Suppression of Immunotherapy on Group 2 Innate Lymphoid Cells in Allergic Rhinitis

Da-Chuan Fan1,2, Xiang-Dong Wang1, Cheng-Shuo Wang2,4, Yang Wang1, Fei-Fei Cao1,2, Luo Zhang1,2,4

1Department of Otolaryngology Head and Neck Surgery, Beijing Tongren Hospital, Capital Medical University, Beijing 100005, China
2Department of Otorhinolaryngology Head and Neck Surgery, The First Affiliated Hospital, Harbin Medical University, Harbin, Heilongjiang 150001, China
3Department of Allergy, Beijing Tongren Hospital, Capital Medical University, Beijing 100005, China
4Beijing Key Laboratory of Nasal Diseases, Beijing Institute of Otolaryngology, Beijing 100005, China

Abstract

Background: Group 2 innate lymphoid cells (ILC2s) are regarded as a novel population of lineage-negative cells that induce innate Type 2 responses by producing the critical Th2-type cytokines interleukin (IL)-5 and IL-13. ILC2s as key players in the development of allergic rhinitis (AR) have been proved, however, the effect of subcutaneous immunotherapy (SCIT) with dermatophagoides pteronyssinus extract (Der p-SCIT) on ILC2s in AR patients is not clear. This study aimed to investigate the response of ILC2s of peripheral blood in house dust mites (HDM)-sensitized Chinese patients with AR who received SCIT with Der P extract.

Methods: Seven healthy controls without symptoms of AR who had negative reactions to any of the allergens from skin-prick testing, nine patients diagnosed with persistent AR according to the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines, and 24 AR patients who received Der p-SCIT for 1.0–3.5 years were recruited for the study. ILC2s in the peripheral blood were evaluated using flow cytometry. The severity of their symptoms of all participants was rated based on the Total 5 symptom score.

Results: Among 40 participants, 9 AR patients were assigned to the untreated group, 24 AR patients receiving Der p-SCIT were assigned to the immunotherapy group, and 7 healthy controls without symptoms of AR were assigned to healthy control group. The mean Total 5 symptom score of immunotherapy group was significantly lower than that of untreated group (4.3 ± 1.4 vs. 10.1 ± 2.5, P < 0.001). Similarly, the levels of ILC2s in the peripheral blood of immunotherapy group were significantly reduced compared with that in untreated group (P < 0.001), but were not significantly different from healthy controls (P = 0.775). Further subgroup analysis based on the duration of SCIT therapy (1.0–2.0 years [SCIT1.0–2.0], 2.0–3.0 years [SCIT2.0–3.0], and 3.0–3.5 years [SCIT3.0–3.5]) showed that the percentage of ILC2s was not significantly different between SCIT1.0–2.0, SCIT2.0–3.0, and SCIT3.0–3.5 groups (SCIT1.0–2.0 vs. SCIT2.0–3.0: P = 0.268; SCIT1.0–2.0 vs. SCIT3.0–3.5: P = 0.635; and SCIT2.0–3.0 vs. SCIT3.0–3.5: P = 0.787).

Conclusions: The present study highlighted the suppression of Der p-SCIT on ILC2s in HDM-AR patients. ILC2s identified in peripheral blood can be used as an effective biomarker for Der p-SCIT.

Key words: Allergic Rhinitis; Group 2 Innate Lymphoid Cell; House Dust Mite; Immunotherapy

INTRODUCTION

Allergic rhinitis (AR) is a chronic inflammatory disorder of the nasal mucosa, characterized by an immunoglobulin E (IgE)-mediated Type 2 immune response.[1] A review of the studies investigating the prevalence, incidence of comorbid allergic diseases, and trends and patterns of sensitizing allergens of AR in adults and children in China has indicated that the prevalence of AR with sensitivity to house dust mites (HDM) has increased dramatically in China.[2] Furthermore, evidence suggested that HDM sensitization in patients with AR was associated with an increased risk for the development of allergic asthma, independent of other

Address for correspondence: Dr. Luo Zhang, Beijing Key Laboratory of Nasal Diseases, Beijing Institute of Otolaryngology, No. 17, Houguohutong, Dongcheng District, Beijing 100005, China
E-Mail: dr.luozhang@139.com

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

© 2016 Chinese Medical Journal | Produced by Wolters Kluwer - Medknow

Received: 31-07-2016 Edited by: Xin Chen
How to cite this article: Fan DC, Wang XD, Wang CS, Wang Y, Cao FF, Zhang L. Suppression of Immunotherapy on Group 2 Innate Lymphoid Cells in Allergic Rhinitis. Chin Med J 2016;129:2824-8.
HDM allergen has been shown to be the most prevalent indoor allergen in China, and therefore avoidance to this allergen is often difficult and rarely achievable. Similarly, the benefits of pharmacotherapy are not sustained after discontinuation of the medication. In this respect, subcutaneous immunotherapy (SCIT) is thought to be an effective and safe approach leading to long-term symptom remission for AR patients, as SCIT induces T-cell tolerance by the generation of Type 1 T-regulatory (Tr1) cells and blocks IgE-mediated responses in favor of serum-specific IgG4 antibodies. Thus, Tr1 cells and IgG4 antibodies may be recognized as potential biomarkers to monitor responses to SCIT.

Dermatophagoides pteronyssinus/Dermatophagoides farinae (EUROBlotMaster 44, Lübeck, Schleswig-Holstein, Germany) for 1.0–3.5 years were enrolled and had received Der p-SCIT (Alutard SQ, ALK-Abelló A/S; Hørsholm, Denmark) and 24 AR patients sensitized to HDM who were allocated to receive a cluster protocol, followed by a dose maintenance phase. In addition, seven healthy controls without symptoms of AR and with negative skin prick test reactions to any of a panel of common allergens (including Der p, D. farinae, Chenopodium album, animal hair, tree mix, grass mix, cereal mix, dandelion, giant ragweed, Humulus species, locust bean, Blattella germanica, mugwort, pine, plantain, Cochliobolus lunatus, Candida albicans, Penicillium notatum, Alternaria tenuis, and Aspergillus fumigatus) were also enrolled in the study.

The severity of symptoms of sneezing, rhinorrhea, nasal obstruction, nasal pruritus, and ocular pruritus were rated on a scale of 0–3 (0 = symptoms not present; 1 = mild symptoms present, but not bothersome; 2 = moderate symptoms bothersome, but easily tolerated; and 3 = severe symptoms difficult to tolerate), which were added and expressed as the Total 5 symptom score. Exclusion criteria were as follows: (1) antihistamines, steroids, or leukotriene receptor antagonist therapy within 4 weeks; (2) allergic reaction to any drug during the past 2 weeks; (3) acute infection within the past 4 weeks; (4) smokers within the past 12 months; or (5) pregnancy.

**Analysis of Group 2 innate lymphoid cells by flow cytometry**

ILC2s were analyzed according to the method reported by Mjösberg et al. Briefly, peripheral blood cells were stained simultaneously using an anti-human fluorescein isothiocyanate-conjugated lineage cocktail, phycoerythrin (PE)-conjugated CRTH2, and PE-CY7-conjugated CD127, or appropriate isotype controls (all from BD Pharmingen, San Diego, CA, USA). The cell lineage cocktail comprised antibodies to CD3, CD16, CD14, CD19, CD34, CD123, CD11c, T-cell receptor (TCR) αβ, and TCRγδ expressed on T-cells, B-cells, monocytes, macrophages, mast cells, dendritic cells, and hematopoietic progenitor cells. Lymphocytes lacking any of these lineage markers, as well as expressing CRTH2 and CD127, were considered to be the ILC2 population [Figure 1]. Cell counts were performed using the FACSAnia II flow cytometry device (BD Biosciences, San Diego, CA, USA), and all data were analyzed using the FACSDiva software (BD Biosciences). The proportion of ILC2s was expressed as a percentage of total lymphocytes.

**Statistical analysis**

All data were shown as mean ± standard deviation (SD) and analyzed using the SPSS software version 19.0 (IBM, Armonk, NY, USA), and graphs were generated using Prism software version 4 (GraphPad, La Jolla, CA, USA). Qualitative data were compared between groups using Chi-square test, Total 5 symptom scores of patients before and after Der p-SCIT, and nonparametric data were analyzed using Mann-Whitney *U*-test. All tests were two-tailed, and a *P* < 0.05 was considered statistically significant.
Among 40 participants, 9 AR patients were assigned to the untreated group, 24 AR patients receiving Der p-SCIT were assigned to the immunotherapy group, and 7 healthy controls without symptoms of AR were assigned to healthy control group. The mean ages of patients in untreated, immunotherapy, and healthy control groups were 29.0 ± 9.4 years, 28.9 ± 13.8 years, and 30.0 ± 9.3 years, respectively. Similarly, the proportion of males in the untreated, immunotherapy, and healthy control groups was 22.2%, 54.2%, and 28.6%, respectively. The mean period of Der p-SCIT in immunotherapy group was 2.2 ± 0.9 years. The differences with respect to age, gender, or diseases among the three groups were not statistically significant (all $P > 0.05$). The mean Total 5 symptom score of immunotherapy group was significantly lower than that of untreated group (4.3 ± 1.4 vs. 10.1 ± 2.5, $Z = -4.367$, $P < 0.01$).

To determine the effect of immunotherapy on ILC2s, we assessed the levels of ILC2s in the peripheral blood of untreated group, immunotherapy group, and healthy controls using flow cytometry. The level of ILC2s was significantly lower in the peripheral blood of immunotherapy group compared with that in untreated

Figure 1: Gating strategy to identify peripheral blood ILC2s. Lymphocytes were detected from peripheral blood mononuclear cells and lineage-negative cells. Lineage-negative cells were further assessed for expression of CD127 and CRTH2 or isotype control staining, and ILC2s were identified as lineage-CRTH2+ CD127+ lymphocytes. The cell lineage cocktail consisted of antibodies to CD3, CD16, CD14, CD19, CD34, CD123, CD11c, TCRαβ, and TCRγδ. Representative flow plots are shown for untreated group (a) and immunotherapy group (b). FSC: Forward scatter; SSC: Side scatter; FITC: Fluorescein isothiocyanate; PE: Phycoerythrin; ILC2s: Group 2 innate lymphoid cells; TCR: T-cell receptor.
group [Figure 2, $Z = -4.320, P < 0.001$], but there was no statistically significant difference between immunotherapy group and healthy controls [Figure 2, $Z = -0.286, P = 0.775$]. In addition, the level of ILC2s in the untreated group was significantly higher compared with that in healthy controls [Figure 2, $Z = -3.342, P = 0.001$]. Moreover, further subgroup analysis based on the duration of SCIT therapy (1.0–2.0 years [SCIT }, 2.0–3.0 years [SCIT }, 3.0–3.5 years [SCIT ]; showed that the percentage of ILC2s was not significantly different between SCIT groups, SCIT groups [SCIT vs. SCIT : $Z = -1.108, P = 0.268$; SCIT vs. SCIT : $Z = -0.475, P = 0.635$; and SCIT vs. SCIT : $Z = -0.270, P = 0.787$; Figure 2).

**Discussion**

This study demonstrated that the proportion of ILC2s was significantly decreased in the peripheral blood of immunotherapy group compared with untreated group, but was not significantly different compared with healthy controls. Moreover, the levels of ILC2s appeared to be decreased in the 1st year of Der p-SCIT to the normal level and were not significantly altered for at least the next 2 years. These findings suggested that the therapeutic strategy of SCIT to target ILC2s was likely to be effective in modifying the course of allergic inflammation in individuals with HDM-AR.

Although the precise molecular mechanisms by which ILC2s are downregulated in allergic inflammatory disorders by SCIT are presently not clear, Salimi et al have reported that E-cadherin, an adhesion protein pivotal for maintaining the integrity of airway epithelia, might play a significant role, as E-cadherin ligation on cultured ILC2s isolated from skin of patients with atopic dermatitis significantly downregulated the expression of GATA3 and transcription of IL-5 and IL-13, as well as reduced the proliferation of the skin ILC2s in vitro.[10] In the absence of E-cadherin, ILC2 cytokine production was unhindered. Other studies have suggested that inhibition of ILC2s may be influenced by E-cadherin-mediated signaling pathway through inhibitory killer cell lectin-like receptor G1 (KLRG1), which was expressed as high levels in activated ILC2s of allergic individuals.[10] As cleavage or loss of E-cadherin in the nasal resulting from eosinophil infiltration has been suggested to trigger the initial step of subsequent epithelial destruction in allergic states[19,20] and administration of exogenous IL-10 has been shown to attenuate the decline in the expression of several intestinal epithelial junctions, including E-cadherin, zona occludens-1, and occluding, as well as a loss of intestinal barrier function,[21] it was tempting to speculate that a mechanism involving IL-10 and E-cadherin may be operative in AR patients receiving SCIT, particularly as IL-10 has been shown to be elevated in AR patients who received SCIT.[5,7,22] Thus, in HDM-sensitized AR patients, SCIT induced IL-10, which resulted in the production of E-cadherin and subsequent E-cadherin-KLRG1-mediated inhibition of ILC2s. Collectively, these findings suggested that ILC2s were a critical target to treat allergic diseases and that the IL-10-E-cadherin-KLRG1 axis may represent a mechanism for suppression of ILC2s in AR patients who received SCIT. However, further research on the inhibitory signals is needed to elucidate the relative contribution of ILC2s to AR as well as SCIT.

The Total 5 symptom scores in patients receiving Der p-SCIT were significantly lower than that in those before SCIT treatment, which indicated that SCIT was an effective treatment for AR patients. A limitation of this study, however, was that baseline data for ILC2s prior to SCIT treatment in immunotherapy group were not available. Despite this limitation, the current study has demonstrated that the levels of ILC2s in AR patients who had received SCIT for 1.0–3.5 years were similar to those in healthy controls, whereas both the Total 5 symptom scores and ILC2s levels in the AR patients who had not received SCIT were significantly higher. The findings of this study reflected the inhibited effect of SCIT therapy on ILC2s in HDM-sensitized AR patients accurately, which was consistent with the study of Lao-Araya et al,[9] but these finding need to be further confirmed in much larger cohort of patients in the future. Furthermore, targeting ILC2s as a potential therapeutic strategy for AR patients in well-controlled trials may provide a valuable insight into the role of these cells in allergic diseases.

In conclusion, this experimental study has suggested that the relatively high level of ILC2s in AR patients sensitized to HDM may be treated by Der p-SCIT, and a reduction of ILC2 levels might contribute to symptom remission and immunologic tolerance in AR. Furthermore, ILC2s identified in peripheral blood might be used as an effective biomarker for therapeutic response to Der p-SCIT in AR patients.
Financial support and sponsorship
This work was supported by grants from the Program for Changjiang Scholars and Innovative Research Team (No. IRT13082), the 12th Five-Year Science and Technology Support Project (No. 2014BA107B04), the National Science Fund for the Major International Joint Research Program (No. 81420108009), National Natural Science Foundation of China (No. 81100704, 81441029, 81441031, and 81570894), Beijing Natural Science Foundation (No. 7131006), Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support (No. ZYLX201310), the Capital Health Research and Development of Special (No. 2011-1017-06), Specialized Research Fund for the Doctoral Program of Higher Education of China (No. 20111107120004), the Special Fund of Sanitation Elite Reconstruction of Beijing (No. 2009-2-007), Beijing Municipal Administration of Hospitals’ Mission Plan (No. SML20150203), and Beijing Health Bureau Program for High Level Talents (No. 2009-2-007, 2011-3-039, 2011-3-043, and 2014-3-018).

Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. 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. doi: 10.1111/j.1398-9995.2007.01620.x.
2. Zhang Y, Zhang L. Prevalence of allergic rhinitis in china. Allergy Asthma Immunol Res 2014;6:105-13. doi: 10.4168/aair.2014.6.2.105.
3. Li J, Sun B, Huang Y, Lin X, Zhao D, Tan G, et al. A multicentre study assessing the prevalence of sensitizations in patients with asthma and/or rhinitis in China. Allergy 2009;64:1083-92. doi: 10.1111/j.1398-9995.2009.01967.x.
4. Shaaban R, Zureik M, Soussan D, Heinrich J, Sunyer J, et al. Rhinitis and onset of asthma: A longitudinal population-based study. Lancet 2008;372:1049‑57. doi: 10.1016/S0140‑6736(08)61446‑4.
5. Lou W, Wang C, Wang Y, Han D, Zhang L. Enhancement of the frequency and function of IL‑10‑secreting type I T regulatory cells after 1 year of cluster allergen‑specific immunotherapy. Int Arch Allergy Immunol 2012;159:391‑8. doi: 10.1159/000338995.
6. Lou W, Wang C, Wang Y, Han D, Zhang L. Responses of CD4(+) CD25(+) Foxp3(+) and IL‑10‑secreting type I T regulatory cells to cluster‑specific immunotherapy for allergic rhinitis in children. Pediatr Allergy Immunol 2012;23:140‑9. doi: 10.1111/j.1399-3038.2011.01249.x.
7. Jetel M, Akdis CA. Immunological mechanisms of allergen‑specific immunotherapy. Allergy 2011;66:725‑32. doi: 10.1111/j.1398‑9995.2011.02589.x.
8. Doherty TA, Scott D, Walford HH, Khorram N, Lund S, Baum R, et al. Allergen challenge in allergic rhinitis rapidly induces increased peripheral blood type 2 innate lymphoid cells that express CD84. J Allergy Clin Immunol 2014;133:1203‑5. doi: 10.1016/j.jaci.2013.12.1086.
9. Lao‑Araya M, Steveling E, Scadding GW, Durham SR, Shamji MH. Seasonal increases in peripheral innate lymphoid type 2 cells are induced by subcutaneous grass pollen immunotherapy. J Allergy Clin Immunol 2014;134:1193‑5.e4. doi: 10.1016/j.jaci.2014.07.029.
10. Salimi M, Barlow JL, Saunders SP, Xue L, Gutowska-Oswiak D, Wang X, et al. A role for IL‑25 and IL‑33‑driven type‑2 innate lymphoid cells in atopic dermatitis. J Exp Med 2013;210:2939‑50. doi: 10.1084/jem.20130351.
11. Bartemes KR, Kephart GM, Fox SJ, Kita H. Enhanced innate type 2 immune response in peripheral blood from patients with asthma. J Allergy Clin Immunol 2014;134:671‑8.e4. doi: 10.1016/j.jaci.2014.06.024.
12. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, et al. Inmate production of T(H) 2 cytokines by adipose tissue‑associated c‑Kit(+) Sca‑1(+) lymphoid cells. Nature 2010;463:540‑4. doi: 10.1038/nature08636.
13. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, et al. Nuocytes represent a new innate effector leukocyte that mediates type‑2 immunity. Nature 2010;464:1367‑70. doi: 10.1038/nature08900.
14. Price AE, Liang HE, Sullivan BM, Reinhardt RL, Eisley CJ, Erle DJ, et al. Systemically dispersed innate IL‑13‑expressing cells in type 2 immunity. Proc Natl Acad Sci U S A 2010;107:11489‑94. doi: 10.1073/pnas.1003988107.
15. Zhang L, Wang C, Han D, Wang X, Zhao Y, Liu J. Comparative study of cluster and conventional immunotherapy schedules with Dermatophagoides pteronyssinus in the treatment of persistent allergic rhinitis. Int Arch Allergy Immunol 2009;148:161‑9. doi: 10.1159/000155747.
16. Mjösberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, et al. Human IL‑25‑and IL‑33‑responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. Nat Immunol 2011;12:1055‑62. doi: 10.1038/ni.2104.
17. Nawijn MC, Hackett TL, Postma DS, van Oosterhout AJ, Heijink IH, E‑cadherin: Gatekeeper of airway mucosa and allergic sensitization. Trends Immunol 2011;32:248‑55. doi: 10.1016/j.it.2011.03.004.
18. Bank C, Fügère C, Brossay L. Immunoregulatory functions of KLRG1 c‑Cadherin interactions are dependent on forward and reverse signaling. Blood 2009;114:5299‑306. doi: 10.1182/blood‑2009‑06‑228353.
19. Kobayashi N, Terada N, Hamano N, Numata T, Konno A. Transepithelial migration of activated eosinophils induces a decrease of E‑cadherin expression in cultured human nasal epithelial cells. Clin Exp Allergy 2000;30:807‑17. doi: 10.1046/j.1365‑2222.2000.00827.x.
20. Kobayashi N, Dezawa M, Nagata H, Yuasa S, Konno A. Immunohistochemical study of E‑cadherin in asthma. J Allergy Clin Immunol 2014;134:671‑8.e4. doi: 10.1016/j.jaci.2014.06.024.
21. Cosmi L, Santarlasci V, Angeli R, Liotta F, Maggi L, Frosali F, et al. Role for IL‑25 and IL‑33‑driven type‑2 innate lymphoid cells in atopic dermatitis. J Exp Med 2014;231:1191‑202. doi: 10.1084/jem.20130215.