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The Regulation of the IL-18 on Group 2 Innate Lymphoid Cells in Allergic Rhinitis

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ABSTRACT

Background: Group 2 innate lymphoid cells (ILC2s) promote allergic inflammation by producing interleukin-4 (IL-4), IL-5, IL-9, and IL-13. IL-18 can promote T helper 2 cell (Th2) response by inducing IL-4, and IL-13 production from mast cells and basophils. However, the regulation of IL-18 on ILC2s remained unknown.

Objective: To investigate the regulatory role of IL-18 in inducing the type 2 innate lymphoid cells.

Methods: Twenty patients with allergic rhinitis (AR) and 20 controls were enrolled. The mRNA and protein levels of IL-18 in serum, as well as the frequencies of ILC2 in peripheral blood mononuclear cells (PBMCs) were measured by real-time polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and flow cytometry. The ILC2s were sorted and the mRNA expression of IL-18 receptor in ILC2 was analyzed by real-time PCR. The effects of IL-18 on the proliferation and type 2 cytokine production were detected by tritiated thymidine incorporation test, real-time PCR, and ELISA, respectively.

Results: The levels of IL-18 mRNA and protein were significantly higher in AR patients than in the controls ($P<0.05$). The frequency of ILC2 in peripheral blood was elevated in the AR patients than in the controls. After stimulation by IL-18 and house dust mite (HDM), the expression of IL-18 receptor (IL-18R) by ILC2 was significantly up-regulated. The tritiated thymidine incorporation results showed that IL-18 promoted the proliferation of ILC2 in a dose-dependent manner. IL-18 also induced the expression of IL-5 and IL-13 proteins by ILC2.

Conclusion: Our results confirmed -for the first time- the effect of IL-18 in innate immunity, which was demonstrated by direct effect on the differentiation and function of ILC2.

Keywords: Allergic Rhinitis, Group 2 Innate Lymphoid Cells, IL-18

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INTRODUCTION

Allergic rhinitis (AR) is characterized by persistent allergic inflammation of nasal mucosa. It affects about 30% of the population, manifested as sneezing, nasal congestion, itchy nose, and watery rhinorrhea (1, 2). Traditionally, T helper 2 cell (Th2) cells are thought to have the dominant role in the pathogenesis of AR. However, recent studies suggested that innate immunity seems to have more important roles in AR (3). For example,

group 2 innate lymphoid cells (ILC2s) is deeply involved in allergy by producing interleukin-5 (IL-5), the IL-13, and some IL-4 in response to IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) (4). Moreover, the allergic response still exist even when the Th2 cells were deficient, suggesting the irreplaceable role of ILC2 (5).

The IL-18, secreted from monocytes and macrophages, belongs to the member of the IL-1 family. IL-18 plays its role by binding with IL-18 receptors expressed by various lymphoid cells, endothelial, and smooth muscle cells (6). The effect of IL-18 on Th response often depends on the co-stimulatory cytokines. In the presence of the IL-12, the IL-18 promotes interferon-γsecretion from Th1 cells and inhibits Th2 cell differentiation (7). However, the IL-18 can promote Th2 response by inducing Th2 cytokines production from mast cells or basophils without IL-12 (8). Previous studies had shown that the IL-18 expression was elevated in Th2 inflammation dominated diseases including asthma, allergic rhinitis, and atopic dermatitis (9-11). However, the effect of the IL-18 on the regulation of the ILC2 was not explored.

In the present study, we aimed to explore the regulation of the IL-18 in AR and its effect on the differentiation and function of the ILC2 to clarify the role of the IL-18 in the innate immunity of AR.

METHODS

Patients

Twenty confirmed AR subjects were recruited in this study according to Allergic Rhinitis and its Impact on Asthma (ARIA) guideline. The AR was diagnosed by disease duration, complaints, and positive atopic status. A skin prick test or specific IgE to dust mites was detected. All patients were non-smokers and had no history of glucocorticoids and anti-histamines use in the past month. Twenty healthy volunteers without allergic symptoms and positive allergen tests were used as controls. The use of human tissue samples was conducted under the ethical approval of local ethical committee boards.

Flow Cytometry and the ILC2 Sorting

Peripheral blood mononuclear cells (PBMCs, 20mL) were purified by Lymph prep (Nycomed) or Ficoll-Paque Plus (GE Healthcare). Depletion of T, B, NK cells, and monocytes was performed via staining lineage negative cocktail kit (eBioscience, CA). After that, about 50% of pure Lin^- cells were left. These lineage negative cells were then treated by PE-conjugated CRTH2 and PE-Cy7 conjugated CD127 antibody (BD Bioscience, NJ) for identification of the ILC2s. Therefore, the ILC2 was defined as $Lin^-CRTH2^+CD127^+$ cells. The isotype-matched antibodies (eBioscience) were used as the controls for nonspecific staining. The Beckman flow cytometer (Beckman Coulter, CA) was used.

Lin-CRTH2+CD127+ cells were purified by a FACS Aria (BD). The sorted Lin-CRTH2+CD127+ cells ($1*10^5$ cells/mL) were incubated in medium and stimulated by the IL-25 (10 ng/ml), IL-33 (10 ng/ml), TSLP (10 ng/ml) and IL-2 (50 ng/ml) for 72 hours. At the same time, various concentrations of the IL-18 or PBS (negative control) were provided in the culture medium. For the ILC2 proliferation assay, the ILC2 were cultured with the medium alone and PBS (negative control) or the IL-18. After five days, the ILC2s were pulsed by 0.5 μCi of ^3H thymidine (PerkinElmer, Waltham, MA) for 24h before harvesting, and counts per minute (cpm) were measured upon harvesting.

Quantitative Real-time PCR

For the ILC2 cells, total RNA was purified using TRIzol reagent (Life Technologies, CA) and nucleospin RNA II kit (Machery-Nagel). The cDNA was synthesized using the MBI Fermentas first-strand cDNA synthesis kit (Fermentas

GmbH, Germany). The qPCR was finished using ABI PRISM 7300 Detection System. Reaction conditions included: Amplification under 95°C for 10 minutes, 40 cycles of denaturation at 95°C for 10 s, annealing extension at 60°C for 60 s. The target genes expression was analyzed using the comparative ΔCt method and corrected for the housekeeping gene. The sequences of primers were listed as follows: IL-18, sense--5′-GCTTGAATCTAAATTATCAGTC-3, anti-sense-; IL-18R, sense- 5′-GAAGATTCAAATTGCATCTTAT-3, anti-sense-5′-GCAATCTTTAGTCTCATTCCCG-3; GATA3 sense, 5′-GCGGGCTCTATCACAAAATGA-3′, antisense, 5′-GCTCTCCTGGCTGCAGACAGC-3′; RORα sense, 5′-AAGGAGCCAGAAGGGATGAAC-3′, antisense, 5′-GGAACA ACAGACGCCAGTAAG-3′; β-actin sense, 5′-CGAAACTACCTTCAACTCCATC-3′, antisense, 5′-AGTGATCTCCTTCTGCAT CCT-3′.

Enzyme-linked Immunosorbent Assay (ELISA)

Protein levels of the IL-18, IL-5, and IL-13 were measured by ELISA kits (R&D Systems, USA). The detection limits was as follows: IL-5, 3.9 pg/mL, IL-13, 125 pg/mL, IL-18, 11.7 pg/mL.

Statistical Analysis

Student's t-test, one-way ANOVA, and Dunnett's post-test were performed for different comparisons. P-value less than 0.05 was defined as statistically significant.

RESULTS

Serum IL-18 Expression and Its Relation to the ILC2 Frequency in AR

The information of study subjects is shown in Table 1. The IL-18 mRNA and protein levels were significantly higher in AR patients than in the controls (Figure 1). The frequency of the ILC2 in peripheral blood was also higher in AR than in the controls (Figure 1). Correlation analysis showed that protein expression of the IL-18 was positively correlated with the proportion of the ILC2 ($r=-0.71$, $P<0.05$) from peripheral blood.

The IL-18 Receptor Expression by ILC2 in AR

After stimulated by IL-18 and HDM, the expression of the IL-18R by the ILC2 was significantly elevated ($P<0.05$) (Figure 2). However, these effects cannot be observed when the IL-12 was added.

The Differentiation and Function of the ILC2 Regulated by IL-18

The tritiated thymidine incorporation results showed that the IL-18 induced the proliferation of the ILC2 in a dose-dependent manner (Figure 3). Similarly, the IL-18 also induced the expression of IL-5 and IL-13 by the ILC2 (Figure 3). We found that the main transcription factors of the ILC2 (GATA3 and RORα) were up-regulated significantly after stimulation of the IL-18 (Figure 3). However, these effects were inhibited significantly when the IL-18R inhibitor or the IL-12 was present.

Table 1. Demographic characteristic of study subjects

Groups	AR group	Controls
Number	20	20
Sex (Male: Female)	10:10	12:8
Age (years)	28.1(18-48)	26.2 (18-46)
TIgE (kU/L)	174.3(31.1-784.3)*	16.2 (8.6-40.6)
Sensitization		
D1 (kU/L)	17.4(1.8-44.0)	-
D2 (kU/L)	26.5(3.8-59.6)	-

*Compared with the control group, P<0.05; D1, Dermatophagoïdes pteronyssinus; D2, Dermatophagoïdes farinae

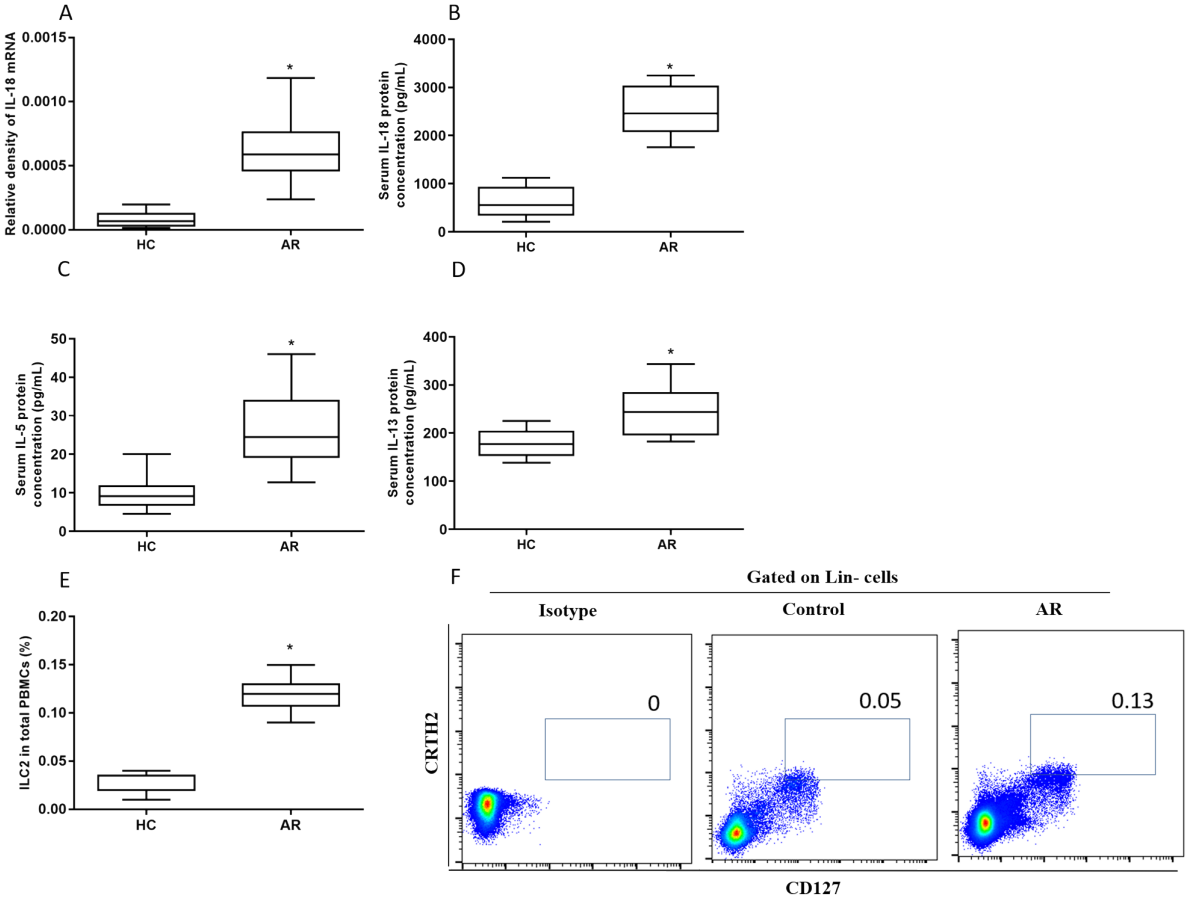

Figure 1. The serum concentration of IL-18, type 2 cytokines, and the ILC2 frequency between AR and controls. The mRNA and protein concentration of the IL-18 (pg/mL) detected by RT-PCR and ELISA between the controls and the AR are shown in Figure a-d. Flow cytometry showed that the ILC2 frequency in AR was significantly compared with the controls (e,f).*, P<0.05, compared with the controls.

DISCUSSION

The ILC2 is a group of cells distributed in lymphoid tissue, intestine, lung, and skin (12). ILC2 secrete type 2 cytokines and are dependent on GATA3 and retinoic acid receptor–related orphan receptors for differentiation (13). In AR patients allergic to cats, nasal stimulation can promote the proliferation of peripheral blood ILC2 (14). Moreover, the ILC2 plays different actions in the AR patients allergic to dust mites or mugwort (15). However, the regulation of the ILC2 was not fully understood.

The IL-18 is believed to be a multi-functional cytokine and its role in AR had been reported in previous studies. Verhaeghe reported that the nasal IL-18 expression was elevated in SAR patients during the

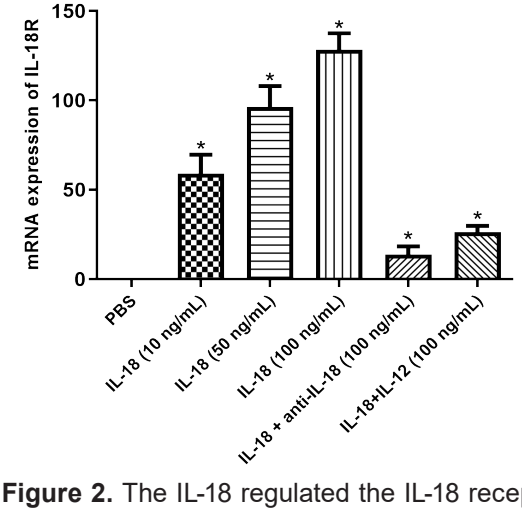

Figure 2. The IL-18 regulated the IL-18 receptor expression by the ILC2. PCR found that the the IL-18R was significantly enhanced by the ILC2 after being stimulated by the IL-18, whereas the addition of the IL-12 or the IL-18R inhibitor inhibits IL-18 receptor expression. Three independent tests were conducted for every experiment. *, P<0.05, compared with the IL-18(10 ng/mL) group.

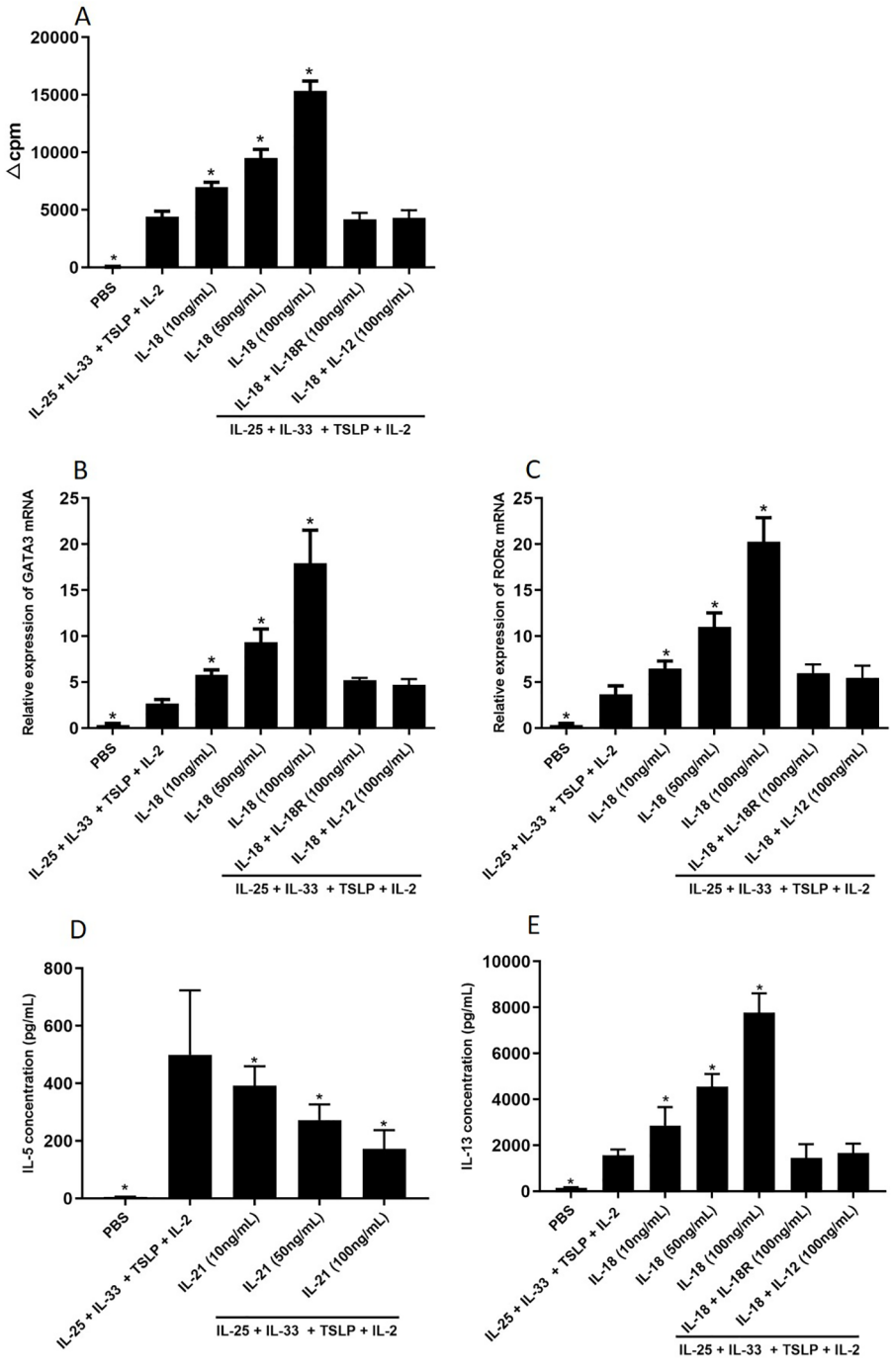

Figure 3. The proliferation and function of the ILC2 cells treated by the IL-18. The proliferation of the ILC2 was detected using tritiated thymidine incorporation (A), the mRNA expression of GATA3 and RORα by ILC2 (B,C), the protein expression of the IL-5, and IL-13(D,E). Three independent tests were done for every experiment. *, P<0.05, compared with the cytokines group. The concentrations of cytokines were as follows: IL-25 (10 ng/ml), IL-33 (10 ng/ml), TSLP (10 ng/ml) and IL-2 (50 ng/ml).

allergen season (16). Kodama's study proved that administration of the IL-18 induced the IL-5 expression in the bronchoalveolar lavage fluid (BALF) in ovalbumin-induced asthmatic mice (17). Moreover, the IL-18 promotes airway response in allergic mice through up-regulating antigen-induced TNF-αproduction (18). Consistently, Yamagata's results suggested that the IL-18-deficient mice had decreased OVA-specific IgE, IL-4, or IL-13 levels in BALF compared with wild type controls (19). All above studies revealed

the positive effect of the IL-18 in allergic inflammation, but the exact mechanism of the IL-18 in the regulation of adaptive or innate immunity was not explored.

As expected, our data also confirmed the increased expression of the IL-18 in AR. We also found a positive correlation between the IL-18 and the frequency of ILC2, suggesting the possible interaction between them. Next, we detected the mRNA level of the IL-18R after the IL-18 stimulation. We found that the IL-18 can regulate the IL-18R by ILC2 directly, thus proving the direct effect between the IL-18R and the ILC2.

To illustrate the regulation of the IL-18 on ILC2, we stimulated the ILC2 by the IL-18. Both the proliferation and function of the ILC2 were enhanced when the IL-18 was added to the culture medium. Moreover, the expression of GATA3 and RORα was up-regulated by the IL-18. As expected, the above effects were inhibited significantly when the IL-18R inhibitor or the IL-12 was added, suggesting that the IL-12 can also inhibit innate immunity response.

In conclusion, for the first time, our results confirmed the effect of the IL-18 in innate immunity, and was proved by a direct effect on the differentiation and function of the ILC2.

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Conflicts of Interest: None declared.

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