Role of B-lymphocyte activating factor (BAFF) in the pathogenesis of systemic lupus erythematosus

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Background
B-lymphocyte activating factor (BAFF) is a new member of the tumor necrosis factor family that promotes B-cell survival, acting as an antiapoptotic factor and thus contributing to the development of autoimmune disease.

Objectives
The aim of the study was to investigate the role of BAFF in the pathogenesis of systemic lupus erythematosus (SLE) by correlating its serum levels to different clinicopathological indices of disease activity.

Methods
This is a prospective study that was conducted on 20 female patients with SLE. Ten healthy controls of matching age and sex were also included in this study. All patients were subjected to full history taking and clinical examination upon presentation, and the following laboratory parameters were evaluated: complete blood picture, erythrocyte sedimentation rate, serum (creatinine, blood urea nitrogen), and complete urine analysis (anti nuclear antibody, anti-dsDNA, C3, C4 in serum). Serum BAFF levels were measured by enzyme-linked immunosorbent assay in all patients. Renal biopsy was performed whenever necessary.

Results
Serum BAFF levels were significantly higher in patients with active SLE than in controls ($P<0.05$). These levels also correlated positively with systemic lupus erythematosus disease activity index (SLEDAI) in a highly significant manner ($P<0.001$). Correlating serum BAFF among patients with photosensitivity and symptoms of central nervous system affection proved to be highly significant ($P<0.001$). In addition, within this study, serum BAFF levels correlated positively with ESR levels among patients and negatively with both C3 and C4 in a significant manner ($P<0.05$ and 0.001, respectively).

Conclusion
Serum BAFF levels were significantly higher among patients with active SLE than among controls. It correlated in a negative manner with both C3 and C4 – significantly with C3 and highly significantly with C4. BAFF levels also correlated with SLEDAI in a highly significant manner, implicating B-cell immunoglobulin production and immune complex formation in the disease activity of lupus patients.

Keywords:
B-lymphocyte activating factor, B-lymphocytes, systemic lupus erythematosus

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Introduction

The pathologic findings of systemic lupus erythematosus (SLE) are seen throughout the body and are manifested by inflammation and blood vessel abnormalities that encompass vasculopathy and vasculitis with immune complex formation and deposition. Antibodies formed may be driven by self-antigens implicating a more generalized immune cell dysfunction, which promotes B-cell hyperactivity [1]. The immunological events triggering the onset of clinical manifestations in SLE have not yet been fully defined, but a central role for B cells in the pathogenesis of this disease has been identified. This includes abnormal expression or function of key signaling molecules and dysregulation of cytokines with key B-cell effects, ultimately influencing B-cell tolerance, rendering both antibody-dependent and antibody-independent mechanisms of B-cell activation important in SLE [2]. The B-lymphocyte activating factor (BAFF) is a key survival factor for B cells and maintains their survival by reducing apoptotic clearance [3]. It belongs to the tumor necrosis factor ligand superfamily comprising B-cell maturation antigen, transmembrane activator and calcium modulator and cyclophilin ligand interactor, and BAFF receptor (BAFF-R); the dysregulation of the BAFF/BAFF-R system is

believed to contribute to the induction and development of autoimmune disease and is an important therapeutic target [4]. It exists in both membrane-bound and soluble forms and was found to be specifically expressed on cells of myeloid lineage and to selectively stimulate B-lymphocyte proliferation and immunoglobulin production [5]. Levels of both the membrane-associated and soluble forms were found to be regulated by cytokines such as interferon-γ and to a lesser extent by interleukin-10 (IL-10) [6].

Patients and methods

This is a prospective study that was conducted on 20 female patients with SLE attending the rheumatology and rehabilitation outpatient clinic and the Internal medicine department at Ain Shams University hospitals. Their ages ranged from 16 to 47 years with a mean age of 28.85 ± 9.626 years. Ten healthy controls of matching age and sex were also included in this study with an age range of 23–40 years with a mean of 30.45 ± 7.342 years.

All patients were subjected to:

(1) thorough history taking and detailed clinical examination;
(2) routine laboratory investigations such as:
   (a) determination of the complete blood picture by the coulter method and erythrocyte sedimentation rate (ESR) by the westergreen method;
   (b) analysis of serum creatinine and blood urea nitrogen levels;
   (c) determination of serum C3 and C4 by rate nephelometry and anti nuclear antibody and anti-dsDNA by the indirect immunofluorescence test;
   (d) urine analysis including (urinary albumin, urinary red blood cells, and urinary white blood cells);
   (e) measurement of serum BAFF levels by enzyme-linked immunosorbent assay and
   (f) renal biopsy when indicated.

Statistical methodology

Data were analyzed using the statistical program for social science, version 12 (SPSS 15.0.1 for windows; SPSS Inc., Chicago, Illinois, USA) and all variables were expressed as mean ± SD; the following tests were used:

(1) Student's *t*-test to compare between two independent means.
(2) Analysis of variance: to test the difference between mean values of some parameters among multiple group.
(3) Correlation matrix and coefficient of correlation *r* for determining the relationship of different variables against each other by Pearson's method:
   (a) $P > 0.05$ = insignificant.
   (b) $P < 0.05$ = significant.
   (c) $P < 0.001$ = highly significant.

Results

Sixteen patients (80%) presented with anemia, two patients (10%) with leukopenia, and another two (10%) patients presented with thrombocytopenia. All patients had elevated ESR levels in the first hour and complementary low serum C3 levels, whereas 19 patients had low serum C4 levels (Tables 1–3).

Out of the 20 patients, 17 had undergone renal biopsy showing that three patients had grade II lupus nephritis (LN), six patients had grade III LN, and eight patients had grade IV LN (Figs 1–2).

There was a statistically highly significant increase in serum BAFF levels in SLE patients in comparison with controls. (Fig. 3). There was significant negative linear correlation and highly significant negative linear between BAFF levels and C3 ($P < 0.05$, $r = -0.456$) and C4 ($P < 0.05$, $r = -0.507$) (Figs 4–5), respectively.

Discussion

SLE is a prototype systemic, autoimmune inflammatory disease that can affect virtually any organ system. The pathogenesis behind the disease remains unclear. The main immunological feature is uncontrolled formation of autoantibodies, leading to excess formation of immune complexes that deposit in different tissues, causing inflammation and tissue damage [7]. BAFF is a new member of the tumor necrosis factor family that promotes B-cell survival and acts as an antiapoptotic factor, thus contributing to the development of autoimmune diseases [8]. In this study, upon

Table 1 Clinical parameters of the studied group

	n (%)	
Clinical features	Number of affected patients (positive)	Number of nonaffected patients (negative)
Fever	9 (45)	11 (55)
Fatigue	16 (80)	4 (20)
Alopecia	18 (90)	2 (10)
Malar rash	19 (95)	1 (5)
Discoid rash	12 (60)	8 (40)
Photosensitivity	17 (85)	3 (15)
Reynaud's phenomenon	11 (55)	9 (45)
Mucosal ulcers	11 (55)	9 (45)
Arthralgia	16 (80)	4 (20)
Peripheral vascular changes	3 (15)	17 (85)
Cerebrovascular stroke	1 (95)	19 (95)
CNS affection	8 (40)	12 (60)
Pleural effusion	10 (50)	10 (50)
Pericardial effusion	5 (25)	15 (75)
Hepatomegaly	7 (35)	13 (65)
Visual disturbance	2 (10)	18 (90)
Renal affection	19 (95)	1 (5)

CNS, central nervous system.

Table 2 Distribution of patients according to SLEDAI score

SLEDAI score	13–24	25–34	35–46
Number of patients	7	5	8

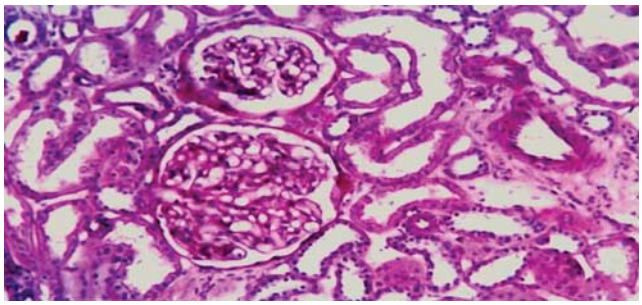
SLEDAI, systemic lupus erthematosus disease activity index.

Table 3 Comparison between patients with and without different clinical manifestations regarding BAFF levels

	Positive cases		Negative cases				
Clinical manifestation	*N*	BAFF (mean ± SD)	*N*	BAFF (mean ± SD)	*t*	*P*	Significance
Fever	9	1633 ± 1291	11	2169 ± 1161	0.976	> 0.05	NS
Fatigue	16	2085 ± 1264	4	1300 ± 875	− 1.162	0.260	NS
Alopecia	18	1942 ± 1286	2	1800 ± 282	− 0.392	0.705	NS
Malar rash	19	1987 ± 1222	1	800	− 0.947	0.356	NS
Discoid rash	12	2300 ± 1486	8	1680 ± 997	− 1.035	0.323	NS
Photosensitivity	17	2138 ± 1204	3	733 ± 57	− 4.781	0.001	HS
Reynaud's phenomenon	11	1923 ± 1248	9	1933 ± 1257	0.017	0.986	NS
Mucosal ulcers	11	1872 ± 1039	9	1995 ± 1473	0.218	0.830	NS
Arthritis	16	1997 ± 1183	4	1650 ± 1511	− 0.500	0.623	NS
Peripheral vascular changes	3	1666 ± 305	17	1974 ± 1318	0.842	0.413	NS
Cerebrovascular stroke	1	3600	19	1840 ± 1185	− 1.447	0.165	NS
CNS affection							
Headache	7	2857 ± 1181	13	1427 ± 938	− 2.971	0.008	HS
Psychosis	1	3200	19	1861 ± 1214	− 1.075	0.297	NS
Seizures	4	3550 ± 838	16	1522 ± 930	− 3.96	0.001	HS
Lung affection	10	2130 ± 1503	10	1726 ± 887	− 0.732	0.474	NS
Cardiac affection	5	1220 ± 1121	15	2164 ± 1191	0.625	0.138	NS

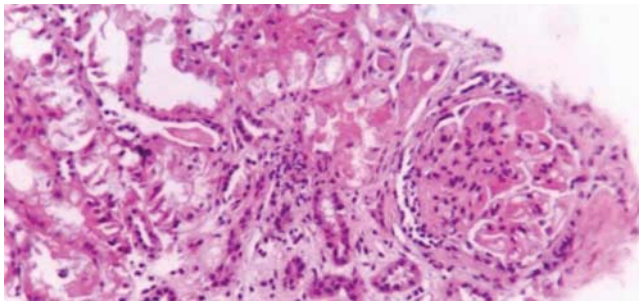
BAFF, B-lymphocyte activating factor; CNS, central nervous system; HS, highly significant; NS, nonsignificant; S, significant.

Figure 1



Focal proliferative lupus nephritis (class IIIa) with foci of glomerular hypercellularity as well as both endothelial and mesangial cell expansion with focal tubular and interstitial changes.

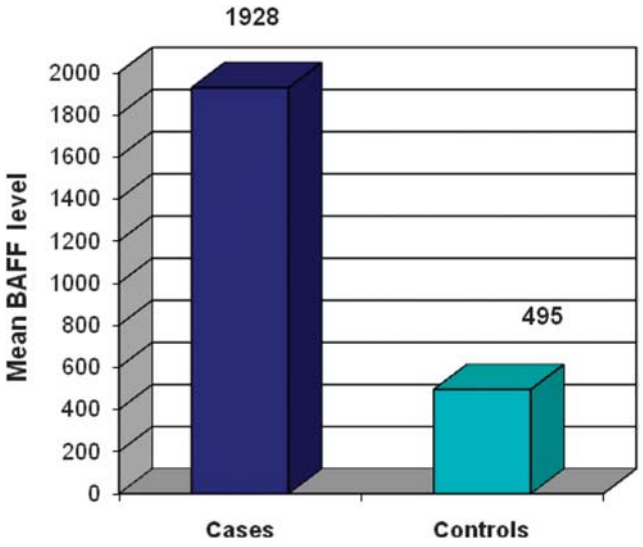
Figure 2



Diffuse proliferative lupus nephritis (class IVb) showing diffuse glomerular hypercellularity with obliteration of capillary lumina, crescent formation, and sclerosing, atrophic, and fibrosing lesions that extend into the tubulointerstitium.

comparing serum levels of BAFF between SLE patients and controls, there was a statistically highly significant difference ($P < 0.001$) with a cutoff value in our study of 985 pg/ml. These results were in agreement with those of Stohl *et al.* [9] and Carter and colleagues, as they proved that BAFF phenotypes in SLE patients were significantly higher than in controls ($P < 0.001$) [10].

Figure 3

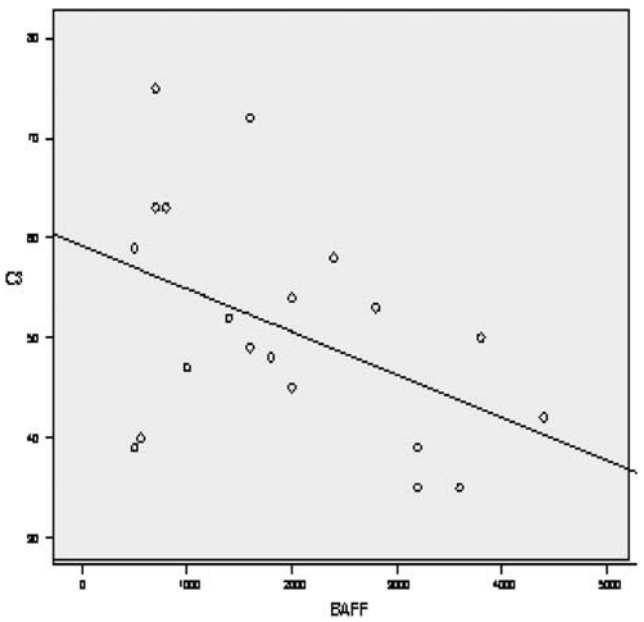


Mean BAFF levels in the studied groups. BAFF, B-lymphocyte activating factor.

In a study by Morimoto and colleagues patients were analyzed by flow cytometry to examine the intracellular expression of BAFF in CD4 + and CD8 + T cells; the investigators found that CD4 + and CD8 + T cells from patients with active SLE expressed intracellular BAFF, whereas those from normal individuals did not, proving that BAFF may play a pathogenic role in SLE by stimulating T-cell-dependent B-cell autoantibodyproduction. Within the same study, patients with LN had significantly higher levels of intracellular BAFF on CD4 + and CD8 + T cells in comparison with SLE patients with no renal involvement [11].

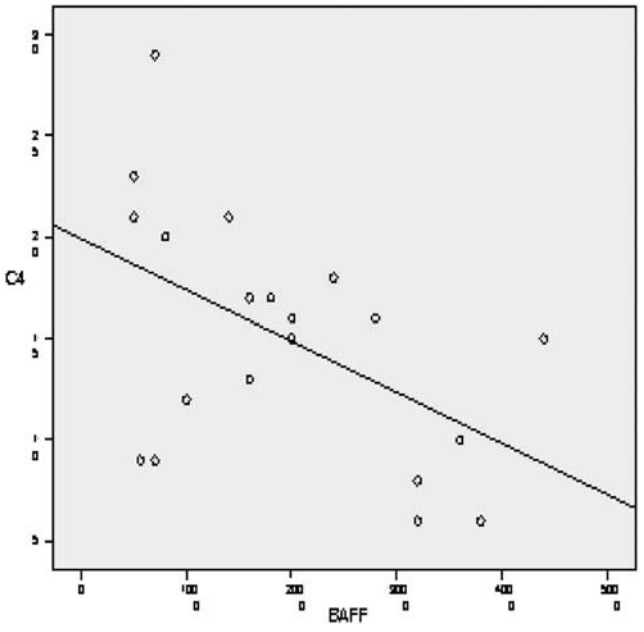
In this study, there was a significant negative correlation between serum BAFF and C3 levels and a highly significant negative correlation between BAFF and C4 levels. These results do agree with those reported by Petri and colleagues that the incidences of immunologic

Figure 4



Significant negative linear correlation between BAFF levels and C3 ($P<0.05$, $r=-0.456$). BAFF, B-lymphocyte activating factor.

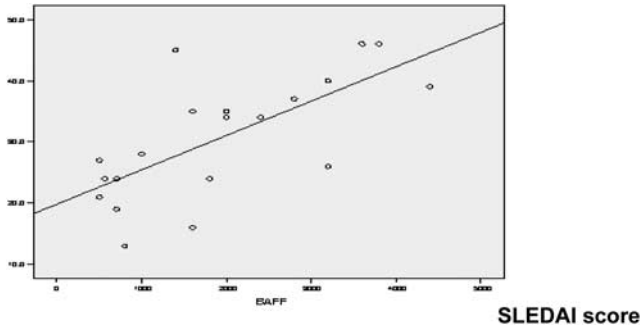
Figure 5



Highly significant negative linear correlation between BAFF levels and C4 ($P<0.05$, $r=-0.507$). BAFF, B-lymphocyte activating factor.

disorders were significantly correlated to serum BAFF levels [12]. Further, on performing a correlation matrix, a statistically highly significant correlation was found between serum levels of BAFF and systemic lupus erythematosus disease activity index (SLEDAI) score. These results were also reported by Carter *et al.* [10], as they proved that increased circulating levels of BAFF correlated with increased disease activity by measuring exogenous BAFF binding to B cells in patients with SLE

Figure 6



Highly significant positive correlation between BAFF levels and SLEDAI score ($r=0.690$, $P<0.001$). BAFF, B-lymphocyte activating factor; SLEDAI, systemic lupus erythematosus disease activity index.

and controls. Also, Ju and colleagues had reported that SLE patients with an SLEDAI score of at least 9.1 had significantly higher levels of BAFF, BAFF-R, and mRNA compared with inactive SLE patients with an SLEDAI score less than 9.0 with *P* value less than 0.05 [13]. This also agrees with the results reported by Becker-Merok *et al.* [3], Morimoto *et al.* [11], and Petri *et al.* [12].

Although in this study there was no statistical significance on correlating serum levels of BAFF in patients with clinical features of organ involvement (arthritis, vasculitis, and cardiac, pulmonary, and renal affection), these findings were similar to those of Becker-Merock and colleagues and Stohl and colleagues, with the exception that in this study, upon correlating symptoms of central nervous system involvement such as headache and seizures with serum BAFF levels among patients, the results were highly significant. Such nonsignificance of organ involvement in this study, as well as in the studies by Stohl and colleagues and Badr ELdin and colleagues, may be attributed to the fact that tissue destruction with lupus activity is not only immune mediated but could also be due to the inflammatory and cytotoxic effects of activated CD + 4 T cells and their released cytokines such as IL-12 [9,14].

In contrast, Zhang and colleagues assumed that there was no correlation between increased BAFF levels and SLE activity. This difference may be due to their assessment for disease activity using the systemic lupus activity measure index (SLAM score) [15].

Also, Stohl *et al.* [9] had proved that clinical disease activity (measured by the SLEDAI) at any point in time was not reliably correlated to the serum BAFF level or to the blood BAFF mRNA level, explaining that, as BAFF has no known direct or immediate proinflammatory properties, increased or decreased serum BAFF levels at any point in time should not be expected to acutely promote increases or decreases in systemic or organ-specific inflammation, which would be reflected in the SLEDAI score.

Interestingly, in the study by Yoshimoto and colleagues, it seems that, as previously observed, BAFF-R expression is

normal or decreased at the surface of B cells. Thus, if there is a decrease in BAFF-R expression on B cells (even if the BAFF level is increased), this BAFF increase cannot induce B-cell activation directly [16]. Yoshimoto *et al*. [16] and Mariette [17] propose an alternative mechanism: in the context of autoimmunity, the effect of BAFF on B-cell activation could be at least partly mediated by a BAFF-driven autocrine secretion of IL-6 by monocytes [17].

In contrast to our findings, Collins and colleagues stated that there is a weak correlation between circulating BAFF levels and disease activity in human SLE, and that disease activity in SLE patients is insensitive to the degree of BAFF overproduction. They explained this by reporting that disease activity in SLE is not solely driven by B cells; systemic inflammation and SLE flares can be triggered through B-cell-independent means [18]. Interestingly, Doreau *et al*. [19] reported in patients with lupus, it has been shown that B-cell activation was dependent on the combination of BAFF and IL-17 [19].

Conclusion

Serum (BAFF) levels were significantly higher among patients with active SLE than among controls, and its correlation with SLEDAI in a highly significant manner reflects its role in the pathogenesis of SLE and in inducing disease exacerbations. BAFF levels also correlated with both C3 and C4 – in a significant manner with C3 and in a highly significant manner with C4 – implicating B-cell immunoglobulin production and immune complex formation in the disease activity of lupus patients.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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