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# Relationship of serum brain-derived neurotrophic factor level with other markers of disease severity in patients with atopic dermatitis

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## KEYWORDS

Brain-derived neurotrophic factor;  
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**Abstract** Elevated serum levels of neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) have been reported in allergic and autoimmune diseases. The aim of this study was to assess serum levels of BDNF in patients with atopic dermatitis (AD) and to investigate the relationship of the BDNF level with other markers of disease severity. Serum BDNF concentration was significantly higher in patients with AD ( $n=62$ ) compared to control subjects ( $n=20$ ) ( $P<0.01$ ). Stepwise multiple regression analysis showed a significant influence of the peripheral blood eosinophil counts ( $F=6.90$ ) and the percentage of CD4<sup>+</sup>IL-4<sup>+</sup> (Th2) cells ( $F=6.61$ ). Moreover, after remission of AD patients with conventionally treated AD patients ( $n=14$ ), serum levels of BDNF, eosinophil counts and percentage of Th2 cells were decreased significantly. These results suggest that serum BDNF may be a useful marker of disease activity in AD and that both eosinophils and Th2 cells are major cellular sources of serum BDNF.

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## Introduction

Atopic dermatitis (AD) is a pruritic, chronic and relapsing inflammatory skin disorder caused by the infiltration of skin lesions by various inflammatory cells including T cells,

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eosinophils, mast cells and macrophages [1–3]. Although the exact etiology and pathogenesis of AD have not yet been entirely elucidated, genetic and environmental factors are implicated in the pathogenesis of the disease [4–6]. A role for genetic factors has been supported by a familial and twin study [7]. Various studies also indicate that AD has a complex etiology with activation of multiple immunologic and inflammatory pathways, and many of the abnormalities found in the acute phase of patients with AD are considered to be the result of a Th2-dominant immune responses [6]. It has been reported that significantly higher frequencies of CD4<sup>+</sup>/IL-4<sup>+</sup> and CD4<sup>+</sup>/CD13<sup>+</sup> T cells were found in patients with AD compared with healthy controls and the eosinophil count correlated closely with increased CD4<sup>+</sup>/IL-4<sup>+</sup> and CD4<sup>+</sup>/CD13<sup>+</sup> T cells [8]. La Grutta et al. have recently reported that CD4<sup>+</sup>/CD13<sup>+</sup> T cells in the patients with AD significantly correlated with SCORAD index [9].

E-selectin, vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) are membrane-bound adhesion molecules which mediate the attachment of leucocytes to endothelial cells, thereby regulating leucocytes migration. These molecules are preferentially expressed on activated endothelium, and the soluble forms of these molecules are present in the circulation as a result of shedding. It has been reported that soluble E-selectin (sE-selectin), but not soluble VCAM-1 or soluble ICAM-1, is significantly correlated with disease severity in AD [10].

Eosinophil-derived products such as ECP are present in increased amounts in the blood and the skin of AD patients. Serum levels of ECP have been described to correlate positively with the disease activity [11,12], although there are conflicting studies [13,14].

Neurotrophins form a large family of dimeric polypeptides that include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, NT-4/5, NT-6 and NT-7 [15–18]. These neurotrophins (NTs) have been shown to promote the growth, survival and differentiation of developing neurons in the central and peripheral nervous systems [19–22]. Numerous recent studies have described NTs as growth factors with a wide spectrum of functions outside the nervous system, including modulation and regulation of immune functions and with roles in inflammatory and autoimmune diseases [23,24]. In relation to allergic disease, increased serum levels of NGF have been found in patients with allergic asthma [25,26] and in patients with AD [27]. Recently, increased blood levels of BDNF were reported in patients with atopic dermatitis [28], and elevated levels of BDNF [26,29] and NT-3 [26] were demonstrated in patients with allergic asthma. Although it has been reported that eosinophils are a cellular source of increased serum levels of BDNF in patients with AD, other cellular sources involved in the regulation and release of BDNF in AD are still not well investigated. In addition, the relationship between serum BDNF levels and other markers of disease severity in AD still remains unknown. The aim of this study was to evaluate serum levels of BDNF and NGF as markers of disease activity in patients with AD and to investigate the relationship of the BDNF level with other markers of disease severity.

## Materials and methods

### Patients

Sixty-two patients with AD (30 males and 32 females; age range 18–41 years, mean±SD 27.2±6.9) diagnosed according to the criteria defined by Hanifin and Rajka [1], 13 sex- and age-matched patients with psoriasis (6 males and 7 females; age range 18–43 years, mean±SD 27.9±8.1) and 20 sex- and age-matched healthy control subjects (10 males and 10 females; age range 18–42 years, mean±SD 27.4±8.0) without a history of allergic disorders were enrolled in this study. AD patients who had previously received systemic anti-inflammatory or immunosuppressive medication were excluded from this study. The disease severity of AD was determined by the SCORAD (SCORing Atopic Dermatitis) index [30]. All patients had been treated with topical corticoids, topical tacrolimus, moisturizers and occasional oral antihistamines, but none had ever taken oral corticoids or immunosuppressants. Blood sample after clinical improvement was obtained from 14 patients (6 males and 8 female). The study protocol was approved by the Kyoto Prefectural University of Medicine institutional review board, and informed consent was obtained from all subjects prior to the study.

### Laboratory investigations

Levels of BDNF and NGF in the serum were determined by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (DuoSet ELISA Development kit; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The detection limits were 5.0 pg/ml for both BDNF and NGF. For the purpose of statistical analysis, if the levels of BDNF or NGF were below the detection limit (i.e., <5.0 pg/ml), they were assigned a numerical value of zero. Serum sE-selectin was also determined by a commercial ELISA kit (R&D Systems) according to the manufacturer's instructions. Total IgE and eosinophil cationic protein (ECP) in the serum were measured by a commercial fluorescence enzyme immunoassay (FEIA, CAP System; Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions.

To assess the percentage of Th1 and Th2 cells in peripheral blood, mononuclear cells (PBMCs; 10<sup>6</sup> cells/ml) isolated by Ficoll-Hypaque density gradients were stimulated for 4 h with phorbol-12-myristate-13-acetate (PMA, 50 ng/ml; Sigma, St. Louis, MO, USA) and ionomycin (500 ng/ml; Sigma) in the presence of Brefeldin A (10 µg/ml; Sigma). After activation, the cells were incubated with peridinin chlorophyll protein (PerCP)-conjugated CD4 monoclonal antibody (Becton-Dickinson Pharmingen, San Diego, CA, USA) for 30 min, permeabilized (1× flow cytometry cell sorter (FACS) Permeabilizing Solution; BD Pharmingen), and stained with fluorescein isothiocyanate (FITC)-conjugated anti-human interferon (IFN)-γ monoclonal antibody (mAb) and phycoerythrin (PE)-conjugated anti-human interleukin (IL)-4 mAb (BD Pharmingen) for 30 min. Isotype matched normal, PerCP, PE, and FITC antibodies were used as negative controls. Cells were analyzed by

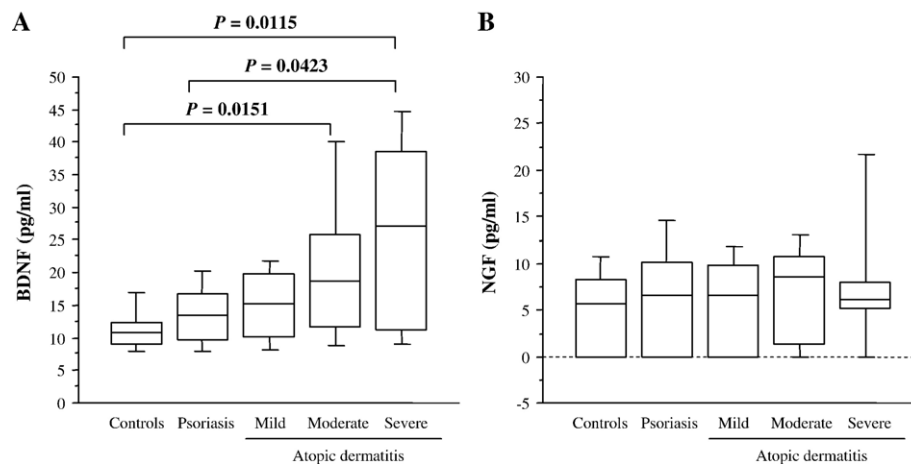


Figure 1.

three-color flow cytometric analysis using FACScalibur (Becton Dickinson).

### Statistical analysis

One-way ANOVA with Scheffe's *post hoc* test was performed to compare the serum levels of BDNF between groups of AD patients and control subjects. Kruskal–Wallis with Dunn's *post hoc* test was performed to compare the serum levels of NGF between groups of AD patients and control subjects. Student's paired *t*-test was performed for comparison of serum BDNF values in AD patients before and after improvement. Correlation coefficients were determined by the Pearson's correlation test. A *P* value < 0.05 was considered statistically significant. A stepwise multiple regression analysis was performed to assess the influence of the following independent variables on serum BDNF concentrations: serum NGF, ECP, log total IgE, sE-selectin, peripheral blood eosinophil count and percentage of Th1 and Th2 cells in PBMC. Variables were excluded from the regression analyses at each step of calculations if the *F* value was < 4.0.

### Results

Serum BDNF was detected in all subjects. On the other hand, NGF was below the detection limit (<5.0 pg/ml) in 8 of 20 healthy controls (40.0%), in 4 of 13 patients with psoriasis (30.8%), in 9 of 21 mild AD patients (42.9%), in 8 of 31 moderate AD patients (25.8%) and in 2 cases of 10 severe AD patients (20.0%). BDNF serum levels were significantly increased in patients with AD (mean ± SE,  $20.2 \pm 1.6$  pg/ml;  $n=62$ ) compared with non-atopic control subjects ( $11.4 \pm 0.7$  pg/ml;  $n=20$ ,  $P<0.0061$ ) and patients with psoriasis ( $12.2 \pm 0.9$  pg/ml;  $n=13$ ,  $P=0.0488$ ). When the patients were subdivided into three groups according to clinical severity, BDNF serum values were significantly higher in moderate AD patients ( $21.8 \pm 14.5$  pg/ml;  $n=31$ ,  $P=0.0151$ ) and in severe AD patients ( $25.9 \pm 14.7$  pg/ml;  $n=10$ ,  $P=0.0115$ ) than in non-atopic controls (Fig. 1A). The BDNF serum levels in severe AD patients were significantly higher than that in patients with psoriasis ( $P=0.0423$ ) (Fig. 1A). No significant difference in NGF serum values was found among non-atopic controls,

patients with psoriasis and each subgroup of patients with AD ( $P=0.2683$ ) (Fig. 1B).

As shown in Table 1, the SCORAD index was significantly and positively related to BDNF ( $r=0.364$ ,  $P=0.0034$ ), eosinophil counts ( $r=0.375$ ,  $P=0.0024$ ), ECP ( $r=0.303$ ,  $P=0.0163$ ), sE-selectin ( $r=0.297$ ,  $P=0.0185$ ) and percentage of Th2 cells ( $r=0.272$ ,  $P=0.0320$ ).

Table 2 shows the Pearson's correlation and stepwise multiple regression analysis between BDNF values and the 7 clinical variables. The BDNF serum levels were positively related to eosinophil counts ( $r=0.387$ ,  $P=0.0017$ ), percentage of Th2 cells ( $r=0.382$ ,  $P=0.0020$ ), SCORAD index ( $r=0.364$ ,  $P=0.0034$ ), sE-selectin ( $r=0.318$ ,  $P=0.0114$ ) and ECP ( $r=0.251$ ,  $P=0.0485$ ). Stepwise multiple regression analysis showed that peripheral blood eosinophil counts ( $F=6.90$ ) and percentage of Th1 cells in PBMC ( $F=6.61$ ) are

**Table 1** Laboratory data and Pearson's correlation between SCORAD index and 7 clinical variables in patients with atopic dermatitis

|                                                 | Mean ± SE (range)           | <i>r</i> | <i>P</i> |
|-------------------------------------------------|-----------------------------|----------|----------|
| SCORAD index                                    | $28.0 \pm 2.0$ (3–66)       | –        | –        |
| Serum brain-derived neurotrophic factor (pg/ml) | $20.4 \pm 1.6$ (3.1–66.9)   | 0.364    | 0.0034   |
| Serum nerve growth factor (pg/ml)               | $8.7 \pm 0.8$ (0–31.0)      | 0.210    | 0.1017   |
| Eosinophil (/ml)                                | $646.0 \pm 66.2$ (68–2750)  | 0.375    | 0.0024   |
| Serum eosinophilic cation protein (ng/ml)       | $20.5 \pm 1.8$ (2.5–66.3)   | 0.303    | 0.0163   |
| Serum total IgE (log IgE)                       | $2.69 \pm 0.09$ (1.18–4.49) | 0.126    | 0.3299   |
| Serum sE-selectin (ng/ml)                       | $33.9 \pm 1.3$ (16.5–61.6)  | 0.297    | 0.0185   |
| Th1 cell (% in PBMC)                            | $31.7 \pm 0.5$ (25.1–42.6)  | –0.154   | 0.2344   |
| Th2 cell (% in PBMC)                            | $2.3 \pm 0.1$ (0.8–4.6)     | 0.272    | 0.0320   |

PBMC, peripheral blood mononuclear cells.

**Table 2** Pearson's correlation and stepwise multiple regression analysis of relationship between serum BDNF levels and 7 clinical variables in patients with atopic dermatitis

| Variables                                 | <i>r</i> | $\beta$ | <i>F</i> |
|-------------------------------------------|----------|---------|----------|
| SCORAD index                              | 0.364**  | –       | –        |
| Serum nerve growth factor (pg/ml)         | 0.036    | –       | –        |
| Eosinophil (/ml)                          | 0.387**  | 0.309   | 6.90     |
| Serum eosinophilic cation protein (ng/ml) | 0.251*   | –       | –        |
| Serum total IgE (log IgE)                 | 0.144    | –       | –        |
| Serum sE-selectin (ng/ml)                 | 0.318*   | –       | –        |
| Th1 cell (% in PBMC)                      | –0.116   | –       | –        |
| Th2 cell (% in PBMC)                      | 0.382**  | 0.303   | 6.61     |

*r*, univariate correlation coefficient.  $\beta$ , standard regression coefficient. PBMC, peripheral blood mononuclear cell. Variables were deleted at each step in the regression analysis if *F* was not significant (i.e., <4.0). \**P*<0.05, \*\**P*<0.01.

relevant variables independently for the serum BDNF concentrations.

We next assessed the changes of serum BDNF levels in 14 clinically improved AD patients after treatment with conventional therapy including topical corticoids, topical tacrolimus, moisturizers and occasional oral antihistamines. The serum BDNF levels decreased significantly in the improved AD patients (*P*<0.001) (Fig. 2A). The change in BDNF concentration correlated significantly with changes in both eosinophil counts (*r*=0.703, *P*=0.0038) (Fig. 2B) and percentage of Th2 cells (*r*=0.658, *P*=0.0089) (Fig. 2C). On the other hand, there were no significant correlations between the changes of serum BDNF and the changes of NGF, ECP, sE-selectin or percentage of Th1 cells ( $-0.023 \leq r \leq 0.246$ ).

## Discussion

It has recently been demonstrated that several immune cells, such as mast cells [31,32], lymphocytes [33–36] and

eosinophils [27,37], have neurotrophin-producing capabilities and express neurotrophin receptors [38–40]. Because the pathophysiology of allergic diseases is characterized by allergic inflammation, the potential role of neurotrophins within the immune system is of great interest. Several recent studies have described effects on neurotrophins, especially NGF, in allergic asthma and AD [25–29].

Toyoda et al. [27] have recently reported that plasma NGF levels were markedly increased in patients with AD compared with non-atopic controls and strongly positively correlated with disease activity. However, the data in this study do not support their results. Although the precise reasons for this discrepancy are not clear, a possible reason may be the difference in the ELISA assay system. The serum NGF values in non-atopic control subjects of the Toyoda study are remarkably higher than the values we and others have found [23,24]. Therefore, further study is needed to clarify this point. On the other hand, we have found that the serum BDNF levels in patients with AD were significantly higher than that in non-atopic healthy subjects and correlated positively with disease severity, which is in agreement with the findings of Raap et al. [28]. In addition, the present study demonstrated for the first time that serum BDNF concentration is independently positively associated with eosinophil counts and with the percentage of Th2 cells. Moreover, the significant reduction of serum BDNF levels in the improved AD patients was also correlated significantly with changes in both the eosinophil counts and the percentage of Th2 cells. These findings strongly suggest that the major cellular source of increased BDNF in the serum of AD patients is Th2 cells and eosinophils, although it cannot rule out possibility of release from other immune/inflammatory component cells, such as keratinocytes, endothelial cells and macrophages. This is in agreement with reports that BDNF production by T cells was increased upon allergen and/or antigen activation [34,41,42].

Many of the immunological abnormalities found in patients with AD are considered the result of a predominant production of Th2 cytokines, including IL-4, IL-5 and IL-13, which promote IgE production and peripheral blood eosinophilia. Although it is well known that BDNF controls the survival, differentiation and maintenance of neurons in the

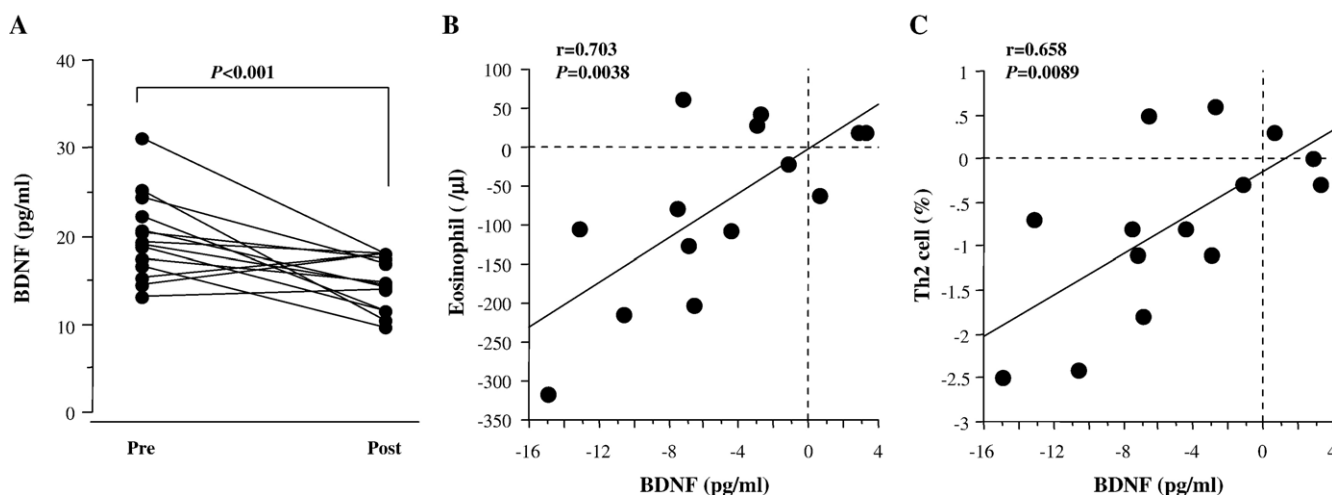


Figure 2.

peripheral and central nervous system, little is known about the pathogenic role of BDNF in AD. Raap et al. [26] have shown that stimulation with BDNF significantly inhibited eosinophil apoptosis and BDNF induced chemotaxis of eosinophils from patients with AD, whereas these effects were not shown in eosinophils from non-atopic control subjects. These findings suggest that BDNF plays an important role in the pathogenesis of AD.

Finally, although the serum levels of BDNF, ECP and sE-selectin were significantly positively related to SCORAD index, there were no significant correlations between the changes of serum BDNF and the changes of ECP or sE-selectin in the improved AD patients. These findings suggests that BDNF, ECP and sE-selectin are useful but distinct serum markers that reflect that different clinical and/or inflammation features of AD.

In conclusion, the present study demonstrates a significant correlation among serum levels of BDNF, SCORAD, Th2 cells and eosinophils. Our data suggest that serum BDNF may be a useful marker of disease activity in AD and that both activated Th2 cells and eosinophils are major cellular sources of serum BDNF.

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