Am J Clin Exp Immunol 2026;15(2):142-147
www.ajcei.us /ISSN:2164-7712/AJCEI0168083

Original Article

IL-21 expression during sublingual immunotherapy for allergic rhinitis

Chunrui Wang, Xiaowei Qin, Li Jiang, Tianhong Zhang

Department of Otolaryngology, The 1st Affiliated Hospital of Harbin Medical University, Harbin 150001, Heilongjiang, The People's Republic of China

Received August 13, 2025; Accepted March 5, 2026; Epub April 25, 2026; Published April 30, 2026

Abstract: Background: Allergic rhinitis (AR) is an inflammatory disorder involving immunoglobulin E (IgE)-mediated immune response. Interleukin (IL)-21 IL-21 can regulate immune cell functions and IgE production. We aimed to explore the modulation of IL-21 expression before and after sublingual immunotherapy (SLIT) in AR patients and its correlation with IgE and Th2 cytokines. Methods: Fifty AR patients were enrolled and treated with HDM allergen extract via SLIT. Serum levels of IL-21 and Th2 cytokines as well as IgE were determined using enzyme-linked immunosorbent assay (ELISA). Statistical analysis was performed to compare changes in cytokine levels and assess correlations. Results: After 3 years of SLIT, serum levels of IL-21, Th2 cytokines and IgE were significantly reduced. Greater decreases were found in the effective treatment group compared to the ineffective group. The expression of IL-21 was correlated to IL-4 and IgE levels. Conclusion: SLIT downregulates the expression of IL-21 and other Th2 cytokines, with IL-21 potentially serving as a biomarker for SLIT efficacy in AR patients.

Keywords: Allergic rhinitis, IL-21, sublingual immunotherapy, biomarker, Th2 cytokines

Introduction

Allergic rhinitis (AR) is a persistent inflammation of the upper airways, characterized with an abnormal immune response involving the immunoglobulin E (IgE) [1]. The main symptoms included sneezing, pruritus, nasal obstruction, and rhinorrhea. Allergen specific immunotherapy (AIT), such as subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT), represents the only disease-modifying treatments that provide enduring clinical benefits and alter the allergic response [2].

Follicular helper T (Tfh) cells, identified within germinal centers (GCs), are a novel CD4+ T-cell subset that has a pivotal role in orchestrating the pathogenesis of B-cell responses [3]. Tfh cells produce high-levels interleukin (IL)-21 (IL-21), which exerts direct regulatory effects on immune cell functions [3].

IL-21 is a cytokine with a 4-helix bundle structure and shares a common receptor gamma-chain with IL-2 [4]. IL-21 has a diverse functions, including regulation of activation, expansion, and function of various immune cells [5]. IL-21 exhibits both stimulatory and inhibitory regulatory effect on allergic inflammation. Previous studied showed that IL-21 receptor-deficient mice had aberrant IgE production, while exogenous IL-21 administration suppressed IgE synthesis [6, 7]. Conversely, experiments using sorted B cells demonstrated elevated IgE production in the presence of IL-21 [8, 9].

Yang et al. [10] confirmed IL-21 as a universal negative regulator of IgE class-switching. Avery et al. [11] found STAT3 phosphorylation as indispensable for IL-21-driven IgE secretion from human naive B cells. Spolski and Leonard [12]'s review showed IL-21 as possessing both therapeutic promise and pathogenic potential depending on immunological context.

Previous studies had reported Tfh cell alterations following AIT. Schulten et al. [13] found restored Tfh/Tfr equilibrium, while Yao et al. [14] observed recovered Tfr function post-SCIT. Zhang [15] implicated Tfh-derived IL-21 production in asthma pathogenesis. However, the

https://doi.org/10.62347/XBRN4628

changes of serum IL-21 during SLIT is unknown. Therefore, this study aimed to track IL-21 concentrations throughout three years of HDM-SLIT and the difference between responders and non-responders. Our study may provide potential predictor of SLIT efficacy.

Methods

Patients and treatment

A cohort of 50 AR adults patients diagnosed according to the Allergic Rhinitis and its Impact on Asthma Guidelines (ARIA) were recruited from January 2020 to January 2024. Inclusion criteria were: (1) typical nasal symptoms for at least 2 years; (2) sensitization to Dermatophagoides farinae (Der f) and/or Dermatophagoides pteronyssinus (Der p) assessed by positive skin-prick test (SPT) with a wheal ≥3 mm in diameter. Patients with coexisting immune system diseases or systematic diseases were excluded.

House dust mite (HDM) allergen extract were provided by Wolwo Bio-Pharmaceutical (CHANLLERNGEN, Zhejiang Wolwo Bio-Pharmaceutical, China). A daily dose of the drugs was given sublingually for 2 minutes before swallowing, following the manufacturer's instructions. During the maintenance phase, patients received No. 5 (1000 μg/mL) solution after completing No. 1-4 solution (1-333 μg/mL) during concentration increment phase.

Treatment efficacy

During the whole treatment period, patients recorded their daily symptom scores. Symptom were scored using a total nasal symptom score (TNSS). The TNSS was the sum of score for nasal rhinorrhea, sneezing, itching, and congestion on a scale of 0-3 (0 = absent, 1 = mild, 2 = moderate, 3 = severe). Patients achieving ≥30% SMS reduction relative to baseline were group as response group.

Enzyme-linked immunosorbent assay (ELISA) for cytokines

Peripheral venous blood samples (5 mL) were collected at baseline (prior to SLIT initiation) and at the three-year treatment endpoint. Samples were processed within 2 hours of collection. Serum was separated by centrifugation (3000 rpm, 10 minutes), aliquoted, and stored at -80°C until analysis. All samples from individual patients were analyzed simultaneously in a single batch to minimize inter-assay variability.

Serum concentrations of IL-21, IL-4, IL-5, IL-13, and total IgE were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) per manufacturer protocols. All samples were analyzed in duplicate, with mean values used for statistical analysis.

Data analysis

SPSS 16.0 (SPSS Inc., USA) was conducted for statistical analysis. The results were reported in terms of mean ± standard deviation (SD) or median accompanied by its range. For quantitative variables, non-parametric statistical tests were employed for comparisons, namely the Mann-Whitney U test for unpaired samples and the Kruskal-Wallis test for multiple independent groups. Comparisons involving paired data at two different time points were analyzed using the Wilcoxon signed-rank test. Associations between IL-21 and other biomarkers were assessed using Spearman rank correlation coefficients, as serum cytokine levels did not follow normal distribution. Statistical significance was established at a p-value threshold of 0.05.

Results

Baseline characteristics and SLIT efficacy

Fifty adult patients (23 males, 27 females; mean age 25.3±5.6 years) were enrolled between January 2020 and January 2021 (**Table 1**). Among these, 42 patients (84%) completed the three-year study protocol. Eight patients discontinued treatment: six (12%) due to perceived therapeutic ineffectiveness, one (2%) due to mild gastrointestinal adverse events, and one (2%) lost to follow-up due to relocation. The final efficacy analysis included 42 patients: 31 (73.8%) classified as responders and 11 (26.2%) as non-responders. Baseline demographic and clinical characteristics are summarized in **Table 2**.

Table 1. Demographic characteristic of study patients

Characteristics	
Cases	50
Sex (Male:Female)	23:27
Age	25.3±5.6
Duration of symptoms (years)	4.5±1.6
Serum sIgE level to Der p (IU/mL)	24.8 (3.2-113.7)
Serum sIgE level to Der f (IU/mL)	12.5 (2.1-89.1)
Total IgE (IU/mL)	354.7 (212-943.1)

sIgE, specific immunoglobulin E, Der p, Dermatophagoïdes pteronyssinus, Der f, Dermatophagoïdes farinae.

Table 2. Clinical efficacy after 3 years' SLIT

	Effective group	Ineffective group
Cases	31 (73.4%)	11 (26.6%)
Baseline TNSS	8.7±2.6	7.9±2.7
Endpoint TNSS	2.1±1.3*	5.7±3.4
Change of TNSS	5.9±2.2*	1.6±0.8

*Compared with ineffective group, $P<0.05$. TNSS, total nasal symptom score, SLIT, sublingual immunotherapy.

Changes in cytokine and IgE levels after 3 years of SLIT

Following SLIT, serum levels of IL-21, IL-4, IL-5, IL-13, and IgE were significantly decreased compared to baseline (**Table 3**). Moreover, the effective treatment group exhibited greater decreases in these biomarkers compared to the ineffective group (**Table 4**).

Correlation analysis

A significant positive correlation was noted between the expression levels of IL-21 protein and those of IL-4, as well as IgE. Conversely, no statistically significant correlation was detected between IL-21 and either IL-5 or IL-13 (**Table 5**).

Discussion

Numerous studies have sought to identify biomarkers that can predict the suitability for AIT or forecast its outcomes. These biomarkers have the potential to greatly improve patient management during AIT treatments and bolster the effectiveness of clinical trials aimed at developing new AIT products [16-18].

IL-21, predominantly secreted by Tfh cells, plays a critical effect in B-cell proliferation and isotype switching, and modulates diverse immune cell functions. IL-21 stimulates high-level secretion of IgM, IgE, and IgA by mature B-cells [19]. IL-21 are reported to play different roles in allergic diseases. Wang's findings indicated significantly elevated expression of both IL-21 mRNA in peripheral blood mononuclear cells (PBMCs) and IL-21 protein in serum of AR patients. Additionally, the increase in IL-21 correlated with increases in Bcl-6 and total serum IgE [20]. Wood et al. reported that IL-21 augmented IgE production by unfractionated PBMCs or B cells stimulated with anti-CD40 plus IL-4 or IL-13 [9]. Magari's study also demonstrated that IL-21, in combination with prostaglandin E2, induced B-cell apoptosis, whereas IL-21 alone maintained the viability of B cells [21]. On the contrary, Sharif's results showed that IL-4 facilitated GCs reactions and class switch recombination (CSR), whereas IL-21 exerted an inhibitory effect on these processes [22]. Nevertheless, IL-21 demonstrated a cooperative interaction with IL-4 in stimulating the differentiation of plasmablasts, IgE-positive cells, and the production of total IgE (tIgE), which plays a pivotal role in potentiating IgE-mediated allergic reactions [23]. Suto and colleagues demonstrated that IL-21 negatively regulated IgE production by B cells, without exerting any influence on the differentiation of T helper 2 (Th2) cells [6]. In contrast, Caven found that IL-21 suppressed IgE production in vitro at elevated cell densities, whereas it exhibited only a minor stimulatory effect when employing lower cell numbers in culture systems. Collectively, these seemingly contradictory findings suggest that IL-21 exerts context-dependent effects on allergic inflammation, with its net impact likely determined by the local cytokine milieu, cellular density, and stage of immune activation. Within the framework of SLIT-induced immune modulation, which is characterized by a shift from Th2-dominant responses toward regulatory T cell (Treg) activity and immune tolerance, the role of IL-21 remains to be fully integrated.

Our data suggested that SLIT reduced the level of serum IL-21 as well as IL-4 or IgE levels, especially in effective group, suggesting that IL-21 may be used as a potential biomarker for SLIT treatment. Despite IL-5 and IL-13 expression were downregulated after SLIT, no relation was found between the expression of IL-21 and IL-5 or IL-13. Similarly, Sharif's study showed

Table 3. Altered cytokine levels after 3 years' SLIT

	Baseline	Three years' SLIT
IL-21 (pg/mL)	235.7±85.6	57.1±12.3*
IL-4 (pg/mL)	95.7±11.6	41.3±9.8*
IL-5 (pg/mL)	21.3±9.2	8.5±3.3*
IL-13 (pg/mL)	368.1±137.5	177.3±50.4*
TIgE (pg/mL)	467.8±212.3	156.3±48.2*

*Compared with baseline, P<0.05. SLIT, sublingual immunotherapy. TIgE, total immunoglobulin E.

Table 4. Comparison cytokine levels between responsive and unresponsive group after 3 years' SLIT

	Responsive group	Unresponsive group
IL-21 (pg/mL)	36.2±10.1*	69.4±22.1
IL-4 (pg/mL)	22.6±7.3*	57.2±19.4
IL-5 (pg/mL)	6.6±2.9*	12.4±4.1
IL-13 (pg/mL)	119.4±21.6*	257.5±108.3
TIgE (pg/mL)	135.8±26.5*	211.8±53.8

*Compared with responsive group, P<0.05. SLIT, sublingual immunotherapy, TIgE, total immunoglobulin E.

that intranasal allergen challenge induced IL-4, IL-21, and IL-6 expression, while SCIT or SLIT inhibited the expression of these cytokines. These results are consistent with the emerging model that SLIT exerts its immunomodulatory effects not only through direct suppression of Th2 cytokines but also via modulation of Tfh cell activity, as reflected by decreased IL-21 levels. The dissociation between IL-21 and the Th2-associated cytokines IL-5/IL-13 in our study suggests that IL-21 may have a distinct regulatory role during SLIT. Moreover, the downregulation of IL-21 may indicate reduced Tfh-mediated B-cell help, contributing to the downregulation in IgE production - a hallmark of successful SLIT. Future studies examining the relationship between IL-21, Tfh cell frequencies, and regulatory immune populations would further elucidate the precise pathways by which IL-21 contributes to clinical outcomes following SLIT.

Our study had several limitations. First, while we observed an association between reduced IL-21 levels and effective SLIT, we did not assess the predictive value of IL-21 prior to treatment initiation. Therefore, we cannot determine whether pre-treatment IL-21 levels could serve as a predictor of therapeutic response. Secondly, the observed correlations between IL-21, IL-4, and IgE indicate associations rather than causal relationships or predictive utility. Third, the lack of association between IL-21 and type 2 cytokines (IL-5, IL-13) suggests that IL-21 may operate through distinct immunological pathways.

Table 5. Relationship between IL-21 and other cytokines after 3 years' SLIT

	IL-21	
	r	*P*
IL-4 (pg/mL)	0.61	0.03
IL-5 (pg/mL)	0.27	0.28
IL-13 (pg/mL)	0.43	0.23
TIgE (pg/mL)	0.58	0.04

SLIT, sublingual immunotherapy, TIgE, total immunoglobulin E.

In summary, this study demonstrates that SLIT is associated with reduced serum IL-21 levels, particularly in patients showing clinical improvement, and that these changes correlate with modifications in IL-4 and IgE. However, the current design establishes association rather than predictive value. Future prospective studies incorporating baseline biomarker measurements and placebo-controlled designs are warranted to determine whether IL-21 could serve as a clinically useful predictor of SLIT efficacy.

Disclosure of conflict of interest

None.

Address correspondence to: Tianhong Zhang, The 1st Affiliated Hospital of Harbin Medical University, No. 23, Youzheng Street, Nangang District, Harbin 150001, Heilongjiang, The People's Republic of China. E-mail: zhangtianhong3856@163.com

References

[1] Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, Zuberbier T, Baena-Cagnani CE, Canonica GW, van Weel C, Agache I, Aït-Khaled N, Bachert C, Blaiss MS, Bonini S, Boulet LP, Bousquet PJ, Camargos P, Carlsen KH, Chen Y, Custovic A, Dahl R, Demoly P, Douagui H, Durham SR, van Wijk RG, Kalayci O, Kaliner MA, Kim YY, Kowalski ML, Kuna P, Le LT, Lemiere C, Li J, Lockey RF, Mavale-Manuel

S, Meltzer EO, Mohammad Y, Mullol J, Naclerio R, O'Hehir RE, Ohta K, Ouedraogo S, Palkonen S, Papadopoulos N, Passalacqua G, Pawankar R, Popov TA, Rabe KF, Rosado-Pinto J, Scadding GK, Simons FE, Toskala E, Valovirta E, van Cauwenberge P, Wang DY, Wickman M, Yawn BP, Yorgancioglu A, Yusuf OM, Zar H, Annesi-Maesano I, Bateman ED, Ben Kheder A, Boakye DA, Bouchard J, Burney P, Busse WW, Chan-Yeung M, Chavannes NH, Chuchalin A, Dolen WK, Emuzyte R, Grouse L, Humbert M, Jackson C, Johnston SL, Keith PK, Kemp JP, Klossek JM, Larenas-Linnemann D, Lipworth B, Malo JL, Marshall GD, Naspitz C, Nekam K, Niggemann B, Nizankowska-Mogilnicka E, Okamoto Y, Orru MP, Potter P, Price D, Stoloff SW, Vandenplas O, Viegi G and Williams D; World Health Organization; GA(2)LEN; AllerGen. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy 2008; 63 Suppl 86: 8-160.

[2] Drazdauskaitė G, Layhadi JA and Shamji MH. Mechanisms of allergen immunotherapy in allergic rhinitis. Curr Allergy Asthma Rep 2020; 21: 2.

[3] Nurieva RI, Chung Y, Hwang D, Yang XO, Kang HS, Ma L, Wang YH, Watowich SS, Jetten AM, Tian Q and Dong C. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. Immunity 2008; 29: 138-149.

[4] Long D, Chen Y, Wu H, Zhao M and Lu Q. Clinical significance and immunobiology of IL-21 in autoimmunity. J Autoimmun 2019; 99: 1-14.

[5] Saadh MJ, Alfattah MA, Ismail AH, Saeed BA, Abbas HH, Elashmawy NF, Hashim GA, Ismail KS, Abo-Zaid MA and Waggiallah HA. The role of Interleukin-21 (IL-21) in allergic disorders: biological insights and regulatory mechanisms. Int Immunopharmacol 2024; 134: 111825.

[6] Suto A, Nakajima H, Hirose K, Suzuki K, Kagami S, Seto Y, Hoshimoto A, Saito Y, Foster DC and Iwamoto I. Interleukin 21 prevents antigen-induced IgE production by inhibiting germ line C(epsilon) transcription of IL-4-stimulated B cells. Blood 2002; 100: 4565-4573.

[7] Ozaki K, Spolski R, Feng CG, Qi CF, Cheng J, Sher A, Morse HC, 3rd, Liu C, Schwartzberg PL and Leonard WJ. A critical role for IL-21 in regulating immunoglobulin production. Science 2002; 298: 1630-1634.

[8] Pène J, Guglielmi L, Gauchat JF, Harrer N, Woisetschläger M, Boulay V, Fabre JM, Demoly P and Yssel H. IFN-gamma-mediated inhibition of human IgE synthesis by IL-21 is associated with a polymorphism in the IL-21R gene. J Immunol 2006; 177: 5006-5013.

[9] Wood N, Bourque K, Donaldson DD, Collins M, Vercelli D, Goldman SJ and Kasaian MT. IL-21 effects on human IgE production in response to IL-4 or IL-13. Cell Immunol 2004; 231: 133-145.

[10] Yang Z, Wu CM, Targ S and Allen CDC. IL-21 is a broad negative regulator of IgE class switch recombination in mouse and human B cells. J Exp Med 2020; 217: e20190442.

[11] Avery DT, Ma CS, Bryant VL, Santner-Nanan B, Nanan R, Wong M, Fulcher DA, Cook MC and Tangye SG. STAT3 is required for IL-21-induced secretion of IgE from human naive B cells. Blood 2008; 112: 1784-1793.

[12] Spolski R and Leonard WJ. Interleukin-21: a double-edged sword with therapeutic potential. Nat Rev Drug Discov 2014; 13: 379-395.

[13] Schulten V, Tripple V, Seumois G, Qian Y, Scheuermann RH, Fu Z, Locci M, Rosales S, Vijayanand P, Sette A, Alam R, Crotty S and Peters B. Allergen-specific immunotherapy modulates the balance of circulating Tfh and Tfr cells. J Allergy Clin Immunol 2018; 141: 775-777, e6.

[14] Yao Y, Wang ZC, Wang N, Zhou PC, Chen CL, Song J, Pan L, Liao B, Zhang XH, Yang YS, Xu XY, Zhu RF, Yu D and Liu Z. Allergen immunotherapy improves defective follicular regulatory T cells in patients with allergic rhinitis. J Allergy Clin Immunol 2019; 144: 118-128.

[15] Gong F, Zheng T and Zhou P. T follicular helper cell subsets and the associated cytokine il-21 in the pathogenesis and therapy of asthma. Front Immunol 2019; 10: 2918.

[16] Brożek JL, Bousquet J, Agache I, Agarwal A, Bachert C, Bosnic-Anticevich S, Brignardello-Petersen R, Canonica GW, Casale T, Chavannes NH, Correia de Sousa J, Cruz AA, Cuello-Garcia CA, Demoly P, Dykewicz M, Etxeandia-Ikobaltzeta I, Florez ID, Fokkens W, Fonseca J, Hellings PW, Klimek L, Kowalski S, Kuna P, Laisaar KT, Larenas-Linnemann DE, Lødrup Carlsen KC, Manning PJ, Meltzer E, Mullol J, Muraro A, O'Hehir R, Ohta K, Panzner P, Papadopoulos N, Park HS, Passalacqua G, Pawankar R, Price D, Riva JJ, Roldán Y, Ryan D, Sadeghirad B, Samolinski B, Schmid-Grendelmeier P, Sheikh A, Togias A, Valero A, Valiulis A, Valovirta E, Ventresca M, Wallace D, Waserman S, Wickman M, Wiercioch W, Yepes-Nuñez JJ, Zhang L, Zhang Y, Zidarn M, Zuberbier T and Schünemann HJ. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016 revision. J Allergy Clin Immunol 2017; 140: 950-958.

[17] Sindher SB, Long A, Acharya S, Sampath V and Nadeau KC. The use of biomarkers to predict aero-allergen and food immunotherapy

responses. Clin Rev Allergy Immunol 2018; 55: 190-204.
[18] Layhadi JA, Lalioti A, Palmer E, van Zelm MC, Wambre E and Shamji MH. Mechanisms and predictive biomarkers of allergen immunotherapy in the clinic. J Allergy Clin Immunol Pract 2024; 12: 59-66.
[19] Kucuksezer UC, Ozdemir C, Cevhertas L, Ogulur I, Akdis M and Akdis CA. Mechanisms of allergen-specific immunotherapy and allergen tolerance. Allergol Int 2020; 69: 549-560.
[20] Zhao Y, Lutalo PM, Thomas JE, Sangle S, Choong LM, Tyler JR, Tree T, Spencer J and D'Cruz DP. Circulating T follicular helper cell and regulatory T cell frequencies are influenced by B cell depletion in patients with granulomatosis with polyangiitis. Rheumatology (Oxford) 2014; 53: 621-630.
[21] Wang XQ, Ke X, Shen Y, Kang HY, Gu Z, Hu GH and Hong SL. Changes in circulating follicular helper T-cells in Chinese patients with allergic rhinitis. Acta Otolaryngol 2016; 136: 199-204.
[22] Magari M, Nishikawa Y, Fujii Y, Nishio Y, Watanabe K, Fujiwara M, Kanayama N and Ohmori H. IL-21-dependent B cell death driven by prostaglandin E2, a product secreted from follicular dendritic cells. J Immunol 2011; 187: 4210-4218.
[23] Sharif H, Acharya S, Dhondalay GKR, Varricchi G, Krasner-Macleod S, Laisuan W, Switzer A, Lenormand M, Kashe E, Parkin RV, Yi Y, Koc M, Fedina O, Vilà-Nadal G, Marone G, Eifan A, Scadding GW, Fear DJ, Nadeau KC, Durham SR and Shamji MH. Altered chromatin landscape in circulating T follicular helper and regulatory cells following grass pollen subcutaneous and sublingual immunotherapy. J Allergy Clin Immunol 2021; 147: 663-676.