

# B- and T-Lymphocyte Attenuator Expression on Regulatory T-Cells in Patients with Severe Sepsis

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To the Editor: Regulatory T-cells (Tregs), a subset of CD4<sup>+</sup> T-cells, have the capacity to actively suppress immune responses and play a pivotal role in sepsis-induced immunosuppression.<sup>[1]</sup> B- and T-lymphocyte attenuator (BTLA) is a co-inhibitory receptor that is known to potentially inhibit CD4<sup>+</sup> T-cell function and to block prosurvival signaling in CD4<sup>+</sup> T-cells.<sup>[2]</sup> Tregs constitutively express BTLA. It has been reported that the absence of BTLA expression on Tregs resulted in reduced interleukin-10 production by Tregs in a model of established experimental autoimmune encephalomyelitis.<sup>[3]</sup> However, the role of BTLA expression on Tregs in patients with sepsis has rarely been investigated. In this study, we investigated the dynamic changes in BTLA expression on Tregs on days 1 and 7 during sepsis and explored the potential role of BTLA expression on Tregs. We used the combination of the surface markers CD4, CD25, and CD127 to identify Tregs.

Results are expressed as the relative percentage of BTLA-positive Tregs and as the numbers of Tregs per microliter of whole blood for absolute count data obtained by flow cytometry. In total, 101 patients with severe sepsis were enrolled in the Emergency Department of Beijing Chao-Yang Hospital from January to December 2016. In addition, 45 healthy volunteers were included as controls. A comparison of healthy controls and patients with severe sepsis revealed significant differences in the percentages of Tregs in total CD4<sup>+</sup> T-cells (mean, 4.97 vs. 6.65,  $F = 10.511$ ,  $P < 0.001$ ) and the numbers of CD4<sup>+</sup> T-cells (median, 897 vs. 430,  $Z = -7.677$ ,  $P < 0.001$ ) and Tregs (median, 44 vs. 28,  $Z = -4.032$ ,  $P < 0.001$ ). On day 1, the percentage of Tregs in total CD4<sup>+</sup> T-cells was not different between survivors and nonsurvivors in patients with severe sepsis [Table 1]. However, nonsurvivors had lower absolute Treg counts than survivors [Table 1]. By day 7, the number of Tregs increased, and the percentage of Tregs decreased in survivors [Table 1]. Although the number of Tregs increased, the percentage of Tregs progressively increased in nonsurvivors [Table 1]. Moreover, survivors had a lower percentage of Tregs and higher number of Tregs than nonsurvivors [Table 1]. At admission, the expression of BTLA on Tregs was significantly higher in patients with severe sepsis compared with that in healthy controls (median, 29.5 vs. 34.7,  $Z = -2.766$ ,  $P = 0.006$ ). However, no differences were found between patients and healthy controls when flow cytometry data were expressed as mean fluorescence

intensity (MFI) values (mean, 4.87 vs. 5.30,  $F = 0.282$ ,  $P = 0.088$ ). BTLA expression on Tregs was higher in nonsurvivors than that in survivors, as were MFI values [Table 1]. On day 7, both BTLA expression on Tregs and MFI values increased in both survivors and nonsurvivors. Moreover, BTLA expression on Tregs increased more in nonsurvivors than that in survivors [Table 1]. Similar results were observed for MFI values [Table 1]. On the contrary, the expression of BTLA on CD4<sup>+</sup> T-cells was significantly lower in patients with severe sepsis compared with that in healthy controls on day 1 (median, 77.9 vs. 68.9,  $Z = -5.961$ ,  $P < 0.001$ ). However, there were no differences in MFI values between patients and healthy controls (mean, 6.90 vs. 6.51,  $F = 3.233$ ,  $P = 0.096$ ). Similar results were observed when comparing survivors and nonsurvivors [Table 1]. On day 7, the expression of BTLA on CD4<sup>+</sup> T-cells increased in both survivors and nonsurvivors [Table 1]. However, no differences were found between survivors and nonsurvivors in flow cytometry data expressed as the percentage of BTLA/CD4<sup>+</sup> T-cells or MFI values [Table 1]. Based on the correlations between BTLA expression on Tregs and outcomes, the correlation coefficients among BTLA expression on Tregs, Sequential Organ Failure Assessment (SOFA) scores, and CD4<sup>+</sup> T-cell counts were evaluated in patients with sepsis at two time points. There was a positive correlation between BTLA expression on Tregs and SOFA scores and an inverse correlation between BTLA expression on Tregs and CD4<sup>+</sup> T-cell counts [Figure 1].

There is predominance of an initial hyperinflammatory phase after sepsis initiation.<sup>[4]</sup> In present study, there was lower BTLA expression on CD4<sup>+</sup> T-cells in patients with sepsis compared with that in healthy controls. Moreover, BTLA expression on CD4<sup>+</sup> T-cells was lower in nonsurvivors than that in survivors on day 1. The reduction in BTLA expression on CD4<sup>+</sup> T-cells may

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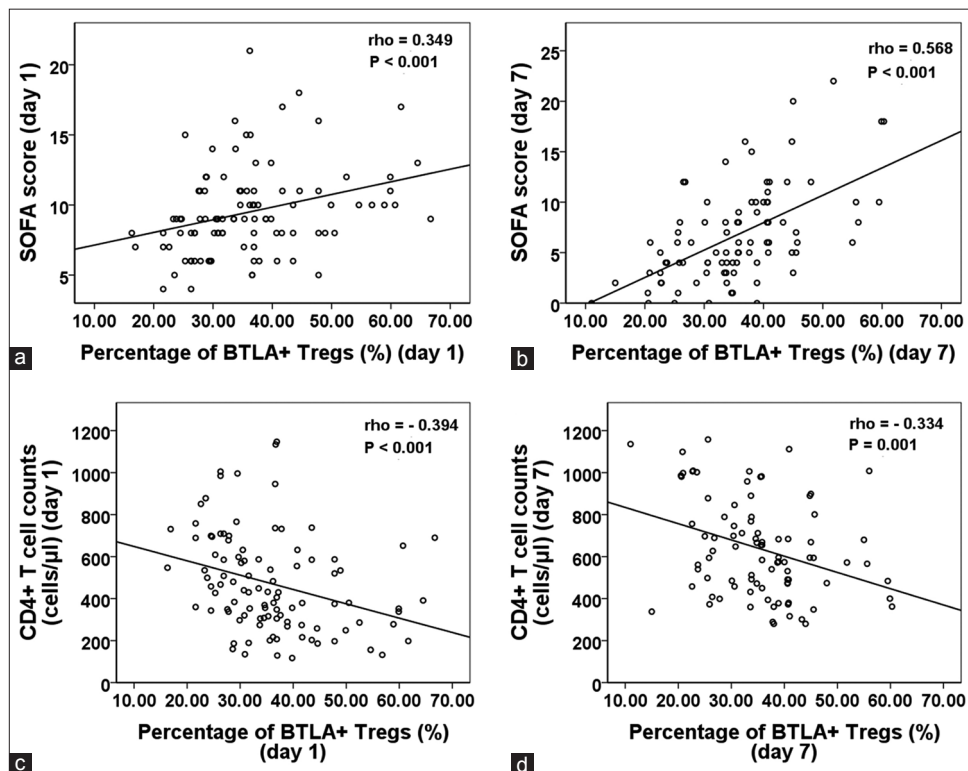
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**Table 1: Comparison of BTLA expression on Tregs and CD4+ T-cells between the survivors and the nonsurvivors on days 1 and 7**

Parameters	Day 1		Statistics	P	Day 7		Statistics	P
	Survivors (n = 65)	Nonsurvivors (n = 36)			Survivors (n = 65)	Nonsurvivors (n = 24)		
Tregs in CD4+ T-cells (%)	6.71 ± 2.49	6.99 ± 3.17	-0.128*	0.898	5.94 ± 1.55	7.51 ± 1.61	-3.789*	<0.001
Number of Treg cells (cells/μl)	31.0 (21.0–47.0)	22.0 (15.0–35.0)	-2.589†	0.010	39.0 (30.0–50.5)	32.0 (21.0–39.8)	-3.067†	0.002
BTLA + Tregs (%)	31.6 (26.3–39.4)	36.8 (30.6–47.0)	-2.457†	0.014	33.7 (25.6–38.9)	40.7 (37.1–45.0)	-3.662†	<0.001
MFI of BTLA + Tregs	5.03 ± 1.18	5.78 ± 1.65	-2.393*	0.020	5.49 ± 1.14	6.26 ± 0.98	-2.924*	0.004
Number of CD4+ T-cells (cells/μl)	534.0 (351.0–697.0)	327.0 (209.3–389.3)	-3.861†	<0.001	683.0 (540.0–894.5)	387 (360.3–521.2)	-5.441†	<0.001
BTLA + CD4+ T-cells (%)	71.6 (66.1–76.4)	64.5 (59.6–73.0)	-3.560†	<0.001	72.8 (68.9–77.9)	71.1 (68.0–78.7)	-0.356†	0.722
MFI of BTLA + CD4+ T-cells	6.48 ± 1.47	6.57 ± 1.22	-0.318*	0.751	6.09 ± 0.88	6.38 ± 1.02	-1.295*	0.199

\*F values; †Z values. Normally distributed data were reported as means and Standard deviation. Comparisons between two groups were performed using independent samples *t*-test. Nonnormal distributions of continuous variables were expressed as medians and interquartile range. Comparisons between two groups were carried out using the Mann-Whitney *U*-test. Tregs: Regulatory T-cells; BTLA: B- and T-lymphocyte attenuator; MFI: Mean of fluorescence intensity.



**Figure 1:** Correlations of the percentage of BTLA+ Tregs with SOFA score and CD4+ T-cell counts on days 1 and 7. Spearman correlation test was used between factors. (a) Correlation of the percentage of BTLA+ Tregs and SOFA score on day 1. (b) Correlation of the percentage of BTLA+ Tregs and SOFA score on day 7. (c) Correlation of the percentage of BTLA+ Tregs and CD4+ T-cell counts on day 1. (d) Correlation of the percentage of BTLA+ Tregs and CD4+ T-cell counts on day 7. BTLA: B- and T-lymphocyte attenuator; Tregs: Regulatory T-cells; SOFA: Sequential Organ Failure Assessment.

contribute to the activation of CD4+ T-cells in the early stage of sepsis.<sup>[5]</sup> Meanwhile, the increase in the percentage of Tregs was accompanied by the initiation of the pro-inflammatory response, which suggests that the immune system works to maintain a balance between effector T-cells and Tregs during sepsis. However, BTLA expression on CD4+ T-cells was upregulated to block T-cell

activation over time, which may lead to immunosuppression during the progression of sepsis.

The most important finding is that BTLA expression on Tregs remained high in patients with sepsis compared with that in healthy controls. Furthermore, BTLA expression on Tregs progressively increased in patients with severe sepsis on day 7. Moreover,

nonsurvivors had higher BTLA expression on Tregs than survivors on days 1 or 7. However, the exact mechanisms of the regulation of BTLA expression on Tregs remain unknown. A study by Tao *et al.*<sup>[6]</sup> showed that BTLA<sup>-/-</sup> CD4<sup>+</sup> CD25<sup>+</sup> Tregs had normal suppressive activity. Albring *et al.*<sup>[7]</sup> demonstrated that Tregs maintain low levels of BTLA after activation compared with CD4<sup>+</sup> T-cells and that anti-BTLA treatment increases the number and frequency of Tregs after allogeneic hematopoietic stem cell transplantation. The present study showed that BTLA<sup>+</sup> Tregs were inversely correlated with CD4<sup>+</sup> T-cell counts. The increased BTLA expression on Tregs may contribute to enhance the suppression function of Tregs or the conversion of these cells in patients with sepsis. Thus, the mechanism of BTLA expression on Tregs merits further investigation. Another intriguing finding was that BTLA/Tregs were positively correlated with the severity of severe sepsis in patients, as assessed by SOFA scores. Moreover, nonsurvivors had higher BTLA expression on Tregs than survivors. These patterns of changes in BTLA expression on Tregs may also contribute to the immunosuppression observed in patients with sepsis. Several studies have demonstrated that BTLA/CD4<sup>+</sup> T-cells are associated with subsequent nosocomial infections and the severity of sepsis.<sup>[5,8]</sup> Further studies are needed to fully elucidate the use of BTLA as a potential biomarker or potential therapeutic target. In conclusion, BTLA expression on Tregs increased in patients with sepsis over time. Moreover, increased expression of BTLA on Tregs was correlated with the severity of sepsis. Additional studies are needed to fully elucidate the mechanisms through which BTLA expression on Tregs is regulated and to determine the specific roles of BTLA expression in these cells.

### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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### Conflicts of interest

There are no conflicts of interest.

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