

Anxiety accelerates T-helper 2-tilted immune responses in patients with atopic dermatitis

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Summary

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Background Stress, which mediates anxiety, worsens skin symptoms in patients with atopic dermatitis (AD). The contribution of anxiety to immune dysfunction, which plays a critical role in the pathogenesis of AD, requires clarification.

Objectives To examine the relationship between anxiety and atopy-relevant immune function in AD.

Methods Eighty-five patients with AD and 58 normal individuals without a history of allergic disorders were enrolled in this study. To assess anxiety, the state-trait anxiety inventory was completed for both groups. In the AD group, measurements were made of SCORAD scores, serum IgE levels, itching (visual analogue scale), blood eosinophil count and T-helper (Th) 1/Th2 ratio in the peripheral blood.

Results Anxiety was significantly higher among the subjects with AD than the normal subjects, and trait anxiety (TA) was higher than state anxiety (SA) in the AD group. Serum total IgE levels were correlated positively with TA and the TA/SA ratio and inversely with SA, and the Th1/Th2 ratio was correlated inversely with TA and the TA/SA ratio.

Conclusions The patients with AD had higher anxiety levels than normal individuals, and those with a stronger perception of TA than SA showed enhanced serum IgE synthesis and Th2 shifting.

Atopic dermatitis (AD) is a chronically relapsing pruritic skin disorder, affecting 10–20% of young people worldwide.^{1,2} Both children and adults with AD have higher anxiety levels than those without, and psychotherapy improves not only the psychological but also the dermatological condition.^{3–6} It is well known that emotional and psychological stresses bring on attacks or exacerbate skin symptoms in daily settings. Although the reason why stress influences skin conditions is not fully understood, recent studies have revealed that it both mediates anxiety and affects immune function.^{7,8} As the pathogenesis of AD has an immunological basis, as exemplified by increased serum IgE levels and blood eosinophilia,¹ persistent immune modulation induced by everyday stressors might be closely related to symptomatic episodes in patients with AD. The present study was designed to clarify the link between anxiety and atopy-relevant immune function in patients with AD.

Materials and methods

Eighty-five patients with AD (33 males and 52 females; age range 12–52 years, mean $\pm$ SD 25 ± 8) diagnosed on the

basis of the criteria of Hanifin and Rajka⁹ and 58 normal individuals (20 males and 38 females; age range 18–31 years, mean $\pm$ SD 24 ± 2) without a history of allergic disorders were enrolled in this study. The study was approved by the University's Ethics Committee, and all subjects gave informed consent before participating. The SCORAD score,¹⁰ a representative means of assessing the severity of AD (maximum 103, minimum 0), ranged from 16.6 to 63.5 (mean $\pm$ SD 38.1 ± 16.0), and itching score, assessed using a visual analogue scale (VAS; maximum 100, minimum 0), ranged from 0 to 70 (mean $\pm$ SD 38.5 ± 23.9). Serum IgE levels, measured by the radioimmunoabsorbent test, ranged from 23 to 32 767 IUa mL⁻¹ (mean $\pm$ SD 7118 ± 8319 ; normal < 250), and blood eosinophil count ranged from 0 to 3596×10^6 L⁻¹ (mean $\pm$ SD $939.0 \pm 712.9 \times 10^6$; normal $< 500 \times 10^6$). All patients had been treated with topical corticosteroids, topical tacrolimus, moisturizers and occasional oral antihistamines, but none had ever taken oral corticosteroids or immunosuppressants.

The state-trait anxiety inventory (STAI), which is a self-administered questionnaire consisting of 20 questions concerning state anxiety (SA) and 20 concerning trait anxiety

(TA), previously translated into Japanese by Iwata *et al.*,¹¹ was completed for the patients with AD and for the normal subjects. These refer to the level of anxiety felt at present (SA) and in general (TA). STAI is scored from a minimum of 20 (the lowest level of anxiety) to a maximum of 80 (the highest level of anxiety) for each scale. The TA/SA ratio was expressed as TA score/SA score.

To assess the T-helper (Th) 1/Th2 balance, peripheral blood mononuclear cells were obtained by Ficoll-Hypaque centrifugation and cultured at 10^6 cells mL^{-1} in 24-well culture plates in complete RPMI 1640 containing 5% autologous plasma and antibiotics in the presence of 1 ng mL^{-1} phorbol myristate acetate and $0.1 \mu\text{g mL}^{-1}$ calcium ionophore for 24 h. One microlitre of Goldplugh (Cytofix/Cytoperm Plus Kit; PharMingen, San Diego, CA, U.S.A.) was added to each well during the last 6 h of culture according to the manufacturer's protocol. The cells were then reacted with $100 \mu\text{L}$ of CytoFix for 15 min at 4°C , washed with phosphate-buffered saline containing 0.1% saponin and stained with fluorescein isothiocyanate-conjugated antihuman interferon (IFN)- γ monoclonal antibody (mAb) (4S.B3; BD PharMingen, San Diego, CA, U.S.A.) and phycoerythrin-conjugated antihuman interleukin (IL)-4 mAb (8D4-8; BD PharMingen) for 30 min, followed by reaction with peridinin chlorophyll protein-conjugated anti-CD4 antibody (SK3; BD PharMingen). The Th1/Th2 CD4+ T-cell ratio was expressed as the number of intracytoplasmic IFN- γ + cells $\times 100$ /number of intracytoplasmic IL-4+ cells.¹²

The data were analysed using Statview (Hulinks Co., Tokyo, Japan). The Mann-Whitney U-test, Spearman's correlation and Kendall's correlation were used for nonparametric statistics, and the χ^2 test and Student's t-test for parametric statistics. $P < 0.05$ was considered significant.

Results

While there was no statistical difference in age distribution between the two groups, the male/female ratio differed significantly between the groups ($P < 0.003$, χ^2 test). In correlation with previous studies,³⁻⁶ the patients with AD had higher anxiety than the normal individuals. The mean $\pm$ SD TA and SA scores were 49 ± 8 and 44 ± 8 in the AD patients, respectively, and were significantly higher than those of the normal subjects (40 ± 7 , $P < 0.0001$ and 40 ± 9 , $P < 0.005$, respectively; Fig. 1A). While TA and SA were comparable in the normal subjects, TA was significantly higher than SA in the AD group ($P < 0.0001$). Individual anxiety profiles expressed as the TA/SA ratio confirmed that patients with AD experience TA more intensely than SA, while normal individuals have comparable levels of TA and SA (Fig. 1B, $P < 0.003$, Mann-Whitney U-test). There was no gender difference between TA and SA in either group.

Serum IgE levels were correlated positively with TA ($n = 78$, $P < 0.01$, Spearman's correlation test; Fig. 2A), inversely with SA ($n = 78$, $P < 0.02$, Spearman's correlation test; Fig. 2B) and positively with the TA/SA ratio ($n = 78$,

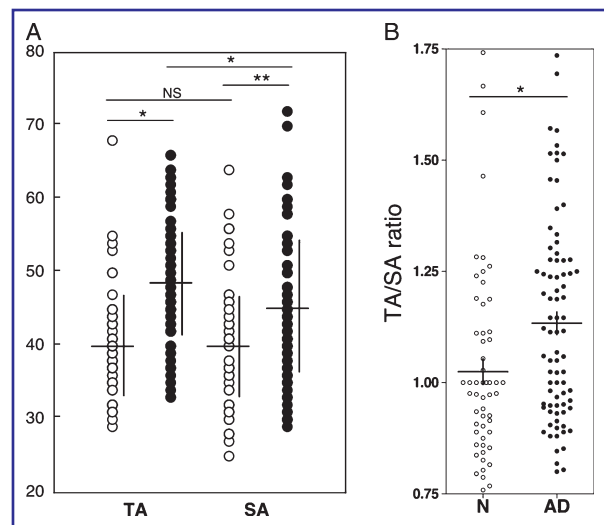


Fig 1. Trait anxiety (TA) and state anxiety (SA) in patients with atopic dermatitis (AD) and normal individuals. (A) The TA and SA values (mean $\pm$ SD) of the AD (●) and normal (○) groups. Vertical bar = SD; horizontal bar = mean value. * $P < 0.0001$, ** $P < 0.005$; NS, not significant. (B) The TA/SA ratio (mean $\pm$ SEM) of the AD (●) and normal (N, ○) groups. Vertical bar = SEM; horizontal bar = mean value. * $P < 0.003$.

$P < 0.0002$, Spearman's correlation test; Fig. 2C), indicating that those with higher TA levels than SA levels had elevated serum IgE.

As IgE production is regulated by a functional balance between Th1 and Th2 cells,¹³ we examined anxiety levels in relation to the ratio of IFN- γ -producing cells (Th1) to IL-4-producing cells (Th2) in consenting patients with AD. There was an inverse correlation between the Th1/Th2 ratio and TA ($n = 20$, $P < 0.05$, Kendall's correlation test; Fig. 3A), reflecting the fact that those with high TA had elevated serum IgE levels. In contrast, we found no correlation between the Th1/Th2 ratio and SA ($n = 20$, $P = 0.42$; Fig. 3B). A strong inverse correlation between the Th1/Th2 ratio and the TA/SA ratio was observed ($n = 20$, $P < 0.005$; Fig. 3C), suggesting that the patients who experienced TA more intensely than SA developed highly Th2-tilted immune responses. There was no correlation between anxiety levels and disease severity as assessed by SCORAD, itching levels as estimated by VAS, or eosinophil counts.

Discussion

According to this study, AD patients with a high TA/SA ratio have elevated serum IgE levels and a low Th1/Th2 ratio. Because of the functional predominance of Th2 cells over Th1 cells, IgE production is more enhanced in patients with high TA relative to SA in comparison with those with comparable TA and SA. The significant correlation between TA levels and both IgE levels and the Th1/Th2 ratio suggests that TA is more critically involved in atopy-relevant immune responses than is SA. Several studies of stress and its impact on immune

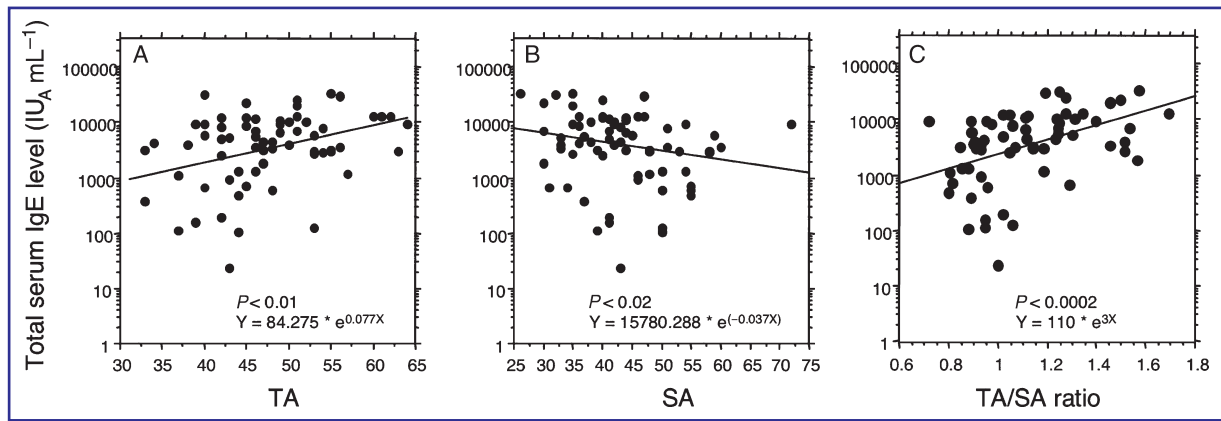


Fig 2. Correlation between serum total IgE levels and anxiety. Data were analysed by Spearman's correlation test. (A) Correlation between serum IgE and trait anxiety (TA). * $P < 0.01$. (B) Correlation between serum IgE and state anxiety (SA). * $P < 0.02$. (C) Correlation between serum IgE and TA/SA ratio. * $P < 0.0002$.

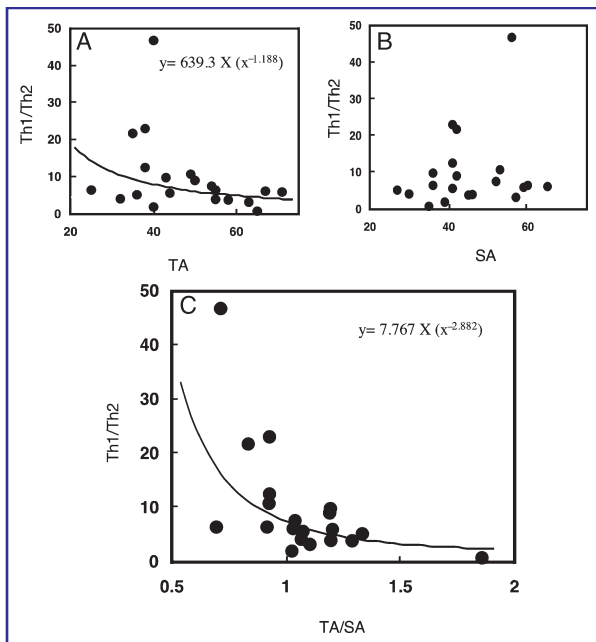


Fig 3. Correlation between T-cell profile and anxiety. Data were analysed by Kendall's correlation test. (A) Correlation between T-helper (Th) 1/Th2 ratio and trait anxiety (TA). * $P < 0.05$. (B) Correlation between Th1/Th2 ratio and state anxiety (SA). * $P = 0.42$. (C) Correlation between Th1/Th2 ratio and TA/SA ratio. * $P < 0.005$.

function have pointed out the possibility that stress mediates anxiety while simultaneously modulating immune reactions in atopic individuals.^{8,14} AD patients with clinically relevant IgE levels show significantly higher levels of excitability and inadequate stress coping compared with those with normal IgE levels.¹⁵ Acute stress is known to result in increased serum IgE while simultaneously influencing the immune system by shifting responses towards the Th2 type.¹⁶ By demonstrating Th2 predominance in individuals with a high TA/SA ratio, the

present study extends these observations and provides further clues as to the impact of stress on immune function.

Acute psychological stress results in increased eosinophil counts in the peripheral blood of patients with AD.^{17,18} However, these previous studies did not address the anxiety levels of patients with acute stress and those without. Therefore, the relevance of these findings to the present study, which showed no correlation between eosinophil counts and anxiety levels, is unknown.

Stressors are known to cause a release of adrenal glucocorticosteroids by activating the hypothalamus–pituitary–adrenal axis, and stimulation of the sympathetic nervous system results in a surge of norepinephrine and epinephrine.⁸ Both hormones have a regulatory effect on the differentiation and function of T cells and other immune cells and, as a result, cellular immunity is inhibited while immunoglobulin production is augmented. Therefore, it is likely that the acute stresses that repeatedly affect patients with AD chronically raise anxiety levels, preferentially TA, and stimulate Th2 responses with enhanced IgE synthesis.

There was no correlation between anxiety and SCORAD scores or pruritus as was previously reported by Linnet and Jemec.⁵ While immunological abnormalities have been implicated in the pathogenesis of AD, other factors irrelevant to immune status also play a critical role in precipitating skin conditions.¹ Therefore, anxiety-related immune dysregulation might not necessarily be related to the severity of eczema.¹⁹

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