Genetics and Molecular Research

Correlation between serum IL-16 and atopic dermatitis

H.Y. Zheng, L. Zhao, C.X. Li and S.H. Li

Department of Dermatology, Shandong Jining First People's Hospital, Jining, Shandong, China

Corresponding author: S.H. Li
E-mail: lishaohuijn@126.com / lshhui168@163.com

Genet. Mol. Res. 15 (1): gmr.15016556
Received August 17, 2015
Accepted November 27, 2015
Published March 24, 2016
DOI http://dx.doi.org/10.4238/gmr.15016556

ABSTRACT. This study aims to investigate the correlation between allergic sensitization of atopic dermatitis (AD) patients and their serum interleukin (IL)-16 levels. AD patients, healthy volunteers, and patients with psoriasis (N = 80, 35, 20, respectively) were tested for serum IL-16 and total and specific IgE levels by enzyme-linked immunosorbent assay, along with eosinophil counts. Serum allergen-specific IgE levels were determined, and skin-prick testing conducted in a subgroup of 45 AD patients. Based on specific IgE levels, AD patients were categorized into non-sensitized group 1 and sensitized group 2. Furthermore, they were sorted as non-sensitized group A and sensitized group B based on skin-prick results. Next, the serum IL-16 and total IgE levels in these subgroups were determined. Compared to levels in healthy volunteers and psoriasis patients, the serum IL-16 levels in AD patients were significantly higher ($P < 0.001$). Additionally, total serum IgE levels were significantly correlated with serum IL-16 levels and eosinophil counts. However, no correlation was observed between serum IL-16 levels and eosinophil counts. The serum IL-16 and total IgE levels in group 2 were also significantly elevated ($P < 0.001$) in contrast to those in group 1. Although we did not observe any significant difference between serum IL-16 levels in groups A and B, the total serum IgE level in group B was significantly higher than that in group A ($P < 0.001$). Thus, allergic

sensitivity in AD patients correlates with total serum IgE as well as serum IL-16; the correlation with IL-6 is weaker.

Key words: IL-16; Atopic dermatitis; Psoriasis

INTRODUCTION

Atopic dermatitis (AD), a highly pruritic, chronic inflammatory skin disease affects 20% of the world's children and can extend to adulthood. The severity of the disease is graded by the AD score (SCORAD) index (Angelova-Fischer et al., 2006). Elevated levels of total IgE and eosinophils in the serum are generally acknowledged as important indicators of this disease. Several reports revealed that total serum IgE plays an important role in the occurrence of AD and progression of dermatitis. Interleukin-16 (IL-16), a cytokine secreted by T cells, eosinophils, and mast, airway epithelial, dendritic, and other cells (Deng and Shi, 2006) was initially identified as a ligand of CD4. IL-16 promotes chemotaxis of all $CD4^+$ cells, especially $CD4^+$ T cells.

Previous studies show that serum IL-16 significantly correlates with the SCORAD index, which reflects the severity of AD. Thus, the serum IL-16 level might be a suitable marker for progression of AD (Angelova-Fischer et al., 2006). However, the correlation between serum IL-16 and degree of allergic sensitization has not been investigated extensively. In this study, we detected the levels of serum IL-16 in AD patients, and analyzed the relationship between serum IL-16 and other parameters that reflect allergen sensitization in AD patients.

MATERIAL AND METHODS

Demographic characteristics of patients

We recruited 80 AD patients (45 males and 35 females), whose ages ranged from 8 to 50 years, with an average age of 19.6 years. All patients met the diagnostic criteria of Hanifin and Rajka (Böhme et al., 2000). The severity of the disease was evaluated by the SCORAD index. The mean SCORAD value was 29, ranging from 8 to 56. Prior to blood sampling, we ensured that none of the patients was treated with any glucocorticoid or other systemic immune-modulators for at least 4 weeks, and any antihistamine or topical corticosteroids for at least 5 days. The subjects were divided into three groups: healthy volunteers (control group, N = 35), AD patients (AD group, N = 80), and psoriasis patients [psoriasis group (patients diagnosed with chronic plaque psoriasis), N = 20]. Subjects in the psoriasis group were not treated with any local or systemic immunomodulatory drug; their average psoriasis area and severity index was 14.2. The age and gender of all subjects in the three groups were matched. All three groups were tested for the serum IL-16 and total IgE levels, and their eosinophil counts recorded. In addition, a subgroup of 45 AD patients underwent serum allergen-specific IgE determination and skin-prick testing. Based on the specific IgE levels, AD patients were divided into two groups: group 1 (non-sensitized, N = 13), including patients with no detectable specific IgE, and group 2 (sensitized, N = 32), including those with one or more IgE types. The skin-prick testing was performed according to standard procedures. Nine different allergens were tested, including house dust mite, house dust mite powder, birch pollen, dander, *Timothy*, *Alternaria*, milk, and egg white and yolk. Based on the results of skin-prick testing, the patients were divided into non-sensitized group A (tested negative, N = 23) and sensitized group B (tested positive, N = 22).

Eosinophil counting

The percentage and absolute number of blood eosinophils were determined by the MEK-7222K automatic hematology analyzer (Nihon Kohden, Tokyo, Japan) as routine clinical tests.

Detection of serum IL-16, and total and specific IgE levels by ELISA

Serum IL-16 levels were determined using the solid-phase sandwich ELISA kit (Shenzhen Jingmei Biological Engineering). The “sensitive screen” allergen detection system and related kits were purchased from the Adaltis Company (Bologna, Italy). Solid-phase sandwich ELISA was also applied to detect total serum IgE, as well as common food and inhalant allergen-specific IgE. All experiments were performed in triplicates.

Statistical analyses

The data were analyzed using the SPSS 15 software (SPSS Company, USA). The measurements of serum concentration of IL-6 and IgE are reported as means ± SD. Differences among subgroups were determined by the Student *t*-test. The variables were analyzed by linear correlation regression. A P value <0.05 was considered statistically significant.

RESULTS

Differential serum IL-16 levels in healthy controls, and psoriasis and AD patients

The levels of serum IL-16 in the AD group were significantly higher than those in control ($P < 0.001$) and psoriasis ($P < 0.001$) groups. The concentrations were 185.03 + 50.51, 60.19 + 5.43, and 68.25 + 2.47 pg/mL for the AD, control, and psoriasis groups, respectively.

Correlation between levels of serum IL-16, total IgE, and the number of eosinophils

A significant correlation was observed between the levels of serum IL-16 and total IgE in AD patients ($r = 0.46$, $P < 0.001$). Results are shown in Figure 1. Total serum IgE levels and the eosinophil counts also correlated significantly ($r = 0.49$, $P < 0.001$; Figure 2). However, serum IL-16 levels and the number of eosinophils did not correlate. The SCORAD index positively correlated with the levels of total serum IgE ($r = 0.35$, $P = 0.088$). Moreover, no correlation was observed between the SCORAD index and serum IL-16 levels.

Analyses of AD patient subgroups

The AD patients were categorized in either sensitized (groups 2 and B) or non-sensitized (groups 1 and A) subgroups. These subgroups were tested further for their serum IL-16 and total IgE levels. The levels of serum IL-16 were significantly higher in group 2 than in group 1 ($P < 0.001$); no significant difference was observed between groups A and B ($P = 0.08$). Meanwhile, the total serum IgE levels in groups 2 and B were also significantly elevated compared with those in groups 1 and A, respectively ($P < 0.001$ in each case), as shown in Table 1.

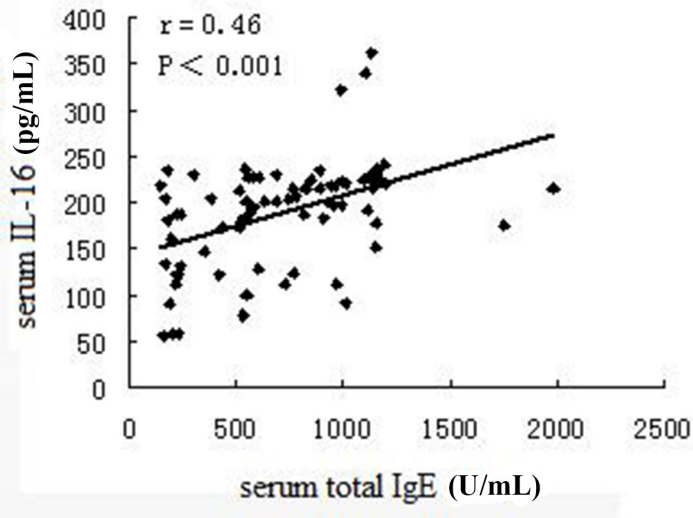

Figure 1. Correlation between serum IL-16 and total IgE levels in AD patients.

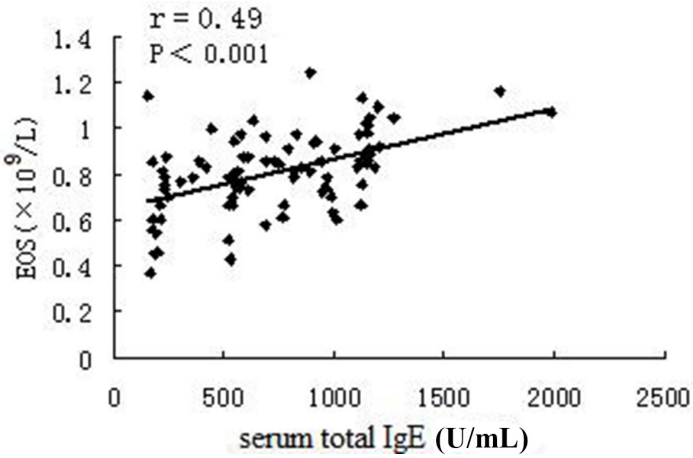

Figure 2. Correlation between eosinophil (EOS) counts and total serum IgE levels.

Table 1. Serum IL-16 and total IgE levels in each subgroup.

Group	No. of cases	IL-16 (pg/mL)	IgE (U/mL)
1	13	103.37 ± 41.13	199.99 ± 26.06
2	32	167.51 ± 49.06*	1083.11 ± 149.02*
A	23	154.87 ± 52.80	516.66 ± 145.83
B	22	182.91 ± 57.25#	959.89 ± 216.74**

Compared with group 1, *P < 0.001; compared with group A, #P = 0.08, **P < 0.001.

DISCUSSION

AD is a chronic inflammatory skin disease. During the process of AD development, imbalanced Th1/Th2 differentiation leads to abnormal secretion of cytokines. These cytokines mainly include IL-4 and IL-13, secreted by Th2 cells in acute skin injury, and IFN-γ secreted by Th1 cells in the chronic stage of AD (Lee et al., 2012).

IL-16 plays different roles in different pathological processes as an immunomodulatory cytokine. As a chemokine, IL-16 drives the chemotactic movement of $CD4^+$ Th cells, monocytes, and eosinophils toward sites of inflammation, promotes expression of the alpha chain of the IL-2 receptor, and activates $CD4^+$ T cells synergistically with either IL-2 or IL-15. Further, as a proinflammatory cytokine, IL-16 promotes inflammatory reactions by stimulating cytokine production by monocytes and mature macrophages.

In this context, IL-16 promotes the secretion of proinflammatory cytokines in allergic diseases. In AD patients, activated epidermal Langerhans cells, induced by allergens, secrete IL-16. IL-16 in turn recruits and activates dendritic cells, T cells, and eosinophils. Therefore, it is evident that IgE-mediated inflammatory response and cell inflammation are related. In addition, allergen-induced eosinophils also secrete IL-16. This IL-16 in turn recruits more eosinophils, capable of producing IL-4, to the sites of inflammation. This is believed to be one of the mechanisms underlying exacerbation of AD (Karaki et al., 2005). In acute and chronic AD lesions, the expression of IL-16 mRNA increases in epidermal keratinocytes and skin T lymphocytes. The number of cells positive for IL-16 mRNA in the acute-lesion stage is significantly higher than that in the chronic period, which might be related to the number of $CD4^+$ cells in the epidermis and dermis. These results indicate that IL-16 plays a potentially important role in the occurrence and development of AD skin inflammation.

The correlation between serum IL-16 levels and severity of AD has been confirmed by several studies. However, to the best of our knowledge, there are no reports on the correlation between serum IL-16 and allergen sensitization.

In this study, we found that levels of serum IL-16 in the AD patients group were significantly higher compared with those in the control group. In addition, IL-16 levels were also significantly different between the AD and psoriasis patients. These results are in accordance with the results of Masuda et al. (2003) who showed that, in contrast to the levels in psoriasis patients, the serum IL-16 levels in AD patients were significantly higher. The authors also concluded that dermal infiltration of monocytes/macrophages in AD patients might be a reason for elevated serum IL-16 levels. In addition, the differential Th1/Th2 imbalance in patients with either psoriasis or AD might lead to the differential serum IL-16 levels between the two kinds of inflammation.

The correlation between elevated serum IL-16 levels and AD is widely studied. Although serum IL-16 levels are generally associated with the SCORAD index (Wu et al., 2011), its relevance remains controversial; they did not find any relationship between total serum IgE and IL-16 levels. However, Masuda et al. (2003) detected positive correlations between serum total IgE and IL-16 levels and between serum IL-16 levels and scratch score in AD patients. In this study, we observed significant correlations between serum IL-16 and total IgE levels, and between total IgE levels and eosinophil counts. A positive, but not significant, association was also found between the SCORAD index and total IgE. In addition, the SCORAD index did not correlate with serum IL-16 levels.

Based on their sensitivities to specific IgE detection and skin-prick test, the AD patients were divided into either non-sensitized or sensitized groups. Both were tested for serum total IgE

and IL-16 levels. The results revealed that compared with the non-sensitized group 1, serum IL-16 and total IgE levels were significantly higher in the sensitized group 2. The serum IL-16 levels did not differ significantly between the non-sensitized group A and sensitized group B; however, group B had a significantly higher level of total serum IgE than did group A. These data indicate that although the serum IL-16 is correlated with allergic sensitization, the extent is not as significant as that between total IgE and allergic sensitization. Compared with serum IL-16, total IgE and either atopic disease activity index (SCORAD, number of eosinophils) or allergen sensitivity are better correlated.

In fact, total serum IgE as a more accurate index to assess disease progression and sensitivity can explain the complicated role of IL-16 in the pathogenesis of AD. Total serum IgE might influence allergic inflammation in different ways. For example, its binding to FcR I on the surface of mast cells and basophils results in immediate hypersensitivity, and in combination with B cell surface FcR II, it initiates T cell-mediated immune responses (Bandeira-Melo et al., 2002; Lugović et al., 2005). IL-16 performs both chemotactic and immunoregulatory functions in inflammation. As a synthetic chemokine of T cells, B cells, and eosinophils, IL-16 recruits cells that initiate cellular and humoral immune responses to the inflammatory sites, leading to allergic diseases. Studies have reported the inhibition of IL-16 upon generation of IgE, suggesting the immunoregulatory function of IL-16 in AD (Trudelle et al., 2007). IL-16 also inhibits the proliferation of Th2 cells and secretion of Th2-type cytokines by enhancing the migration ability of CTLA-4$^+$CD25$^+$Foxp3$^+$ T cell subsets (McFadden et al., 2007).

In conclusion, this study demonstrates that although serum IL-16 levels are associated with the degree of allergenic sensitization in AD patients, the total serum IgE level holds potential as a more accurate indicator.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Shanghai Xiwen Biological Technology Co. Ltd. for their academic advice on this study.

REFERENCES

Angelova-Fischer I, Hipler UC, Bauer A, Fluhr JW, et al. (2006). Significance of interleukin-16, macrophage-derived chemokine, eosinophil cationic protein and soluble E-selectin in reflecting disease activity of atopic dermatitis--from laboratory parameters to clinical scores. *Br. J. Dermatol.* 154: 1112-1117.http://dx.doi.org/10.1111/j.1365-2133.2006.07201.x

Bandeira-Melo C, Sugiyama K, Woods LJ, Phoofolo M, et al. (2002). IL-16 promotes leukotriene C(4) and IL-4 release from human eosinophils via CD4- and autocrine CCR3-chemokine-mediated signaling. *J. Immunol.* 168: 4756-4763.http://dx.doi.org/10.4049/jimmunol.168.9.4756

Böhme M, Svensson A, Kull I and Wahlgren CF (2000). Hanifin's and Rajka's minor criteria for atopic dermatitis: which do 2-year-olds exhibit? *J. Am. Acad. Dermatol.* 43:785-792.

Deng JM and Shi HZ (2006). Interleukin-16 in asthma. *Chin. Med. J.* 119: 1017-1025.

Karaki M, Dobashi H, Kobayashi R, Tokuda M, et al. (2005). Expression of interleukin-16 in allergic rhinitis. *Int. Arch. Allergy Immunol.* 138: 67-72.http://dx.doi.org/10.1159/000087359

Lee J, Noh G, Lee S, Youn Y, et al. (2012). Atopic dermatitis and cytokines: recent patents in immunoregulatory and therapeutic implications of cytokines in atopic dermatitis - part I: cytokines in atopic dermatitis. *Recent Pat. Inflamm. Allergy Drug Discov.* 6: 222-247.http://dx.doi.org/10.2174/187221312802652820

Lugović L, Lipozencić J and Jakić-Razumović J (2005). Prominent involvement of activated Th1-subset of T-cells and increased expression of receptor for IFN-gamma on keratinocytes in atopic dermatitis acute skin lesions. *Int. Arch. Allergy Immunol.* 137: 125-133.http://dx.doi.org/10.1159/000085468

Masuda K, Katoh N, Okuda F and Kishimoto S (2003). Increased levels of serum interleukin-16 in adult type atopic dermatitis. *Acta Derm. Venereol.* 83: 249-253.http://dx.doi.org/10.1080/00015550310016472

McFadden C, Morgan R, Rahangdale S, Green D, et al. (2007). Preferential migration of T regulatory cells induced by IL-16. *J. Immunol.* 179: 6439-6445.http://dx.doi.org/10.4049/jimmunol.179.10.6439

Trudelle A, El Bassam S, Pinsonneault S, Mazer B, et al. (2007). Interleukin-16 inhibits immunoglobulin e production by B lymphocytes. *Int. Arch. Allergy Immunol.* 143: 109-118.http://dx.doi.org/10.1159/000098659

Wu KG, Li TH, Chen CJ, Cheng HI, et al. (2011). Correlations of serum Interleukin-16, total IgE, eosinophil cationic protein and total eosinophil counts with disease activity in children with atopic dermatitis. *Int. J. Immunopathol. Pharmacol.* 24: 15-23.