

Association between Serum IL-16 Levels and the Degree of Sensitization in Patients with Atopic Dermatitis

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Key Words

Interleukin-16 · Atopic dermatitis · Cytokines

Abstract

Background: Interleukin (IL)-16 has been characterized as an immunomodulatory cytokine. Besides its chemotactic properties, IL-16 amplifies inflammatory processes and possesses immunoregulatory functions. Our aim was to investigate the association between serum IL-16 levels and the degree of allergic sensitization in patients with atopic dermatitis (AD). **Methods:** The serum level of IL-16 was measured by immunoenzymatic assays. Eosinophil cell count, serum total and specific IgE levels were assessed; prick tests were also carried out. Based on specific IgE levels and prick tests, AD patients were divided into sensitized and nonsensitized subgroups, and correlations among serum IL-16, total IgE levels and eosinophil cell counts were measured in the total patient group and in subgroups. **Results:** In the total patient group, significantly higher levels of IL-16 were found in the sera of patients with AD, compared to healthy individuals and patients with psoriasis. A significant correlation was detected between serum levels of IL-16 and total IgE, total IgE and eosinophil counts, but not between IL-16 and eosinophils.

When sensitized and nonsensitized subgroups were compared, IL-16 levels showed a significant difference in subgroups that were divided based on specific IgE measurements, but not in those subgroups which were divided based on prick tests. On the other hand, serum total IgE levels showed a significant difference between sensitized and nonsensitized subgroups, assessed by the specific IgE method and also by prick test. **Conclusion:** Serum IL-16 levels of AD patients correlate to some extent with sensitization. This correlation is not as strong as the correlation between total IgE levels and allergic sensitization.

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Introduction

Atopic dermatitis (AD) is a highly pruritic, chronic inflammatory skin disease that affects up to 20% of children worldwide and can persist into adulthood [1]. The severity of the disease is usually graded by the Scoring Atopic Dermatitis (SCORAD) index in studies and clinical trials. Although significant interobserver variability has been reported, this score is considered a standard reference for assessment of AD severity [2, 3]. There are also

laboratory parameters for monitoring disease activity and allergic sensitization, including serum IgE levels, lactate dehydrogenase levels and degrees of eosinophilia [4]. Increased levels of circulating eosinophils and eosinophil granule proteins in the sera are characteristic features of AD [5]. However, new serological parameters are needed both in clinical studies of AD and therapeutic trials for AD.

CD4+ helper T (Th) cells infiltrating skin lesions have been shown to play a pivotal role in the pathogenesis of AD. The exacerbation of AD eruption is driven by Th2 cells, while the maintenance of chronic lesions is associated with both Th1 and Th2 cells [6–8]. Among chemotactic factors, interleukin (IL)-16 has been fully characterized as an immunomodulatory cytokine, playing a role in the recruitment of CD4-expressing Th cells, monocytes and eosinophils to the site of inflammation. Besides its chemotactic properties, IL-16 can trigger IL-2 receptor α -chain expression and activate CD4+ T cells in synergy with IL-2 or IL-15 [9]. In addition, IL-16 amplifies the inflammatory reactions by stimulating cytokine production in monocytes and maturing macrophages [10].

Several studies showed a clear correlation between serum IL-16 levels and the SCORAD index, which reflects AD severity. Therefore, IL-16 seems to be a proper marker for measuring AD activity [2, 4, 9], although little is known about the correlation between serum IL-16 levels and the degree of allergic sensitization. The aims of this study were to (1) assess serum levels of IL-16 in patients with AD and nonatopic healthy controls and (2) analyze the possible correlation between IL-16 and several markers, reflecting the state of sensitization within these patients.

Patients and Methods

Patients

Overall, 85 patients with AD were studied (46 males, 39 females, mean age 18.6 years, range 7–49 years). All patients fulfilled the diagnostic criteria established by Hanifin and Rajka. The study was approved by the local Ethics Committee of the University of Debrecen, Hungary. The severity of the disease was determined by the SCORAD index: the mean SCORAD score was 32 (range 8–56). Patients who were enrolled in this study had not been treated with oral glucocorticosteroids or other systemic immunomodulatory agents for at least 4 weeks. They also had not received antihistamines or topical corticosteroids for at least 5 days prior to blood sampling. In all AD patients, serum IL-16 levels, total IgE levels and eosinophil cell counts were determined, as described. In 48 AD patients, allergen-specific serum IgE was measured and prick tests were performed. Based on the specific IgE levels, we divided AD patients into the following two sub-

groups: group 1 (nonsensitized, n = 13), which consisted of patients with no detectable specific IgE, and group 2 (sensitized, n = 35), which consisted of patients in which 1 or more IgEs were detected. The prick test was performed according to a standardized procedure [11]. Thirty-two different aeroallergens, including perennial, seasonal [house dust mite (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), birch pollen, cat dander, grass pollen (*Phleum pratense*)] and food allergens were applied. Based on prick test results, patients were also divided into a non-sensitized, prick test-negative (group A, n = 24), and a sensitized, prick test-positive (group B, n = 24), subgroup. Blood samples from 36 healthy age-matched volunteers and 20 psoriasis patients that were suffering from chronic plaque type psoriasis (mean age 28.2 years; mean Psoriasis Area Severity Index 15.2) but were not being treated with local or systemic immunomodulatory agents served as controls.

Laboratory Methods

Eosinophil Cell Count

The rate and absolute count of eosinophils in whole blood were determined by Advia 120 hematology analyzer (Siemens, Deerfield, Ill., USA) as part of the routine clinical investigation of the patients. The eosinophils in the analyzer were identified based on their scatter properties and myeloperoxidase positivity.

ELISA

Serum levels of IL-16 were determined by solid phase sandwich enzyme-linked immunosorbent assay (ELISA; Biosource International Inc., Camarillo, Calif., USA) according to the manufacturer's instructions. Blood samples were also evaluated for total IgE and specific IgE for common food and aeroallergens. Serum levels of total IgE were determined by solid-phase sandwich ELISA (Adaltis Italia S.p.A., Bologna, Italy) according to the manufacturer's instructions. All assays were performed in duplicates. Serum levels of specific IgE were determined by the ALLERgen Systems kit, which is based on the immunoenzyme capture system (Adaltis Italia S.p.A.).

Statistical Analysis

Statistical analyses were performed using SPSS 15.0 software. Nonparametric Mann-Whitney and Kruskal-Wallis tests were used to compare the data of AD patients to healthy and psoriasis controls in the subgroup analysis. The nonparametric Spearman rank correlation test was performed between serum total IgE and serum IL-16 levels, between serum total IgE and eosinophils and also between SCORAD score and the levels of serum IL-16 or total IgE. $p < 0.05$ was considered statistically significant. Data are presented as means $\pm$ standard deviation.

Results

Serum IL-16 Levels in AD Patients, in Healthy Individuals and in Patients with Psoriasis

Serum IL-16 levels in patients with AD were compared to those of healthy individuals and patients with psoriasis. We found that IL-16 levels in the sera of patients with AD (median 206.8 pg/ml; minimum 1.4 pg/ml; maxi-

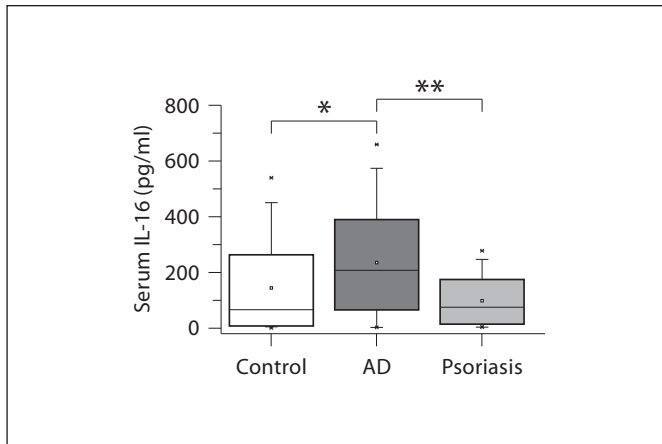


Fig. 1. Serum IL-16 levels in AD patients, in healthy individuals and in patients with psoriasis. We found that IL-16 levels in the sera of patients with AD were significantly elevated compared to those in healthy individuals or those in patients with psoriasis. * $p = 0.013$; ** $p = 0.005$.

mum 839.4 pg/ml) were significantly elevated compared to those in healthy individuals (median 62.8 pg/ml; minimum 1.7 pg/ml; maximum 540.1 pg/ml) and those of patients with psoriasis (median 69.45 pg/ml; minimum 3.1 pg/ml; maximum 277.9 pg/ml) ($p = 0.013$ and $p = 0.005$, respectively; fig. 1).

Association among Serum Levels of IL-16, Total IgE and Eosinophil Counts

In the total patient group, significant correlations were found between serum levels of total IgE and IL-16 ($r = 0.425$; $p = 0.002$; fig. 2) and between total IgE and eosinophil counts ($r = 0.364$; $p = 0.007$; data not shown), but there was no correlation between IL-16 and eosinophils. In the case of the SCORAD index, we detected a positive correlation between SCORAD score and total IgE ($r = 0.444$; $p = 0.091$). No association was found between SCORAD score and serum IL-16 even though the value of the R coefficient was 0.27, which is very close to the statistical limit of correlation.

Subgroup Analysis of Patients with AD

According to specific IgE levels and prick test results, we divided AD patients into sensitized (group 2 and group B) and nonsensitized (group 1 and group A) subgroups. Serum IL-16 and total IgE levels were further assessed within these groups. Serum IL-16 levels were significantly elevated in group 2 compared to those in group 1. No significant differences between groups A and B

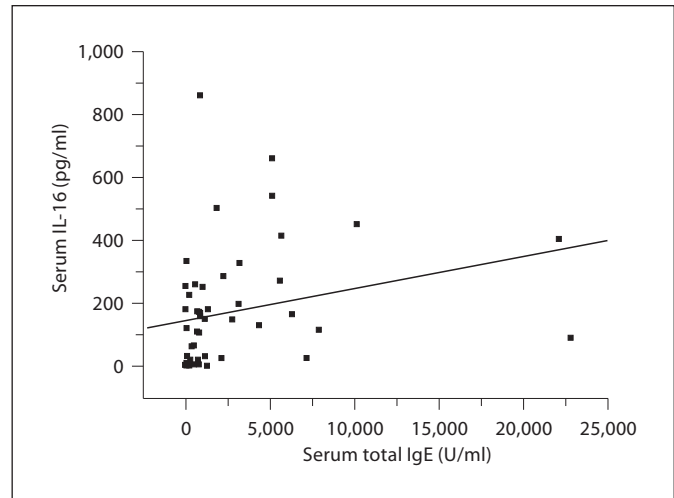


Fig. 2. Correlation between serum IL-16 and total IgE. In patients with AD, a significant correlation was found between serum levels of IL-16 and serum levels of total IgE ($p = 0.002$).

were detected [group 1: median 10.5 pg/ml; minimum 1.4 pg/ml; maximum 255.0 pg/ml; group 2: median 149.9 pg/ml; minimum 5.4 pg/ml; maximum 540.4 pg/ml; $p = 0.038$ (fig. 3a); group A: median 112.25 pg/ml; minimum 2.6 pg/ml; maximum 540.4 pg/ml; group B: median 172.25 pg/ml; minimum 1.4 pg/ml; maximum 659.3 pg/ml (fig. 4a)].

Serum total IgE levels were significantly increased both in group 2 and group B compared to those in group 1 and group A [group 1: median 185.0 U/ml; minimum 1.3 U/ml; maximum 1,279.0 U/ml; group 2: median 1,160.0 U/ml; minimum 50.8 U/ml; maximum 22,791 U/ml; $p < 0.0001$ (fig. 3b); group A: median 606.2 U/ml; minimum 1.3 U/ml maximum 7,170 U/ml; group B: median 1,158.5 U/ml; minimum 50.8 U/ml; maximum 22,791 U/ml; $p = 0.021$ (fig. 4b)].

Discussion

AD is a chronic inflammatory skin disease, characterized by a shift in the cytokine pattern from Th2 to Th1 type during the development of skin lesions. In acute skin lesions, the predominance of Th2 lymphocytes that produce IL-4 and IL-13 is replaced by Th1 lymphocytes with predominant IFN- γ production in the chronic phase of AD [9, 12]. Previous studies also demonstrated impaired Th1 and dominant Th2 cytokine expression in both CD4+ and CD8+ T cells in peripheral blood of AD pa-

Fig. 3. Serum IL-16 and serum total IgE levels in the subgroups divided according to the degree of sensitization, detected by specific IgE measurement. **a** Serum IL-16 levels were significantly elevated in the sensitized subgroup compared to those in non-sensitized subjects. * $p = 0.038$. **b** Serum total IgE levels were significantly different between sensitized and nonsensitized subgroups. *** $p < 0.0001$.

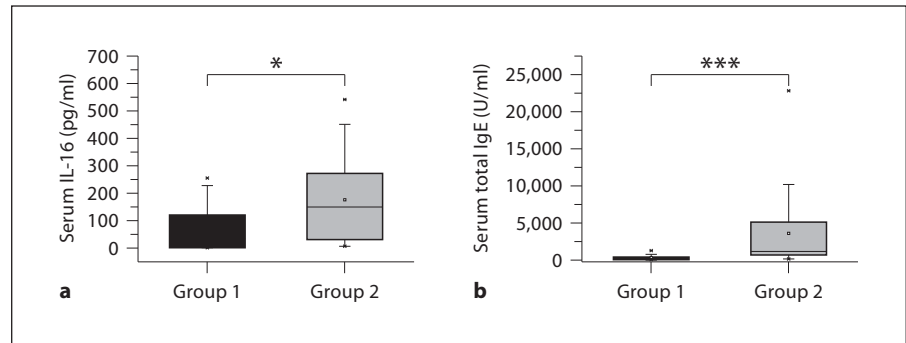
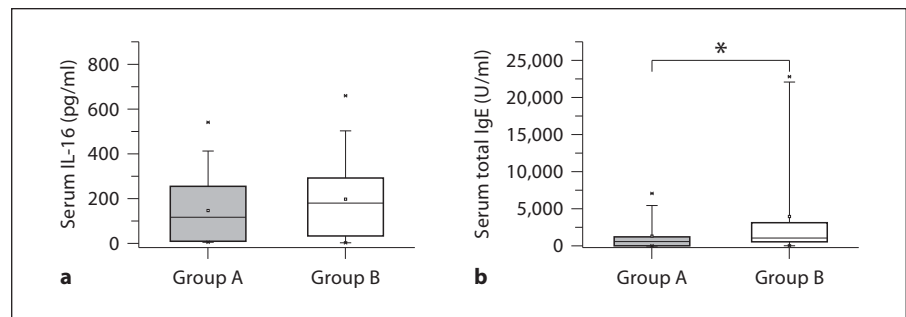


Fig. 4. Serum IL-16 and serum total IgE levels in the prick test-negative and prick test-positive subgroups. **a** Although IL-16 levels were elevated in group B compared to those in group A, the difference was not statistically significant. **b** Serum total IgE levels were significantly elevated in group B compared to those in group A. * $p = 0.021$.



tients [8]. Recent findings indicated that distinct IL-22 producing CD4+ and CD8+ T cell populations were significantly elevated in AD skin and the increased IL-22 signal suggested an immune-driven hyperplasia by IL-22 [13].

Different cell types, including T and B lymphocytes and a variety of innate immune cells, eosinophils, mast cells, airway epithelial cells, dendritic cells and keratinocytes, are able to synthesize IL-16 [14–20]. In T cells, IL-16 is generated as a precursor molecule, pro-IL-16, which is cleaved by caspase-3, yielding the secreted, biologically active cytokine [14, 21]. Secreted IL-16 primes CD4+ T cells for IL-2 and IL-15 responsiveness and has a preferential effect on Th1 cells [21]. Nevertheless, IL-16 was recognized as a chemoattractant for all peripheral immune cells expressing CD4, such as CD4+ T lymphocytes, monocytes and dendritic cells [2, 21, 22].

In allergic diseases, IL-16 has been shown to promote secretion of pro-inflammatory cytokines, which suggests a pathophysiological role for IL-16 as a mediator of inflammation [14]. The secretion of IL-16 from allergen-activated epidermal Langerhans cells may contribute to the recruitment and activation of dendritic cells, T cells and eosinophils in AD, providing a possible link between IgE-mediated and cellular inflammatory responses [23].

In AD it has been shown that one mechanism for the exacerbation of the disease may be the allergen-induced IL-16 production by eosinophils, which recruits additional eosinophils producing IL-4 [4, 20, 24]. Increased expression of IL-16 mRNA has been detected in epidermal keratinocytes and in dermal T lymphocytes both in acute and chronic AD lesions [20]. The number of IL-16 mRNA+ cells was significantly higher in acute lesions compared to that in chronic lesions and correlated with the number of CD4+ cells in both the epidermis and dermis, underlying the important role of IL-16 in the initiation and perpetuation of skin inflammation in AD [2].

The connection between IL-16 and AD has also been confirmed by clinical studies, which show significant correlations between serum IL-16 levels and the severity of the disease [2, 9]. On the other hand, the association between serum IL-16 levels and the degree of sensitization in AD has not been described previously.

In this study, we demonstrated significantly higher serum IL-16 levels in AD patients compared to those in healthy controls. Moreover, a significant difference was detected between AD and psoriatic patients. Our results are in accordance with the findings of Masuda et al. [4] because they also demonstrated significantly increased serum IL-16 levels in patients suffering from AD com-

pared to those in patients with psoriasis. They concluded that the more pronounced monocyte/macrophage infiltration in the dermis of AD patients could be one of the reasons for the high IL-16 serum levels. Also, the difference between psoriasis and AD in a Th1/Th2 balance may contribute to the dissimilarity in IL-16 levels in the two inflammatory disorders [4].

In AD, the association between high serum IL-16 levels and different clinical and laboratory parameters of the disease has been investigated extensively. Although serum IL-16 seems to correlate with SCORAD score [2, 4, 9], the relationship between IL-16 and laboratory parameters is controversial in the literature. Frezzolini et al. [9] did not detect an association between total IgE and serum IL-16 levels, while Masuda et al. [4] found a positive correlation between IgE and IL-16 and also between IL-16 and the eruption score of AD patients. In our study, we found a significant correlation between serum IL-16 and serum total IgE levels, and also between total IgE and eosinophil cell counts. In addition, a positive correlation was found between the SCORAD index and the total IgE level, although this association was not significant. On the other hand, no association was found between SCORAD score and serum IL-16, yet the value of the R coefficient was 0.27, which is very close to the statistical limit of correlation. This shows a modest correlation between SCORAD score and serum IL-16, and this correlation could probably have been increased by more patients.

Based on the degree of sensitization detected by specific IgE measurements and prick tests, AD patients were divided into sensitized and nonsensitized subgroups, and serum total IgE and IL-16 levels were further assessed within these subgroups. According to our results, serum IL-16 levels showed significant elevation in the sensitized subgroup compared to those of the nonsensitized subset only when sensitization was determined by specific IgE. Serum total IgE levels were significantly increased in both sensitized subgroups compared to those of nonsensitized subjects. Our data suggest that although serum IL-16 levels show a correlation with the degree of allergic

sensitization, this association is not as strong as the relationship between serum total IgE and allergic sensitization. Serum total IgE levels, when compared to serum IL-16 levels, correlate better with markers of atopy connected to disease activity (SCORAD, eosinophil cell count) and degree of allergic sensitization.

The fact that total IgE levels seem to be more accurate markers of disease activity and sensitivity can be explained by the complex role of IL-16 in the pathogenesis of AD. Total IgE may influence atopic inflammation through different pathways, such as interaction with FcεRI on mast cells and basophils, resulting in immediate hypersensitivity reactions, or interactions with FcεRII on B cells, resulting in activation of immune responses by T cells [25, 26]. IL-16 can take part in the development of inflammation as a chemoattractant, but it also has immunoregulatory functions. As a chemoattractant, synthesized by T cells, B cells and eosinophils, IL-16 may have a role in the initiation of cellular and humoral immunity by mediating the cellular crosstalk between T cells, B cells, dendritic cells and eosinophils, which leads to the recruitment of these cells at the site of inflammation in allergic diseases [14]. In addition, an inhibitory effect of IL-16 on IgE production was described, suggesting the regulatory role of IL-16 in AD [15]. IL-16 stimulation results in enhanced migration of the CD25+ CTLA-4+ FOXP3+ T cell subpopulation and is capable of suppressing the proliferation and production of Th2 cytokines by autologous T cells [27].

In summary, we can demonstrate that although serum IL-16 levels showed some correlation with the degree of sensitization in patients with AD, serum total IgE levels were found to be more accurate markers of this phenomenon.

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