

IL-16 SERUM LEVEL IN CHILDREN WITH ATOPIC DERMATITIS

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IL-16 is a natural ligand of CD4 molecules and induces chemotaxis in CD4-expressing cells. It amplifies the inflammatory reaction by stimulating cytokine production in monocytes and activating T-cells. There is evidence that IL-16 plays a role in the pathogenesis of atopic dermatitis, and increased serum levels of IL-16 have been detected in allergic diseases. However, few data are available on IL-16 serum levels in atopic dermatitis. The aim of our study is to measure IL-16 serum levels in childhood atopic dermatitis before and after treatment and to evaluate a possible correlation between IL-16 serum levels and disease severity. IL-16 serum levels were measured by an ELISA approach in 34 children (19 males and 15 females; mean age 6.8 years) with moderate to severe atopic dermatitis, at their first visit and after 3 months of treatment, and in 10 non-atopic healthy controls of the same age group. The severity of atopic dermatitis was measured by SCORAD index. IL-16 serum levels were significantly higher in patients affected by atopic dermatitis than in controls before and after treatment with tacrolimus ointment. No clear correlation was found between IL-16 serum levels and atopic dermatitis severity. IL-16 serum levels are increased in atopic dermatitis but do not seem to correlate with disease severity.

There is evidence that IL-16 plays a role in the pathogenesis of atopic diseases such as allergic asthma and atopic dermatitis (AD) (1-3). Several studies suggest CD4⁺ helper T-cells infiltrating the lesional skin play a pivotal role in the pathogenesis of AD (4). Among chemotactic factors, interleukin-16 (IL-16) has been fully characterized as a main immunomodulatory cytokine in the recruitment of CD4⁺ T-cells at sites of inflammation (5-6). IL-16 is a natural soluble ligand to the CD4 molecule and induces activation of CD4⁺ T-cells. Furthermore, it promotes chemotaxis in CD4-expressing cells such as CD4⁺ T-cells, monocytes and eosinophils (7) and amplifies inflammatory reaction by stimulating cytokine production in monocytes and activating T-cells.

Increased serum levels of IL-16 have been detected in allergic diseases, including asthma (8). In AD lesions and positive atopy patch test reactions increased storage of IL-16 has been observed in epidermal dendritic cells. Furthermore, stimulation of dendritic cells, derived from patients with AD through the high-affinity IgE receptor, was shown to enhance the production of IL-16 in these cells (2, 9). Three recent studies reported elevated serum levels of IL-16 in children (10) and adults (11-12) with AD, suggesting that IL-16 serum level may be a marker of AD severity and activity. As a matter of fact, at present there is no objective laboratory parameter for AD severity and activity.

In order to understand if IL-16 serum level can

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be used in clinical practice as an objective laboratory parameter for AD severity and activity we evaluated IL-16 serum levels in children with AD both before and after treatment with tacrolimus ointment, and the results were correlated with disease severity as measured by SCORAD index.

MATERIALS AND METHODS

Patients

34 patients (19 males and 15 females; aged 2-13 years, mean age 6.8 years) with moderate to severe AD according to Hanifin & Rajka criteria were recruited from the Dermatology Unit of the Pediatric Department of the University of Padua. Informed consent was obtained from the patients' parents. The control group consisted of 10 non-atopic healthy subjects of the same age group. Peripheral blood samples from all subjects were obtained at the first visit and serum was separated and stored at -80°C until use. Serum was again obtained and stored after 3 months of treatment with tacrolimus ointment.

Evaluation of disease severity and activity

Clinical severity scores of AD and disease activity were calculated by SCORAD index (13) at the beginning of the study and after 3 months of treatment with tacrolimus ointment.

ELISA for IL-16

Serum levels of IL-16 were measured by an enzyme-linked immunosorbent assay (ELISA) using a commercially available kit according to the manufacturer's instructions (Interleukin-16 Human Biotrak ELISA system, Amersham Biosciences). Briefly, a monoclonal antibody specific for IL-16 was pre-coated onto a microplate. Standards and samples were added to the wells and any IL-16 present was bound by the immobilized antibody. After being washed to remove the unbound substances, an enzyme-linked polyclonal antibody specific for IL-16 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells, and colour developed in proportion to the amount of IL-16 bound. The intensity of the colour was measured with an automated plate reader (Sclavo). The sensitivity of the kit in detecting IL-16 was $<8\text{pg/ml}$. All serum samples were assayed in duplicate.

Statistical analysis

Statistical analysis was performed with commercially available statistical software (SPSS, Chicago, IL, USA), and $p < 0.05$ was considered to indicate statistical significance. Data were evaluated by non-parametric tests.

Mann-Whitney test was performed to compare the serum levels of IL-16 between patients with AD, before and after treatment, and control subjects. The severity scores and serum levels of IL-16 at the beginning of the observation and after 3 months of treatment were compared using the Wilcoxon test, and correlation analysis was performed by Spearman rank-order method.

RESULTS

At the beginning of the study all patients were affected by moderate to severe AD according to SCORAD index. After 3 months of treatment with tacrolimus ointment all the patients showed significant clinical response, with reduction of erythematous, papulo-desquamative, lichenoid lesions. Mean SCORAD index was 61.87 (range 40-87.1) at the beginning of the study; while at the follow-up examination, after 3 months of treatment, the median value of SCORAD index had dropped to 20.4 (range 0-57.1), and the decrease was statistically significant ($p < 0.0001$) compared with day 0.

Serum IL-16 was detectable in all AD patients. Mean value at the beginning of the study was 963.85 pg/ml (range 118-2900 pg/ml) and after 3 months of treatment was 1223.26 pg/ml (range 144-3000 pg/ml) ($p = 0.03$). In healthy controls the mean value was 313 pg/ml (range 50-650 pg/ml). Therefore, the serum levels of IL-16 in patients with AD were significantly higher compared with controls at the beginning of the study ($p = 0.002$) and after 3 months of treatment ($p = 0.002$) (Fig. 1). No clear correlation was found between IL-16 levels and SCORAD index (Fig. 2).

DISCUSSION

IL-16 is a chemoattractant for a variety of CD4-bearing immune cell (lymphocytes, monocytes, activated eosinophils, dendritic cells, neuronal cells) (7). It is produced by several immune and non immune cell types (CD8+ and CD4+ T-cells, mast cells, eosinophils, dendritic cells, epithelial cells, fibroblasts) both constitutively and under stimuli such as histamine, antigen, mitogen, cytokines and complement fragments (4, 11). In addition to cell migration, IL-16 induces functional high-affinity IL-2 receptors on CD4 cells, thus contributing to cellular activation and proliferation. Moreover, it is able

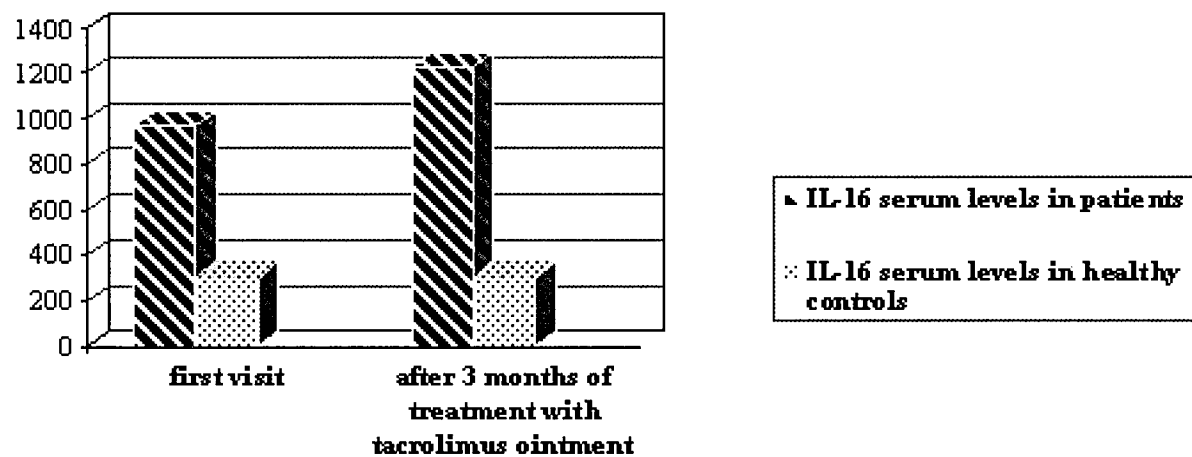


Fig. 1. IL-16 serum levels (pg/ml) in patients with respect to healthy control subjects at the beginning of the study and after 3 months of treatment with tacrolimus ointment.

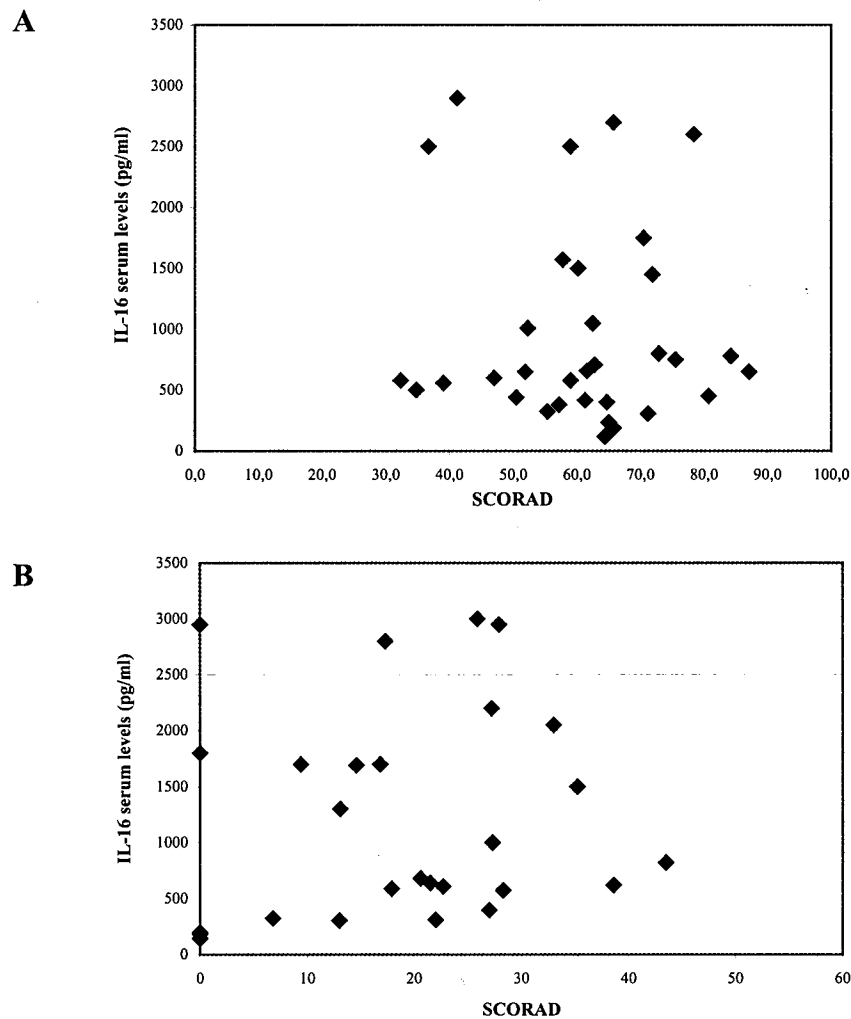


Fig. 2. Not significant correlation between SCORAD index and the serum levels of IL-16 (pg/ml) at the beginning of the study (A) and after 3 months of treatment with tacrolimus ointment (B).

to activate expression and production of proinflammatory cytokines such as IL-1 β , IL-6, IL-15 and tumor necrosis factor (TNF)- α in human monocytes. The role of IL-16 in human diseases has been largely investigated, although the specific biological function is still a matter of debate. IL-16 has been described as the main cytokine able to induce CD4+ cell recruitment and activation at sites of inflammation during asthma and allergic disease.

Three recent studies show increased IL-16 serum levels in patients with AD and a positive correlation between IL-16 levels and disease severity (10-12). In our study, IL-16 serum levels were significantly higher in patients with AD than in healthy controls, both at the beginning of the study and after 3 months of treatment ($p=0.002$, Fig. 1), but we could not demonstrate a positive correlation with disease severity (Fig. 2). We could not show any decrease of IL-16 serum levels at time 0 and after 3 months of treatment, although there was an improved SCORAD index after treatment. IL-16 levels were generally higher in patients than in healthy controls, but in patients did not show any significant change in relation to disease activity. Our results raise doubts on the opportunity of using IL-16 serum levels as an objective laboratory parameter of AD severity.

Our results show increased IL-16 serum levels in AD. These elevated levels of IL-16 could be explained by a genetic predisposition in AD patients to enhanced production of IL-16 and may not be directly correlated to disease activity. The biochemical mechanisms underlying the excessive production of certain pro-inflammatory mediators are probably multiple. A disregulated cytokine production can be primarily involved in the pathophysiology of atopic disease. Experimental evidence indicates that keratinocytes in AD have a disregulated activity of specific constituents of the AP-1 complex, known to be critically involved in the control of the expression of numerous inflammatory genes (14). Furthermore, functionally relevant polymorphisms (i.e. polymorphisms that affect cytokine production) have been identified in a number of human cytokine genes, and the results of epidemiologic studies indicate an association between such polymorphisms and chronic inflammatory and autoimmune disorders (15). There is also evidence that cytokine gene polymorphisms contribute to the genetic basis of inflammatory skin conditions (16-19).

In conclusion, the elevated levels of IL-16 in patients with AD could be explained by a genetic predisposition to enhanced production of IL-16. Further investigations are required to clarify the pathogenic role and clinical significance of IL-16 in AD.

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