

# Increased serum B cell-activating factor level in children with atopic dermatitis

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## Summary

**Background.** B cell-activating factor (BAFF) is a tumour necrosis factor superfamily member best known for its role in the survival and maturation of B cells. BAFF activity is seen in naïve and effector/memory T cells.

**Aim.** To investigate the level and role of BAFF in serum of patients with atopic dermatitis (AD).

**Methods.** Levels of serum BAFF, a proliferation-inducing ligand (APRIL) and total serum IgE level, and total eosinophil count were measured in 245 children.

**Results.** Patients were characterized as having atopic eczema (AE) ( $n = 90$ ) or non-AE ( $n = 77$ ); the remainder were healthy control subjects ( $n = 78$ ). Serum BAFF level in children with AE ( $1625.04 \pm 708.32$  pg/mL) was significantly higher than in non-AE children ( $1194.69 \pm 448.44$  pg/mL,  $P < 0.0001$ ) or healthy controls ( $1062.89 \pm 444.74$  pg/mL,  $P < 0.0001$ ). Serum APRIL level was not different between the three groups. Serum BAFF level significantly correlated with total serum IgE level ( $\gamma = 0.42$ ,  $P < 0.0001$ ) and total eosinophil count. It was also positively correlated with serum BAFF and egg-specific IgE level ( $\gamma = 0.252$ ,  $P = 0.045$ ) in AE.

**Conclusions.** Serum BAFF level is high in AE and might be a useful marker for AE.

## Introduction

Atopic dermatitis (AD) is a chronic inflammatory pruritic skin disease that often presents with flares of disease and erythematous papules associated with excoriation and serous exudation,<sup>1–3</sup> and is often associated with increased serum IgE level and eosinophilia.<sup>4</sup> In addition, it is well known that T cells contribute to the pathogenesis of AD.<sup>5</sup>

It has been largely shown that allergic diseases are driven by activated T-helper (Th)2 lymphocytes, leading

to IgE production and eosinophil activation,<sup>6</sup> and some recent studies have shown that Th1-type cytokines are also associated with chronic lesions of AD.<sup>7,8</sup> The onset of acute AD is strongly associated with the production of Th2-type cytokines,<sup>8</sup> and the maintenance of chronic AD involves production of Th1-type cytokines and several remodelling-associated cytokines.<sup>9</sup>

B cell-activating factor (BAFF), also known as BLyS, TALL-1 and THANK, is a tumour necrosis factor (TNF) superfamily member (TNFSF13B) best known for its role in the survival and maturation of B cells.<sup>10</sup> Its biological role is mediated by the specific receptors B-cell maturation antigen (BCMA), transmembrane activation and calcium modulator and cyclophilin ligand interactor (TACI), and BAFF receptor (BAFF-R).<sup>10–12</sup> In humans, BAFF level is high in patients with systemic autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome and systemic sclerosis, and it has been shown that BAFF plays an important role in the pathogenesis of autoimmune diseases.<sup>12,13</sup> Recently, it has been reported that BAFF-R

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also provides a costimulatory signal to T cells and is produced by antigen-presenting cells (APC).<sup>12</sup> Bacterial products upregulate BAFF production in T cells, and a low level of BAFF transcription, upregulated after T cell receptor (TCR) stimulation, has also been detected in T cells. BAFF may regulate T-cell immunity during APC–T-cell interactions and may function as an autocrine factor once T cells have detached from the APC. Given the ability of BAFF to regulate T-cell activation and its association with autoimmune diseases, previous studies investigated whether BAFF affects the outcome of Th1-mediated and Th2-mediated T-cell responses, and finally how BAFF can play a role in allergic diseases such as asthma or AD.<sup>14</sup> Serum BAFF level was high in patients with asthma and could be used to monitor severity of asthma symptoms.<sup>11</sup> However, in mice, overexpressing BAFF inhibits allergic airway inflammation.<sup>14</sup> Thus, the immunopathogenesis of BAFF in patients with allergic diseases remains unknown.

A proliferation-inducing ligand (APRIL) is also a TNF superfamily member, with close homology to BAFF. BAFF and APRIL share TNF receptors and have similar functions in B-cell survival,<sup>15</sup> however, the function of BAFF and APRIL in allergic diseases remains unknown. A previous study reported that serum APRIL level might have a role in the development of AD.<sup>16</sup>

In the present study, we investigated the role of BAFF in patients with AD by studying the relationship between AD and serum BAFF level and presence. We also measured serum BAFF level in children with atopic and non-atopic eczema (AE and non-AE) and analysed the correlation between serum BAFF level and disease severity.

## Methods

This study was approved by the institutional review board of Severance Hospital and informed consent was obtained from the children's parents.

### Subjects

Sera were obtained from 245 children (mean  $\pm$  SD age  $4.9 \pm 3.4$  years, range 0–14 years) who visited the allergy clinic for AD or a general health check-up at Severance Hospital, Yonsei University. All patients with AD were given a diagnosis according to the criteria of Hanifin and Rajka.<sup>17</sup> Disease severity of AD was determined using the SCORing Atopic Dermatitis (SCORAD) Index system.<sup>18,19</sup> We excluded children with AD who also had respiratory allergies. In this study, we divided patients with AD into AE and non-AE.<sup>20</sup> Children with

AE were sensitive to > 1 of 6 common allergens, and total IgE level was > 150 IU/mL. A specific IgE test was performed with *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, egg whites, cow's milk, peanuts and soybeans, which are six common allergens in Korea. For sera of children with non-AE, total IgE level was < 150 IU/mL with no detectable specific IgE antibodies to any of the six common allergens. Controls were children who attended for general health checkups, who did not have any history of allergic or inflammatory disease. In total, 90 children with AE, 77 with non-AE and 78 controls were included in this study. There were no significant differences in age and gender between groups (Table 1).

### Measurement of blood eosinophil, serum total IgE and specific IgE

After peripheral blood samples were obtained from subjects, total IgE and IgE levels against the six specific common antigens were measured using a fluorezyme-immunoassay (CAP system FEIA; Pharmacia & Upjohn Diagnostic AB, Uppsala, Sweden). According to the manufacturer's instructions, specific IgE levels > 0.35 kU<sub>A</sub>/L should be considered positive. A haematology analyser (NE-8000; Sysmax, Japan) was used to count eosinophils automatically in peripheral blood.

### Enzyme-linked immunosorbent assay

The levels of BAFF and APRIL in sera were assessed by ELISA using commercial kits for the assay of human BAFF (R & D Systems, Minneapolis, MN, USA) and APRIL (Bender MedSystems, Vienna, Austria) according to the manufacturers' protocols. The minimum detectable dose of BAFF and APRIL were < 16 pg/mL and < 0.4 ng/mL, respectively. Absorbance was measured at 450 nm.

### Statistical analysis

Data were analysed by ANOVA, ANCOVA and the Pearson correlation coefficients test.  $P < 0.05$  was considered significant.

## Results

### Demographics of subjects

SCORAD Index, total IgE level and eosinophil count of children with AE were greater than were those of patients with non-AE and of controls.

**Table 1** Demographics of subjects.

|                           | Control (n = 78) | Non-AE (n = 77) | AE (n = 90)         | Total (n = 245) |
|---------------------------|------------------|-----------------|---------------------|-----------------|
| Gender, M/F               | 46/32            | 39/38           | 47/43               | 132/113         |
| Age, years                | 4.97 ± 3.19      | 3.90 ± 2.81     | 5.73 ± 3.79         | 4.91 ± 3.39     |
| Eosinophil count/ $\mu$ L | 203.52 ± 137.29  | 305.53 ± 241.90 | 807.51 ± 956.45*†   | 459.73 ± 657.64 |
| Total IgE, IU/mL          | 34.99 ± 27.79    | 27.75 ± 23.96   | 1090.42 ± 1360.28*† | 426.85 ± 974.75 |
| SCORAD                    | –                | 34.31 ± 22.80   | 52.03 ± 27.49*      | 44.13 ± 26.92   |

AE, atopic eczema; SCORAD, SCORing Atopic Dermatitis. Results are means ± SD. \* $P < 0.05$  between AE and non-AE; † $P < 0.05$  between AE and controls.

### Comparison of serum B cell-activating factor and a proliferation-inducing ligand levels between subject groups

Serum BAFF level (mean ± SEM) was significantly higher in patients with AE ( $1625.04 \pm 708.32$  pg/mL) than in those with non-AE ( $1194.69 \pm 448.44$  pg/mL,  $P < 0.001$ ) and in healthy controls ( $1062.89 \pm 444.74$  pg/mL,  $P < 0.001$ ). There were no significant differences between serum BAFF level in control subjects and those with non-AE (Fig. 1). After correcting serum BAFF level for age and gender, the pattern was still significant.

There were no significant differences between the groups for serum APRIL level:  $8.82 \pm 4.68$  ng/mL for healthy controls;  $8.49 \pm 4.12$  ng/mL for non-AE and  $7.63 \pm 2.04$  ng/mL for AE. Serum APRIL did not correlated with either BAFF or total IgE levels (data not shown).

### Correlation of serum B cell-activating factor level with disease severity, total IgE level, specific IgE levels and eosinophil count

We found that serum BAFF level was correlated with disease severity (SCORAD) in children with AD (Fig. 2a). Serum BAFF level was significantly correlated with total IgE level (Fig. 2b) and eosinophil count in children with AD (Fig. 2c), whereas SCORAD and total IgE were not correlated (data not shown). In the AE group, we explored correlations with serum BAFF levels and allergen-specific IgE levels. We found that egg-specific IgE level was positively correlated with serum BAFF level ( $\gamma = 0.25$ ,  $P < 0.05$ ) but other IgE specificities were not. After correcting for age and gender, the correlations were still significant.

## Discussion

We found that serum BAFF level was increased in children with AE and correlated with disease severity in children with AD. Additionally, serum IgE level and

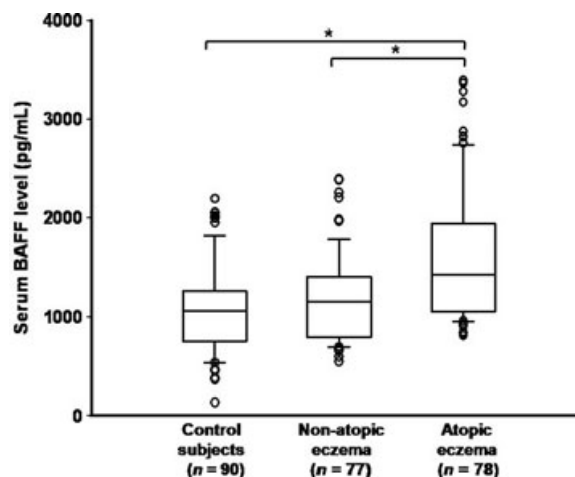


**Figure 1** Serum BAFF level in children with atopic eczema (AE) was significantly increased compared with the level in patients with non-AE and controls. There were no significant differences in serum BAFF level between non-AE patients and controls. Serum level of BAFF in controls, non-AE and AE was  $1062.89 \pm 444.74$ ,  $1194.69 \pm 448.44$  and  $1625.04 \pm 708.32$  pg/mL, respectively. Data are shown as means ± SEM. \* $P < 0.005$ .

total eosinophil count were positively correlated with serum BAFF level in children with AD.

BAFF is an important regulator of B-cell immunity and there are many reports of increased serum BAFF level in systemic autoimmune diseases. Recently, several studies found that BAFF also provides a costimulatory signal to T cells, and its activity is seen in both naive and effector/memory T cells.<sup>12</sup> As for allergic diseases, Kang *et al.*<sup>11</sup> reported that serum BAFF level was increased in patients with asthma and that disease severity correlated with serum BAFF level. They suggested that BAFF might be an effective additional indicator to monitor severity of asthma symptoms. In addition, scavenging of BAFF was found to improve asthmatic symptoms.<sup>21</sup> In contrast, Matsushita *et al.*<sup>16</sup> reported that BAFF was not significantly increased or associated with serum IgE production in patients with AD. Our results also conflict with the results of this study, but the number of cases we enrolled was much

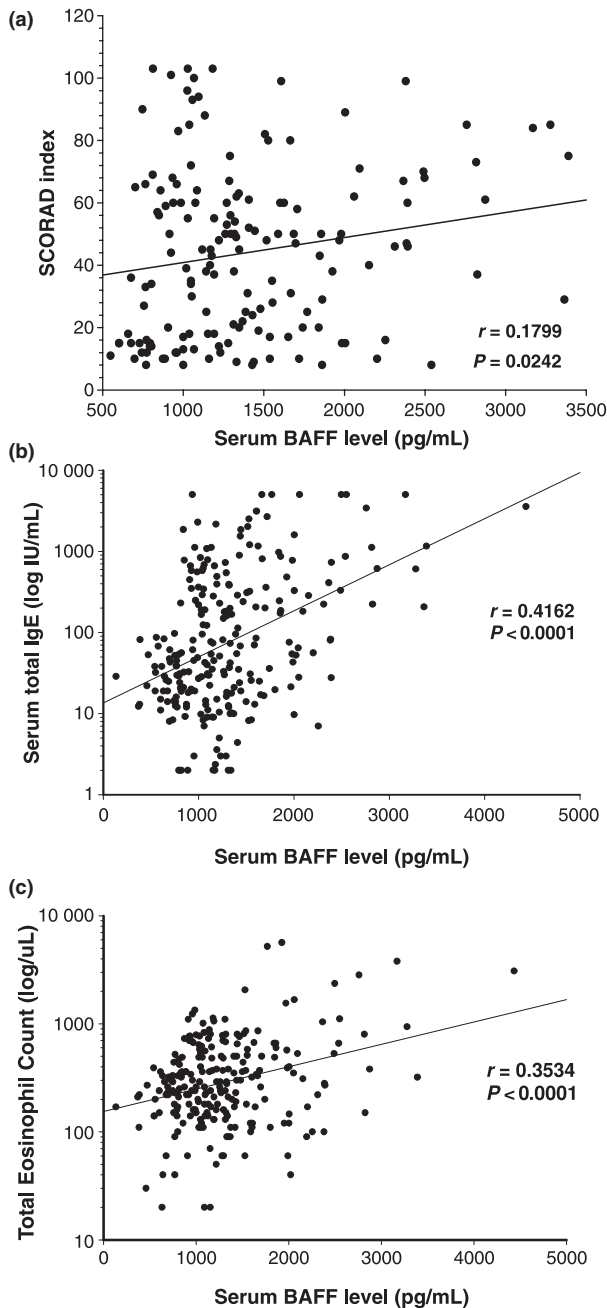


**Figure 2** Correlation of serum B cell-activating factor (BAFF) level with (a) disease severity of atopic dermatitis (AD), (b) total IgE and (c) serum total eosinophil count in children with AD. (a) Serum BAFF level significantly correlated with disease severity [SCORing Atopic Dermatitis (SCORAD) Index];  $r = 0.18$ ;  $P = 0.02$ . (b) Serum BAFF level was significantly correlated with serum total IgE:  $r = 0.42$ ;  $P < 0.0001$ ; (c) Serum BAFF level was positively correlated with serum total eosinophil count:  $r = 0.35$ ;  $P < 0.0001$ .

larger than used in other studies. In addition, we focused on young children (aged 0–14 years) and have to consider the differences in immune mechanisms

between adults and children, which could affect the results. However, the precise mechanisms of how BAFF is involved in AD are controversial, and further investigation is required.

In this study, we divided patients into two groups: AE and non-AE. The immunopathogenesis of non-AE is different from that of AE. The cytokine products are different and there is lower FcεRI expression by epidermal dendritic cells in non-AE. T cells from patients with non-AE produce similar amounts of interleukin (IL)-5 and interferon- $\gamma$  but produce lower amounts of Th2 cytokines such as IL-4 and IL-13, which increase IgE synthesis.<sup>22</sup> The skin of patients with non-AE contains a large number of epidermal dendritic cells which have decreased amounts of high-affinity receptors for IgE.<sup>23</sup> Thus, we investigated children with AE and non-AE separately and found different patterns. Serum BAFF level was increased in patients with AE compared with healthy controls but there was no significant difference between patients with non-AE and healthy controls. Serum BAFF level was positively associated with serum IgE level and blood eosinophil count. Our results show that serum BAFF level is correlated with both serum IgE and eosinophil count, which can be explained by the Th2 paradigm. An allergen triggers Th2 activation, leading to IgE production by B cells in early-phase reaction and activates late-phase eosinophils at the same time.<sup>6</sup> Kato *et al.*<sup>24</sup> reported that nasal epithelial cells produce BAFF, and that patients with chronic rhinosinusitis with nasal polyps have increased levels of BAFF, B cells and IgA in polypoid tissue. Increased IgA triggers eosinophils so that their numbers are increased in patients with allergic rhinitis. This supports the suggestion that BAFF contributes to the development of allergic diseases. BAFF also had positive correlation with AD severity, but when we investigated the correlation with AD severity and serum BAFF level in children with atopic and non-AE separately, neither group had a significant correlation (data not shown).

APRIL has close sequence homology with BAFF but their role in allergic diseases is still under discussion.<sup>10</sup> In this study, there was no difference in serum APRIL level between the groups, and there was no significant correlation between serum APRIL and IgE levels (data not shown). This is interesting because it is completely opposed to a previous study apart from the lack of significant correlation between serum BAFF and APRIL levels (data not shown).<sup>16</sup> Because we were focusing on atopic conditions, we classified subjects as AE or non-AE based not only on serum IgE level but also on the results of a specific IgE test, which might be more helpful and sensitive for exploring the mechanism of allergic

diseases. The results of this study may have been influenced by these differences, thus further studies focused on atopic diseases are necessary to confirm our results.

In terms of correlations between serum BAFF and allergen-specific IgE levels, there were no significant correlations with most allergens but there was a weakly positive correlation with egg allergen. Although this did not reach significance, it did reveal patterns of food allergens that were more closely correlated with serum BAFF level (data not shown), indicating that food allergens are more important than aeroallergens in the development of AD.<sup>25</sup>

In spite of some limitations, our results suggest that BAFF might be involved in the pathogenesis of AD, especially AE. It means that BAFF affects T-cell activation in IgE-mediated AD, and regulates IgE production through BAFF-mediated T-cell activation. Although the BAFF-mediated signalling pathways have not been fully identified, BAFF is expected to play a role in AE and may be an effective additional indicator of atopic diseases. Of course, further studies must be conducted on the mechanism of action in BAFF-mediated allergic reactions.

In conclusion, we have shown that serum BAFF level is significantly increased in patients with AE and is associated with AD severity. This strongly suggests that BAFF may have an important role in the pathogenesis of AE.

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## References

- 1 Akdis CA, Akdis M, Bieber T *et al.* Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Report. *J Allergy Clin Immunol* 2006; **118**: 152–69.
- 2 Boguniewicz M, Leung DY. Atopic dermatitis. *J Allergy Clin Immunol* 2006; **117**: S475–80.
- 3 Boguniewicz M, Schmid-Grendelmeier P, Leung DY. Atopic dermatitis. *J Allergy Clin Immunol* 2006; **118**: 40–3.
- 4 Eigenmann PA. Clinical features and diagnostic criteria of atopic dermatitis in relation to age. *Pediatr Allergy Immunol* 2001; **12** (Suppl. 14): 69–74.
- 5 Leung DY. Pathogenesis of atopic dermatitis. *J Allergy Clin Immunol* 1999; **104**: S99–108.
- 6 El Biaze M, Boniface S, Koscher V *et al.* T cell activation, from atopy to asthma: more a paradox than a paradigm. *Allergy* 2003; **58**: 844–53.
- 7 Grewe M, Gyufko K, Schopf E, Krutmann J. Lesional expression of interferon-gamma in atopic eczema. *Lancet* 1994; **343**: 25–6.
- 8 Hamid Q, Boguniewicz M, Leung DY. Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 1994; **94**: 870–6.
- 9 Toda M, Leung DY, Molet S *et al.* Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions. *J Allergy Clin Immunol* 2003; **111**: 875–81.
- 10 Mackay F, Browning JL. BAFF a fundamental survival factor for B cells. *Nat Rev Immunol* 2002; **2**: 465–75.
- 11 Kang JS, Yoon YD, Ahn JH *et al.* B cell-activating factor is a novel diagnosis parameter for asthma. *Int Arch Allergy Immunol* 2006; **141**: 181–8.
- 12 Huard B, Arlettaz L, Ambrose C *et al.* BAFF production by antigen-presenting cells provides T cell co-stimulation. *Int Immunol* 2004; **16**: 467–75.
- 13 Krumbholz M, Theil D, Derfuss T *et al.* BAFF is produced by astrocytes and up-regulated in multiple sclerosis lesions and primary central nervous system lymphoma. *J Exp Med* 2005; **201**: 195–200.
- 14 Sutherland AP, Ng LG, Fletcher CA *et al.* BAFF augments certain Th1-associated inflammatory responses. *J Immunol* 2005; **174**: 5537–44.
- 15 Mackay F, Schneider P. TACI, an enigmatic BAFF/APRIL receptor, with new unappreciated biochemical and biological properties. *Cytokine Growth Factor Rev* 2008; **19**: 263–76.
- 16 Matsushita T, Fujimoto M, Echigo T *et al.* Elevated serum levels of APRIL, but not BAFF, in patients with atopic dermatitis. *Exp Dermatol* 2008; **17**: 197–202.
- 17 Hanifin JM. Diagnostic criteria for atopic dermatitis: consider the context. *Arch Dermatol* 1999; **135**: 1551.
- 18 Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* 1993; **186**: 23–31.
- 19 Kunz B, Oranje AP, Labreze L *et al.* Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. *Dermatology* 1997; **195**: 10–19.
- 20 Johansson SG, Bieber T, Dahl R *et al.* Revised nomenclature for allergy for global use. Report of the Nomenclature Review Committee of the World Allergy Organization, October, 2003. *J Allergy Clin Immunol* 2004; **113**: 832–6.
- 21 Moon EY, Ryu SK. TACI:Fc scavenging B cell activating factor (BAFF) alleviates ovalbumin-induced bronchial asthma in mice. *Exp Mol Med* 2007; **39**: 343–52.
- 22 Bardana EJ Jr. Immunoglobulin E-(IgE) and non-IgE-mediated reactions in the pathogenesis of atopic eczema/

- dermatitis syndrome (AEDS). *Allergy* 2004; **59** (Suppl. 78): 25–9.
- 23 Akdis CA, Akdis M, Simon D *et al.* Role of T cells and cytokines in the intrinsic form of atopic dermatitis. *Curr Probl Dermatol* 1999; **28**: 37–44.
- 24 Kato A, Peters A, Suh L *et al.* Evidence of a role for B cell-activating factor of the TNF family in the pathogenesis of chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol* 2008; **121**: 1385–92. 92 e1–2.
- 25 Eigenmann PA, Sicherer SH, Borkowski TA *et al.* Prevalence of IgE-mediated food allergy among children with atopic dermatitis. *Pediatrics* 1998; **101**: E8.