Short Report: Elevation of Serum B-Cell Activating Factor Levels During Visceral Leishmaniasis

Yasuyuki Goto,*† Satoko Omachi,† Chizu Sanjoba, and Yoshitsugu Matsumoto

Laboratory of Molecular Immunology, Department of Animal Resource Sciences, Graduate School of Agricultural and Life Sciences,
The University of Tokyo, Tokyo, Japan

Abstract. Elevation of serum B-cell activating factor (BAFF) is one of the characteristics of immunological disorders, including autoimmunity, but the levels of BAFF in infectious diseases have not been studied well. Here, we showed the elevation of serum BAFF in patients with visceral leishmaniasis (VL). The mean serum BAFF value in VL patients (4.65 ng/mL) was 4.3 times higher than that of healthy controls (1.08 ng/mL), and 90% of VL patients showed serum BAFF above the cutoff that was calculated as the mean + 3 SDs of the controls. This report is the first on elevation of serum BAFF during VL.

Leishmaniasis is a spectrum of diseases caused by protozoan parasites of the genus *Leishmania*. Visceral leishmaniasis (VL) is the most severe form, generally caused by *L. donovani* and *L. infantum*. Clinical manifestations of VL include fever, anemia, and splenomegaly, and the disease is fatal if left untreated. Protective immunity against VL is associated with antigen-specific cell-mediated responses represented by lymphoproliferation and delayed-type hypersensitivity¹ and production of type 1 helper T cell (Th1) cytokines, like interferon- γ (IFN- γ) and interleukin-2 (IL-2), on antigen recall.² In contrast, IL-10, which is associated with T-cell hyporesponsiveness, is the predominant cytokine during active VL.³ Other than the suppressed Th1 responses, immunological characteristics of active VL include strong humoral responses. In fact, high immunoglobulin G (IgG) antibodies and delayed type hypersensitivity to parasite antigens are dichotomic factors in VL,⁴ and excess B-cell activation in those patients can also be presumed by the manifestation of hypergammaglobulinemia.⁵

B-cell activating factor (BAFF), also known as B lymphocyte stimulator (BLyS), tumor necrosis factor- and ApoL-related leukocyte expressed ligand 1 (TALL-1) and tumor necrosis factor ligand superfamily 13B (TNFSF13B), is a critical regulator of B-cell development and differentiation.⁶ Although the molecule is indispensable in maintaining B-cell functions, aberrant expression of BAFF is associated with autoimmune diseases.^{7,8} Because B-cell activation is also the characteristic of VL, it is speculated that BAFF is associated with the disease. This study examined serum levels of BAFF in VL patients for the first time and showed the elevation of serum BAFF levels in those patients.

Serum samples from VL patients ($N = 20$), Chagas' disease patients ($N = 10$), and healthy endemic controls (HCs; $N = 9$) were collected with informed consent from each donor at Prof. Edgard Santos University Hospital, Universidade Federal da Bahia, Salvador, Bahia, Brazil. Collection and usage of these human samples were approved by the Institutional Review Board Ethical Committee at Universidade Federal da Bahia. All samples were coded and archived at the hospital, and personal information of the donors, including the name, sex, and age, was not available with the exception of the

sample identification describing the classification of the donor group (i.e., VL, Chagas' disease, etc.). This study was approved by the Institutional Review Board at The University of Tokyo (Approval No. 11-63).

Quantification of serum BAFF levels was performed using the Human BAFF/BLyS/TNFSF13B Quantikine ELISA Kit (R&D Systems, Inc., Minneapolis, MN). Quantification of serum IgG levels was performed using Human IgG Total ELISA Ready-SET-Go! (eBioscience, Inc., San Diego, CA). Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA). Gaussian distribution of each test group was examined by D'Agostino and Pearson omnibus normality tests. Differences in serum levels of BAFF and IgG among groups were analyzed by either one-way analysis of variance (ANOVA) coupled with Bonferroni's multiple comparison test or Kruskal–Wallis test coupled with Dunn's multiple comparison test based on the nature of the analyzed dataset. Spearman's rank correlation test was used for a correlation analysis between serum levels of IgG and BAFF.

Serum BAFF levels of HC ranged from 0.504 to 1.48 ng/mL with the mean $\pm$ SD of 1.08 ± 0.34 ng/mL (Figure 1A). Those of VL patients ranged from 2.01 to 8.33 ng/mL with the mean $\pm$ SD of 4.65 ± 2.16 ng/mL. Those of Chagas' disease patients ranged from 0.519 to 2.88 ng/mL with the mean $\pm$ SD of 1.19 ± 0.672 ng/mL. The levels of VL patients were statistically higher than those of HC and Chagas' disease patients ($P < 0.0001$), whereas there was no statistical difference between HC and Chagas' disease patients ($P > 0.05$). When the mean + 3 SDs of HC was set as a cutoff (2.089 ng/mL), 90% of VL patients showed elevated serum BAFF, whereas 10% of Chagas' diseases patients showed elevated serum BAFF. The magnitude of elevation found in VL patients (odds ratio = 4.3) was equivalent to or higher than that previously reported for other diseases (Table 1).^{9–12} Those diseases include systemic lupus erythematosus, rheumatoid arthritis, and Sjögren's syndrome, where inhibitors of BAFF signaling, such as belimumab and blisibimod, are approved and/or being evaluated for treatment. In this study, significant elevation of serum BAFF was not observed in Chagas' disease patients. Although a recent study showed the elevation of serum BAFF in mice infected with *Trypanosoma cruzi*, the causative agent of Chagas' disease, the magnitude of increase was less than twofold.¹³ Together, activation of BAFF signaling may be more prominent in VL than Chagas' disease.

VL patients also showed high levels of serum IgG (Figure 1B). The mean $\pm$ SD values of serum IgG in VL patients, Chagas' disease patients, and HCs were 24.97 ± 18.34 , 10.26 ± 4.660 ,

* Address correspondence to Yasuyuki Goto, Laboratory of Molecular Immunology, Department of Animal Resource Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. E-mail: aygoto@mail.ecc.u-tokyo.ac.jp

† These authors contributed equally.

FIGURE 1. Elevated serum levels of BAFF in VL patients. Serum levels of (A) BAFF and (B) total IgG in VL patients, Chagas' disease patients, and HCs were measured by sandwich enzyme-linked immunosorbent assay. Solid lines represent the mean value of individual groups. Dotted lines represent cutoff values calculated as the mean + 3 SDs of HC. (C) Correlation between serum levels of BAFF and total IgG in the tested individuals. Dotted lines represent cutoff values calculated as the mean + 3 SDs of HC.

and 4.677 ± 2.609 mg/mL, respectively. The levels in VL patients were statistically higher than those in Chagas' disease patients ($P < 0.05$) and HCs ($P < 0.01$), whereas no difference was found between Chagas' disease patients and HCs ($P > 0.05$). When the mean + 3 SDs of HC was set as a cutoff (12.50 mg/mL), 75% of VL patients showed elevated serum IgG, whereas 30% of Chagas' diseases patients did. Although other classes of Igs, such as IgM and IgA, were also elevated in VL patients, the degree was much less significant than IgG (data not shown). The majority of such high levels of IgG in VL patients seemed to be induced in an antigen-specific manner rather than non-specific pan-B-cell activation, because both VL and Chagas' disease patients showed selective antibody responses to species-specific antigens.^{14,15} Although the majority of VL patients had high levels of both BAFF and IgG, a clear positive correlation between those values within the patient group was not found ($P > 0.05$ by Spearman's rank correlation) (Figure 1C). The elevation of both IgG and BAFF is not restricted by the geographical backgrounds or the infecting *Leishmania* species, because the elevation was also found in VL patients from Bangladesh, where *L. donovani* is a causative species (Omachi S and others, unpublished data). However, a clear positive correlation between IgG and BAFF within VL patients was not found in either region. In contrast, we have observed a statistically significant positive correlation between serum IgG and BAFF during *L. donovani* infection in mice

(Omachi S and others, unpublished data). A previous report has shown the elevation of serum Igs, with an emphasis on IgG, in BAFF transgenic mice.¹⁶ Taken together, BAFF may be involved in hyper-IgG syndrome during VL but is not the sole factor in a clinical setting. In fact, BAFF synergizes with other cytokines, resulting in different outcomes on B-cell activation compared with the sole molecule.¹⁷

Only a few studies have examined serum BAFF levels in patients with infectious diseases. In malaria, BAFF levels were higher in patients with more severe disease and decreased during recovery from the acute episode.¹¹ Such a decrease in BAFF was also observed in treated individuals with experimental malaria infection.¹⁸ In both studies, parasitemia did not show a clear correlation with BAFF levels, suggesting that BAFF is an indicator for disease severity rather than parasite burden. Those studies also indicate that the molecule can be used for a diagnostic/prognostic marker for malaria.^{11,18} For human VL, a scoring system for disease severity has not been well-established. Consequently, it is still unclear whether BAFF levels in VL patients also increase with disease severity. However, we observed a decrease in serum BAFF by treating *L. donovani*-infected mice with AmBisome (Sumitomo Dainippon Pharma Co., Ltd., Osaka, Japan; Omachi S and others, unpublished data). Therefore, it is intriguing to examine if BAFF is a useful diagnostic/prognostic marker for VL and how the molecule is involved in pathogenesis of the disease.

TABLE 1
Degree of BAFF elevation in various diseases

Disease	Patient (ng/mL)	Control (ng/mL)	Ratio	P value	Reference
Visceral leishmaniasis	4.65 ± 2.16	1.08 ± 0.34	4.3	$P < 0.0001$	This study
Chagas' disease	1.19 ± 0.67	1.08 ± 0.34	1.1	NS	This study
Malaria	5.8	2.6	2.2	$P < 0.0001$	11
Systemic lupus erythematosus	10.74 ± 1.04	4.48 ± 0.45	2.4	$P < 0.0001$	7
Systemic lupus erythematosus	12.7 ± 24.4	10.4 ± 13	1.2		8
Systemic lupus erythematosus	1.63	0.78	2.1	$P < 0.001$	12
Rheumatoid arthritis	6.68 ± 0.43	4.48 ± 0.45	1.5	$P < 0.001$	7
Rheumatoid arthritis	23 ± 47	10.4 ± 13	2.2		8
Sjögren's syndrome	53 ± 67	10.4 ± 13	5.1		8
Systemic sclerosis	1.26	0.78	1.6	$P < 0.001$	12
Follicular lymphoma	13.4 ± 5.6	4.6 ± 0.7	2.9	$P < 0.0001$	9
Waldenström macroglobulinemia	13.66 ± 2.75	6.68 ± 2.64	2.0	$P < 0.01$	10

NS = not significant.

Received April 27, 2014. Accepted for publication July 20, 2014.

Published online August 25, 2014.

Acknowledgments: The authors thank Dr. Eduardo Netto (Laboratório de Pesquisa em Infectologia, Hospital University, Prof. Edgard Santos Universidade Federal da Bahia) for providing the serum samples.

Financial support: This study was supported by KAKENHI Grants 23689024 and 23256001 from the Japan Society for the Promotion of Science and a grant from the Japan Science and Technology Agency/ the Japan International Cooperation Agency, SATREPS.

Authors' addresses: Yasuyuki Goto, Satoko Omachi, Chizu Sanjoba, and Yoshitsugu Matsumoto, Laboratory of Molecular Immunology, Department of Animal Resource Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan, E-mails: aygoto@mail.ecc.u-tokyo.ac.jp, satoko.bbk@gmail.com, asanjoba@mail.ecc.u-tokyo.ac.jp, and aysmatsu@mail.ecc.u-tokyo.ac.jp.

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