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Original Article

Serum IL-21 levels are elevated in atopic dermatitis patients with acute skin lesions

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Abbreviations:
AD, atopic dermatitis; EASI, eczema area and severity index; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; LDH, lactate dehydrogenase; Th, T helper; TARC, thymus and activation-related chemokine; TSLP, thymic stromal lymphopoietin

ABSTRACT

Background: Interleukin (IL)-21 is a member of the type I cytokine family and plays a role in the pathogenesis of T helper type 2 allergic diseases. It has been reported that IL-21 expression is upregulated in acute skin lesions in atopic dermatitis (AD) patients; however, little is known about the serum IL-21 levels of AD patients. The aim of this study was to quantify the serum IL-21 levels of AD patients and to evaluate the relationships between the serum IL-21 level and disease severity, laboratory markers, and eruption type in AD patients.

Methods: We measured the serum IL-21 levels of adult AD patients and healthy control subjects using an enzyme-linked immunosorbent assay.

Results: The adult AD patients exhibited significantly higher serum IL-21 levels than the healthy control subjects. A comparison of the patients' serum IL-21 levels based on the clinical severity of their AD revealed that the patients with severe AD demonstrated significantly higher serum IL-21 levels than those with mild AD and the healthy control subjects. The serum IL-21 levels were significantly correlated with the skin severity score, and especially with the degree of acute lesions such as erythema and edema/papules. The serum IL-21 level was not associated with laboratory markers, such as the serum IgE level, the serum thymus and activation-related chemokine level, blood eosinophilia, and the serum lactate dehydrogenase level.

Conclusions: These results suggest that IL-21 might be involved in the pathogenesis of AD, especially the development of acute skin lesions.

Introduction

Atopic dermatitis (AD) is a common allergic skin disease that is caused by genetic and environmental background factors.[1] It is characterized by the disturbance of epithelial barrier function and IgE-mediated sensitization to food and environmental allergens. Genetic sequencing has identified several mutations in genes that encode epidermal structural proteins, such as filaggrin,[2–4] and cytokines related to IgE synthesis, such as interleukin (IL)-4, IL-5, IL-12, IL-13, or granulocyte/macrophage-colony stimulating factor.[5] T helper (Th) 2 cytokines, including IL-4, IL-5, and IL-13, predominate in the acute phase of AD, leading to increased expression of interferon-γ, IL-12, and IL-5 in the chronic phase of the condition.[1]

IL-21 is a member of the type I cytokine family and is produced by lymphoid cells, such as activated $CD4^+$ T cells, natural killer T cells, and Th17 cells.[6–10] The effects of IL-21 are mediated by the binding of IL-21 to a heterodimeric receptor, which consists of common γ-chain subunit and a unique receptor (named IL-21R).[6–10] The common γ-chain subunit is shared with the IL-2, IL-4, IL-7, IL-9, and IL-15 receptors. The IL-21R is expressed on immune cells, such as naïve and activated T cells, B cells, natural killer cells, dendritic cells, and macrophages, as well as certain types of non-immune cells, including keratinocytes and endothelial cells.[6–10]

Several studies have shown that IL-21 contributes to the pathogenesis of allergic diseases. Serum IL-21 levels increase during the acute exacerbation of asthma, and they fall again after treatment.[11] In a recent study by Jin *et al.*, it was found that IL-21 and IL-21R expression were upregulated in the acute skin lesions of AD patients.[12] These findings suggest that IL-21 signaling plays an important role in skin inflammation in AD. Until now, no detailed

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clinical data about the serum IL-21 levels of AD patients or the relationship between the serum IL-21 level and the severity of AD have been reported.

Methods

Subjects

A total of 79 adults patients (49 males, 30 females; median age: 36.3 years old; range: 16–62 years old) with AD were randomly selected and registered for this study. All patients met the diagnostic criteria outlined by Hanifin and Rajka.[13] The severity of their AD was evaluated using the eczema area and severity index (EASI) score[14] (median score: 22.5; range: 1.5–58.1). Based on the EASI score, the AD patients were divided into 3 groups: the mild (score: <10), moderate (score: 10–20), and severe (score: >20) groups. Skin lesion severity (the scores for erythema, edema/papules, oozing/crusting, excoriation, lichenification, and xerosis) and itching severity were assessed in the worse affected areas of skin, as described previously.[15,16] Peripheral blood samples were obtained from each patient during the exacerbation of their eruptions. The peripheral eosinophil count (median: 662/μl; range: 0–3220/μl; reference range: 50–590/μl), and the serum levels of IgE (median: 12,846 IU/ml; range: 125–80,400 IU/ml; reference range: 0–380 IU/ml), lactate dehydrogenase (LDH) (median: 288 IU/L; range: 114–762 IU/L; reference range: 140–243 IU/L), and thymus and activation-related chemokine/chemokine ligand 17 (TARC/CCL17) (median: 5172 pg/mL; range: 125–46,160 pg/mL; reference range: 0–449 pg/mL) were measured.

Each patient had been suffering from AD for more than three years. The patients were treated with conventional therapies, including topical corticosteroids and calcineurin inhibitors and systemic antihistamines. Patients that were taking systemic corticosteroids or immunosuppressive agents were not included in the study. The control group consisted of 17 healthy subjects (9 males, 8 females; median age: 35.5 years old; range: 28–51 years old) who had not taken any medication for at least 2 weeks. All of the subjects provided written consent, and the study protocol was approved by the university ethics committee and was conducted in accordance with the Declaration of Helsinki.

Measurement of the serum IL-21 level using an enzyme-linked immunosorbent assay (ELISA)

The subjects' serum IL-21 levels were measured using an ELISA kit (human IL-21 platinum ELISA eBioscience, San Diego, CA, USA). The reference range for the ELISA kit was 0–5000 pg/mL. When a sample's value fell outside of the reference range for this kit, the sample was reanalyzed at a higher dilution. All measurements were conducted in duplicate, and mean values were obtained.

Statistical analysis

Data are expressed as mean ± SD values unless otherwise indicated. The Mann–Whitney U test was used to compare the serum IL-21 levels of the AD patients with those of the healthy controls. The Steel test or Steel–Dwass test was used for comparisons among three or more groups because most of the data were not normally distributed. Spearman's rank correlation analysis was used to evaluate the relationships between the serum IL-21 level and the EASI score; each eruption score; the peripheral eosinophil count; or the serum levels of IgE, LDH, or TARC. Differences were considered to be statistically significant when the associated *P*-value was <0.05.

Results

Serum IL-21 levels of AD patients and healthy controls

The AD patients' serum IL-21 levels (median: 454 pg/mL, n = 79) were significantly higher than those of the healthy control subjects (median: 50.6 pg/mL, n = 17) ($P < 0.05$) (Fig. 1a).

Correlation between IL-21 and disease severity in patients with AD

To evaluate the relationship between the serum IL-21 level and skin lesion severity scores, the patients were divided into 3 groups, the mild, moderate, and severe groups, based on their EASI scores. When the serum IL-21 levels of these groups were compared, it was found that the patients with severe AD exhibited significantly higher IL-21 levels than the healthy controls and the patients with mild AD ($P < 0.05$) (Fig. 1b). Serum levels of IL-21 were significantly correlated with EASI scores in patients with AD (n = 79, $r = 0.24$, $P = 0.034$; Fig. 2).

Correlation between IL-21 and eruption type in patients with AD

The correlations between the serum IL-21 level and each skin lesion severity score (erythema, edema/papules, oozing/crusting, excoriation, lichenification, and xerosis scores) were evaluated in the worst affected areas of skin. Among six types of eruptions, erythema, edema/papules, oozing/crusting, excoriation, lichenification, and xerosis, serum IL-21 levels were significantly correlated with the erythema score ($r = 0.24$, $P = 0.034$) and edema/papules score ($r = 0.23$, $P = 0.043$), but not with the oozing/crusting score ($r = -0.12$, $P = 0.33$), excoriation score ($r = 0.15$, $P = 0.20$), lichenification score ($r = -0.082$, $P = 0.48$), or xerosis score ($r = 0.075$, $P = 0.56$) (Fig. 3). The itching score was also not correlated with the serum IL-21 level ($r = 0.15$, $P = 0.24$) (Fig. 3).

Correlation between IL-21 and laboratory markers in patients with AD

Relationship were evaluated between serum levels of IL-21 and laboratory markers that known to be elevated in patients with AD. There was no significant correlation between IL-21 and serum IgE (n = 72, $r = -0.036$, $P = 0.763$), peripheral eosinophil count (n = 71, $r = 0.030$, $P = 0.804$), serum LDH (n = 73, $r = -0.076$, $P = 0.517$), or TARC (n = 75, $r = 0.0080$, $P = 0.945$) (Table 1).

Discussion

The results of our study showed that AD patients displayed significantly higher serum IL-21 levels than healthy controls (Fig. 1a). In a comparison based on disease severity, it was found that only patients with severe AD (EASI > 20) had significantly elevated serum IL-21 levels than healthy controls and patients with mild AD (EASI < 10) (Fig. 1b), and serum IL-21 levels were significantly associated with the EASI score (Fig. 2). The serum levels of IL-21 were also correlated with the erythema score and the edema/papules score among six types of eruptions, erythema, edema/papules, oozing/crusting, excoriation, lichenification, and xerosis (Fig. 3).

In our study, serum IL-21 levels were significantly elevated in patients with severe AD, and they were significantly correlated with the disease severity of AD. These results suggest that IL-21 may contribute to the aggravation of AD lesions. It has been reported that IL-21 and IL-21R expression were markedly upregulated by mechanical injuries induced by subjecting to mouse skin to tape stripping, and such injuries mimic AD lesions.[12] In addition, a

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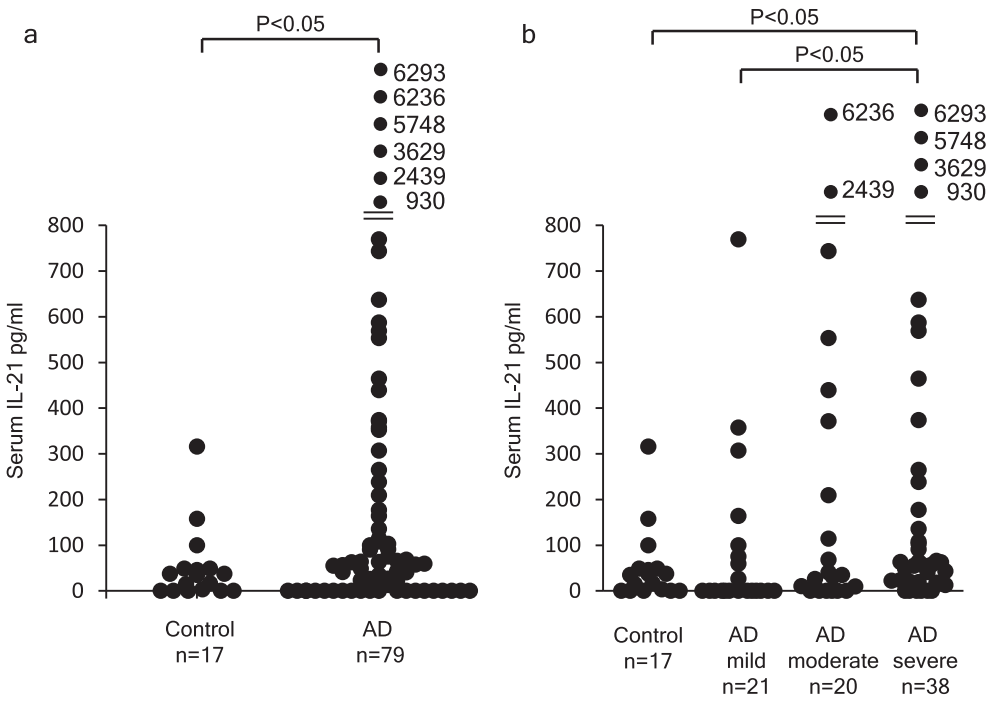

Fig. 1. Serum IL-21 levels of AD patients and healthy control. The serum IL-21 levels of AD patients (n = 79) and healthy controls (n = 17) were quantified (**a**). Mann–Whitney's U test was used to assess the significance of differences. The serum IL-21 levels of AD patients were compared among 3 disease severity groups, the mild (score: <10) (n = 21), moderate (score: 10–20) (n = 20), severe (score: >20) (n = 38) groups (**b**). The Steel test and the Steel–Dwass test were used to assess the significance of differences. The data are expressed as mean ± SD values. $^{*}P < 0.05$.

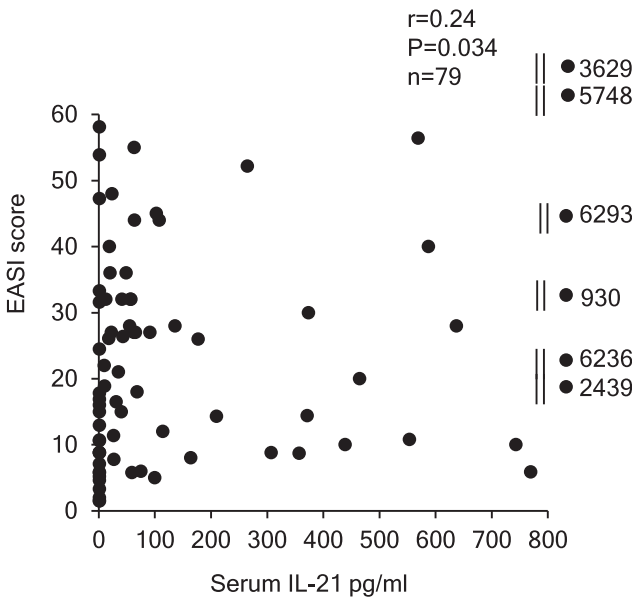

Fig. 2. Correlation between the serum IL-21 level and disease severity in AD patients The correlation between the serum IL-21 level and disease severity (according to the EASI score) was evaluated using Spearman's rank correlation analysis (n = 79).

lack of IL-21R was shown to inhibit the trafficking of skin dendritic cells to draining lymph nodes and Th2-driven allergic skin inflammation induced by epicutaneous sensitization with experimental antigen chicken ovalbumin after tape stripping.[12] Furthermore, IL-21 deficiency suppresses the development of pathogen-induced Th2 responses and Th2 cytokine-dependent fibrosis.[17–19] In our study, serum levels of IL-21 were also correlated with the erythema score and the edema/papules score. Erythema, edema and papule are the main symptoms of acute phase of AD.[20] Our data are consistent with previous findings that IL-21 and IL-21R expressions are upregulated in acute skin lesions of AD.[12] On the other hand, serum IL-21 was low or not detected in AD patients with low degree of acute lesions although their skin severity was severe. Taken together, these findings suggest that it can be stated that IL-21 may increase Th2-type allergic inflammation, leading to the development of acute lesions in AD.

The administration of IL-21 suppresses Th2-driven allergic inflammation, including IgE production, while IL-21 deficiency inhibits the development of Th2-dependent responses. The administration of IL-21 might downregulate IgE production by inhibiting Cε germline transcription and restricting the switching of $IgG1^{+}$ B cells to IgE^{+} B cells in mice.[21,22] Negative correlations between the serum levels of IL-21 and IgE have been observed in a mouse model of allergic rhinitis and in human atopic asthma.[23] Moreover, the administration of IL-21 suppressed mast cell granulation in murine skin affected by immediate-type hypersensitivity.[24] Therefore, IL-21 is considered to be a pleiotropic cytokine that has different effects on immune reactions depending on experimental conditions. Contrary to our findings, a previous study showed that children with AD had lower serum IL-21 levels than the controls.[25] Therefore, the effect of IL-21 on immune reactions in adult AD patients might differ from that seen in atopic children, possibly because of differences between the pathogenic mechanisms of AD between adults and children.

IL-21 is produced by various subsets of activated $CD4^{+}$ T cells. Several studies have suggested that Th2 and T follicular helper cells function in close co-operation during Th2-driven immune responses.[19,26] Interestingly, common allergens, house dust mites were shown to induce IL-21 expression by T follicular helper cells in the lymph nodes of allergic model mice.[19] In AD, the production of thymic stromal lymphopoietin (TSLP) is known to increase in injured skin and to induce Th2-type allergic inflammation.[15,27] It

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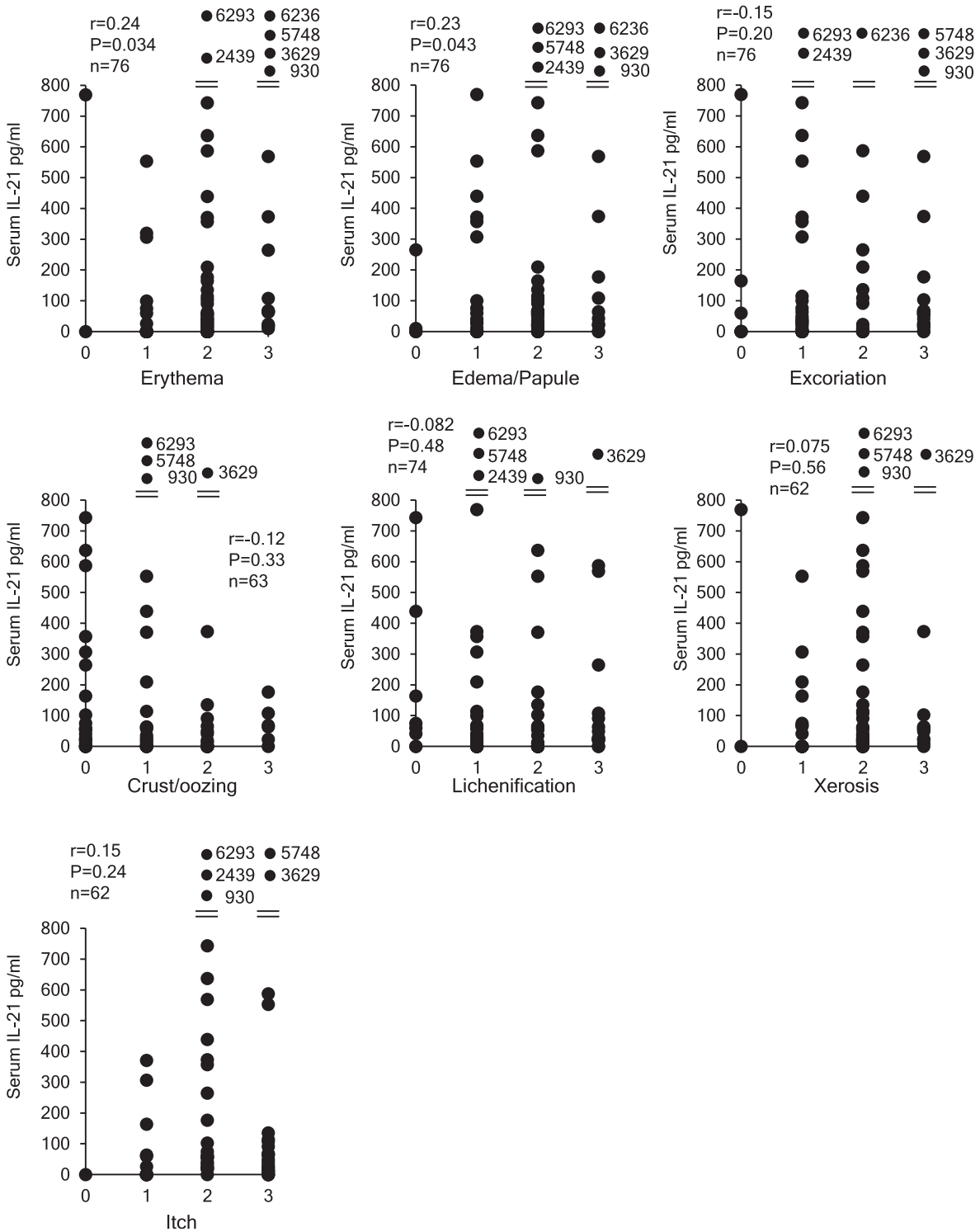

Fig. 3. Correlations between the serum IL-21 level and eruption type in AD patients The correlations between the serum IL-21 level and disease severity (the scores for erythema, edema/papules, oozing/crusting, excoriation, lichenification, and xerosis) were evaluated using Spearman's rank correlation analysis.

Table 1
Correlation between serum interleukin 21 levels and laboratory markers.

	r	*P*
Serum IgE levels (n = 72)	−0.036	0.763
Peripheral eosinophil count (n = 71)	0.030	0.804
Serum LDH (n = 73)	−0.076	0.517
Serum TARC (n = 75)	0.0080	0.945

has been reported that TSLP increases IL-21 production in mast cells.[12] Moreover, exogenous stimuli, such as ultraviolet B, can induce IL-21 production in keratinocytes.[28] Furthermore, it has been reported that serum IL-21 levels increase in patients with other inflammatory skin diseases such as psoriasis.[29] Taken together, these findings suggest that various factors, including exogenous allergens and mechanical injury, can induce IL-21

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production in several types of cells in cutaneous inflammatory conditions.

In conclusion, this study showed that AD patients, especially those with acute lesions, exhibited elevated serum IL-21 levels. Therefore, IL-21 might be associated with the acute aggravation of AD. This cytokine could be an appropriate therapeutic target in adult patients with severe AD symptoms.

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Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

HM and RTM designed the study, collected data, and wrote the manuscript. KM and NN contributed to sample collection. NK wrote the manuscript.

References

1. Bieber T. Atopic dermatitis. *N Engl J Med* 2008;**358**:1483–94.
2. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;**38**:441–6.
3. Sandilands A, Terron-Kwiatkowski A, Hull PR, O'Regan GM, Clayton TH, Watson RM, et al. Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet* 2007;**39**:650–4.
4. Nomura T, Sandilands A, Akiyama M, Liao H, Evans AT, Sakai K, et al. Unique mutations in the filaggrin gene in Japanese patients with ichthyosis vulgaris and atopic dermatitis. *J Allergy Clin Immunol* 2007;**119**:434–40.
5. Morar N, Willis-Owen SA, Moffatt MF, Cookson WO. The genetics of atopic dermatitis. *J Allergy Clin Immunol* 2006;**118**:24–34. quiz 35-6.
6. Gong F, Su Q, Pan YH, Huang X, Shen WH. The emerging role of interleukin-21 in allergic diseases (Review). *Biomed Rep* 2013;**1**:837–9.
7. Spolski R, Leonard WJ. The Yin and Yang of interleukin-21 in allergy, autoimmunity and cancer. *Curr Opin Immunol* 2008;**20**:295–301.
8. Sarra M, Cupi ML, Pallone F, Monteleone G. Interleukin-21 in immune and allergic diseases. *Inflamm Allergy Drug Targets* 2012;**11**:313–9.
9. Spolski R, Leonard WJ. Interleukin-21: basic biology and implications for cancer and autoimmunity. *Annu Rev Immunol* 2008;**26**:57–79.
10. Monteleone G, Pallone F, Macdonald TT. Interleukin-21 as a new therapeutic target for immune-mediated diseases. *Trends Pharmacol Sci* 2009;**30**:441–7.
11. Geng S, Gao S, Hu H, Hu Y, Yu S, Zhao S. [The differentiation and clinical significance of follicular helper T cells during acute exacerbation in asthma patients]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 2015;**31**:1383–6 (in Chinese).
12. Jin H, Oyoshi MK, Le Y, Bianchi T, Koduru S, Mathias CB, et al. IL-21R is essential for epicutaneous sensitization and allergic skin inflammation in humans and mice. *J Clin Invest* 2009;**119**:47–60.
13. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980;**92**:44–7.
14. Hanifin JM, Thurston M, Omoto M, Cherill R, Tofte SJ, Graeber M. The eczema area and severity index (EASI): assessment of reliability in atopic dermatitis. EASI Evaluator Group. *Exp Dermatol* 2001;**10**:11–8.
15. Sano Y, Masuda K, Tamagawa-Mineoka R, Matsunaka H, Murakami Y, Yamashita R, et al. Thymic stromal lymphopoietin expression is increased in the horny layer of patients with atopic dermatitis. *Clin Exp Immunol* 2013;**171**:330–7.
16. Tamagawa-Mineoka R, Okuzawa Y, Masuda K, Katoh N. Increased serum levels of interleukin 33 in patients with atopic dermatitis. *J Am Acad Dermatol* 2014;**70**:882–8.
17. Pesce J, Kaviratne M, Ramalingam TR, Thompson RW, Urban Jr JF, Cheever AW, et al. The IL-21 receptor augments Th2 effector function and alternative macrophage activation. *J Clin Invest* 2006;**116**:2044–55.
18. Frohlich A, Marsland BJ, Sonderegger I, Kurrer M, Hodge MR, Harris NL, et al. IL-21 receptor signaling is integral to the development of Th2 effector responses in vivo. *Blood* 2007;**109**:2023–31.
19. Coquet JM, Schuijs MJ, Smyth MJ, Deswarte K, Beyaert R, Braun H, et al. Interleukin-21-producing CD4(+) T cells promote type 2 immunity to house dust mites. *Immunity* 2015;**43**:318–30.
20. Beltrani VS. The clinical spectrum of atopic dermatitis. *J Allergy Clin Immunol* 1999;**104**:S87–98.
21. Hiromura Y, Kishida T, Nakano H, Hama T, Imanishi J, Hisa Y, et al. IL-21 administration into the nostril alleviates murine allergic rhinitis. *J Immunol* 2007;**179**:7157–65.
22. Kishida T, Hiromura Y, Shin-Ya M, Asada H, Kuriyama H, Sugai M, et al. IL-21 induces inhibitor of differentiation 2 and leads to complete abrogation of anaphylaxis in mice. *J Immunol* 2007;**179**:8554–61.
23. Chatterjee R, Batra J, Ghosh B. A common exonic variant of interleukin 21 confers susceptibility to atopic asthma. *Int Arch Allergy Immunol* 2009;**148**:137–46.
24. Tamagawa-Mineoka R, Kishida T, Mazda O, Katoh N. IL-21 reduces immediate hypersensitivity reactions in mouse skin by suppressing mast cell activation or IgE production. *J Invest Dermatol* 2011;**131**:1513–20.
25. Lin SC, Chuang YH, Yang YH, Chiang BL. Decrease in interleukin-21 in children suffering with severe atopic dermatitis. *Pediatr Allergy Immunol* 2011;**22**:869–75.
26. Baumjohann D, Okada T, Ansel KM. Cutting edge: distinct waves of BCL6 expression during T follicular helper cell development. *J Immunol* 2011;**187**:2089–92.
27. Angelova-Fischer I, Fernandez IM, Donnadieu MH, Bulfone-Paus S, Zillikens D, Fischer TW, et al. Injury to the stratum corneum induces in vivo expression of human thymic stromal lymphopoietin in the epidermis. *J Invest Dermatol* 2010;**130**:2505–7.
28. Kim H, Kang JS, Lee WJ. The production IL-21 and VEGF in UVB-irradiated Human keratinocyte cell line, HaCaT. *Immune Netw* 2010;**10**:75–80.
29. He Z, Jin L, Liu ZF, Hu L, Dang EL, Feng ZZ, et al. Elevated serum levels of interleukin 21 are associated with disease severity in patients with psoriasis. *Br J Dermatol* 2012;**167**:191–3.

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