMİR

TAMER DEMİR

AHMET GÖDEKMERDAN

Follow this and additional works at: <https://journals.tubitak.gov.tr/medical>

 Part of the [Medical Sciences Commons](#)

Recommended Citation

İLHAN, NAFİYE FULYA; DEMİR, NESRİN; DEMİR, TAMER; and GÖDEKMERDAN, AHMET (2012) "BTLA (C272) expression on CD4+ T cells in Behçet patients," *Turkish Journal of Medical Sciences*: Vol. 42: No. 5, Article 5. <https://doi.org/10.3906/sag-1105-32>

Available at: <https://journals.tubitak.gov.tr/medical/vol42/iss5/5>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Medical Sciences by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

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

Nafiye Fulya İLHAN¹, Nesrin DEMİR¹, Tamer DEMİR², Ahmet GÖDEKMERDAN¹

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).

Received: 16.05.2011 – Accepted: 10.06.2011

¹ Department of Immunology, Faculty of Medicine, Firat University, Elazığ - TURKEY

² Department of Ophthalmology, Faculty of Medicine, Firat University, Elazığ - TURKEY

Correspondence: Nafiye Fulya İLHAN, Department of Immunology, Faculty of Medicine, Firat University, Elazığ - TURKEY

E-mail: fulhan23@yahoo.com

* This work was supported by the Research Foundation of Firat University (Project No: 1572). It was presented at the World Immune Regulation Meeting-III 2009, Switzerland, as a poster.

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 Firat 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: $13.01 \pm 1.51\%$, healthy controls: $2.90 \pm 0.814\%$; $P < 0.001$) and CD3⁺CD272⁺ T cell percentages (BD patients: $23.79 \pm 2.27\%$, healthy controls: $6.27 \pm 1.27\%$; $P < 0.001$) 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: $11.43 \pm 2.41\%$, remission phase: $14.11 \pm 1.95\%$; $P > 0.05$). However, the CD3⁺CD272⁺ values were significantly different in BD patients in the active and remission phases (active phase: $1.06 \pm 3.48\%$, remission phase $27.06 \pm 2.74\%$; $P < 0.05$) (Figures 1-3).

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

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

Table 2. Activation criteria in patients with active BD.

Activation criteria	Patients with active BD	
	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

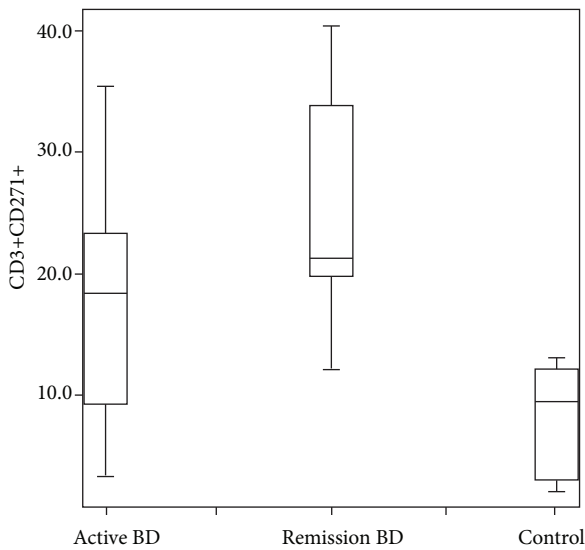


Figure 1. The distribution of BTLA levels in the BD patient and control groups.

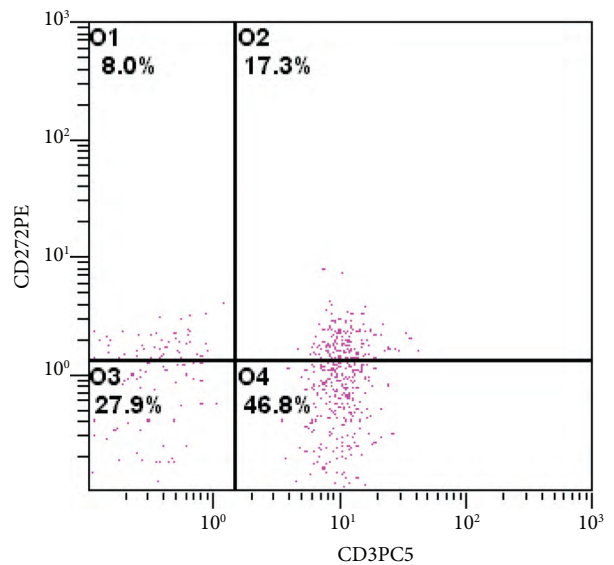


Figure 2. CD272 expression on CD3⁺ T cells in BD patients.

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).

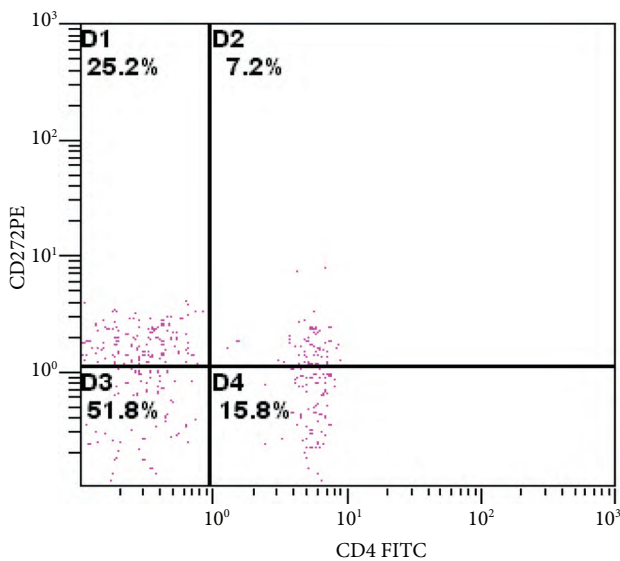


Figure 3. CD272 expression CD4⁺ T cells in BD patients.

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.

References

1. Saito T, Yamasaki S. Negative feedback of T cell activation through inhibitory adapters and costimulatory receptors. *Immunol Rev* 2003; 192: 143-60.
2. Watanabe N, Gavrieli M, Sedy JR, Yang J, Fallarino F, Loftin SK et al. BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1. *Nat Immunol* 2003; 4: 670-79.
3. Sedy JR, Gavrieli M, Potter KG, Hurchla MA, Lindsley RC, Hilder K et al. B and T lymphocyte attenuator regulates T cell activation through interaction with herpes virus entry mediator. *Nat Immunol* 2005; 6: 90-8.
4. Chang YH, Hsieh SL, Chao Y, Chou YC, Lin WW. Proinflammatory effects of LIGHT through HVEM and LTβR interactions in cultured human umbilical vein endothelial cells. *J Biomed Sci* 2005; 12: 363-75.

5. Croft M. The evolving cross talk between co-stimulatory and co-inhibitory receptors: HVEM-BTLA. *Trends Immunol* 2005; 26: 292-4.
6. Hurchla MA, Sedy JR, Gavrieli M, Drake CG, Murphy TL, Murphy KM. B and T lymphocyte attenuator exhibits structural and expression polymorphisms and is highly induced in anergic CD4⁺ T cells. *J Immunol* 2005; 174: 3377-85.
7. Krieg C, Han P, Stone R, Goularte OD, Kaye J. Functional analysis of B and T lymphocyte attenuator engagement on CD4⁺ and CD8⁺ T cells. *J Immunol* 2005; 175: 6420-7.
8. Owada T, Watanabe N, Oki M, Oya Y, Saito Y, Saito T et al. Activation-induced accumulation of B and T lymphocyte attenuator at the immunological synapse in CD4⁺ T cells. *J Leukoc Biol* 2010; 87: 425-32.
9. Deppong C, Juehne TI, Hurchla M, Friend LD, Shah DD, Rose CM et al. B and T lymphocyte attenuator and programmed death receptor-1 inhibitory receptors are required for termination of acute allergic airway inflammation. *J Immunol* 2006; 176: 3909-13.
10. Steinberg MW, Turovskaya O, Shaikh RB, Kim G, McCole DF, Pfeffer KA et al. Crucial role for HVEM and BTLA in preventing intestinal inflammation. *J Exp Med* 2008; 205: 1463-76.
11. Oya Y, Watanabe N, Owada T, Oki M, Hirose K, Suto A et al. Development of autoimmune hepatitis-like disease and production of autoantibodies to nuclear antigens in mice lacking B and T lymphocyte attenuator. *Arthritis Rheum* 2008; 58: 2498-510.
12. Hegele RG. The pathology of asthma: brief review. *Immunopharmacology* 2000; 48: 257-62.
13. Greenwald RJ, Latchman YE, Sharpe AH. Negative co-receptors on lymphocytes. *Curr Opin Immunol* 2002; 14: 391-6.
14. Deppong C, Degnan JM, Murphy TL, Murphy KM, Green JM. B and T lymphocyte attenuator, BTLA, regulates T cell survival in the lung. *J Immunol* 2008; 181: 2973-9.
15. Liu X, Alexiou M, Martin-Orozco N, Chung Y, Nurieva RI, Ma L et al. A critical role of B and T lymphocyte attenuator in peripheral T cell tolerance induction. *J Immunol* 2009; 182: 4516-20.