

# Altered expression of IL-18, IL-18 binding protein and IL-18 receptor in blood monocytes of patients with allergic rhinitis

**Junling Wang**

China Medical University; the First Affiliated Hospital of Jinzhou Medical University

<https://orcid.org/0000-0002-4937-0665>

**Fangqiu Gu**

China Medical University; the First Affiliated Hospital of Jinzhou Medical University

**Zhi Li**

the First Affiliated Hospital of Jinzhou Medical University

**Mengmeng Zhan**

Shenyang Medical College

**Ling Wang**

Shenyang Medical College

**Huiyun Zhang**

the First Affiliated Hospital of Jinzhou Medical University; Shenyang Medical College

**Dong Chen**

the First Affiliated Hospital of Jinzhou Medical University

**Hua Xie**

General Hospital of Shenyang Military Area Command

**Ruonan Chai**

General Hospital of Shenyang Military Area Command

**Nan Zhao**

First Affiliated Hospital of Jinzhou Medical University

**Ruiming Yang**

the First Affiliated Hospital of Jinzhou Medical University

**Yalin Hu**

the First Affiliated Hospital of Jinzhou Medical University

**Shaoheng He (✉ [shoahenghe@126.com](mailto:shoahenghe@126.com))**

China Medical University and the First Affiliated Hospital of Jinzhou Medical University and Shenyang Medical College <https://orcid.org/0000-0002-4230-7639>

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## Abstract

Background: Interleukin (IL)-18 is emerging as an attractive participant in allergic rhinitis (AR). However, correlation of IL-18 with IL-18 binding protein (BP) in plasma, and expression of IL-18, IL-18BP and IL-18 receptor (R) in AR blood monocytes remain obscure.

Methods: We investigated IL-18, IL-18BP and IL-18R expression in monocytes by using flow cytometric analysis, murine AR model, and quantitative real-time PCR in the present study.

Results: It was found that plasma IL-18 and IL-1 $\beta$  in AR patients was higher than those in healthy control subjects. Free (f)IL-18 had a high correlation with IL-18BP, IL-1 $\beta$  and TNF- $\alpha$  in AR plasma. Proportion of IL-18 + monocytes was increased, whereas IL-18BP + monocytes were decreased in blood of patients with AR. It was found that Platanus pollen allergen extract provoked the elevated expression of IL-18 and IL-18R in AR blood monocytes. Dermatophagoides pteronyssinus, Artemisia sieversiana wild and Platanus pollen allergen extracts enhanced IL-18R protein and mRNA expression in primary monocytes from AR patients. Moreover, numbers of macrophages and IL-18R + macrophages in nasal lavage fluid (NLF) were increased, and levels of IL-18 in both plasma and NLF were elevated in AR mice.

Conclusions: IL-18 is likely to participate in the development of AR as a causative factor, therefore, it could be a therapeutic target for AR.

## Background

IL-18, initially discovered as interferon (IFN)- $\gamma$ -inducing factor constitutively expressed by monocytes and macrophages, and plays regulatory roles in both innate and adaptive immunity. It is reported that polymorphisms of IL-18 gene was associated with AR [1–5], and up-regulated IL-18 is found in both nasal secretion and serum of AR patients [6, 7]. It is also observed that elevated serum IL-18 during natural pollen exposure is closely associated with bronchial hyperresponsiveness in seasonal AR (sAR) patients [8]. These implicate that monocyte- and macrophage-derived IL-18 likely contributes to the pathogenesis of AR.

IL-18 binds initially to IL-18R then initiates MyD88-dependent signal pathway and exert immunomodulatory functions [9]. IL-18 binding protein (IL-18BP) is an endogenous soluble antagonist that specifically inhibits IL-18 action by binding to IL-18 with high affinity [10]. Free serum IL-18BP is present at 20fold higher levels than free IL-18 in physiological status [11]; however, under allergic conditions IL-18 may be in excess [12]. These suggest an imbalance between IL-18 and IL-18BP expression may account for increased IL-18 activity in AR. Indeed, we reported recently that the role of IL-18 in atopic asthma is determined by the balance of IL-18/IL-18BP/IL-18R [13]. Since level of circulating IL-18BP in AR plasma/serum has not been reported, we examined level of IL-18BP in AR plasma in the present study.

It has been reported that pollen allergen specific subcutaneous immunotherapy induced increased serum IL-18 in AR patients [14], pollen allergen extract could provoke IL-18 mRNA expression in PBMCs of patients with AR undergoing allergen immunotherapy [15–17], suggesting that allergens may contribute to AR through PBMCs and IL-18-related mechanisms. Since monocytes are one of the major cell types of PBMCs, and little is known about expression of IL-18, IL-18BP and IL-18R at protein and mRNA levels in monocytes of AR, particularly upon allergen challenge. Therefore, the aim of the present study is to investigate levels of IL-18 and IL-18BP in AR plasma, expression of IL-18, IL-18BP and IL-18R in monocytes of patients with AR, and influence of allergens on their expression.

## Materials And Methods

### Reagents

The following reagents were purchased from Biolegend (San Diego, CA, USA): human red blood cell lysis buffer, Brilliant Violet 510™ (BV510)-conjugated donkey anti-rabbit IgG polyclonal antibody, PE/Cy7-conjugated mouse anti-human CD14 monoclonal antibody, BV510-conjugated rat anti-mouse Ly-6G/Ly-6C (Gr-1) monoclonal antibody, BV510-conjugated rat anti-mouse F4/80 monoclonal antibody, PerCP-conjugated rat anti-mouse CD11b monoclonal antibody, human Fc receptor blocking solution, rat anti-mouse CD16/32 antibody, Brefeldin A, Zombie NIR™ Fixable Viability Kit, and Zombie Green™ Fixable Viability Kit. Mouse IL-18BPd DuoSet ELISA kit, APC-conjugated mouse anti-human IL-18Ra monoclonal antibody, PE-conjugated mouse anti-human IL-18 monoclonal antibody, APC-conjugated rat anti-mouse IL-18Ra monoclonal antibody and their respective isotype controls (catalog #: IC002A, IC002P, IC005A) were supplied by R&D Systems (Minneapolis, MN, USA). Rabbit anti-human IL-18BPa and rabbit IgG isotype control were obtained from Novus Biologicals (Minneapolis, MN, USA). IFNy was supplied by PeproTech (Rocky Hill, NJ, USA). Trypan blue dye and Ovalbumin (OVA, Grade V) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Human IL-18BPa and IL-18 ELISA kits were from ImmunoWay Biotechnology Company (Plano, TX, USA) and ExCell Bio (Shanghai, China), respectively.

Cytotix/Cytoperm™ Fixation/Permeabilization Solution Kit was bought from BD Biosciences (Belford, MA, USA). Fetal bovine serum (FBS, Hyclone), penicillin-streptomycin antibiotic mixture and RPMI 1640 medium were obtained from Gibco BRL (Grand Island, NY, USA). Alhydrogel® adjuvant was bought from InvivoGen (San Diego, CA, USA). RBC Lysis Buffer (Multi-species), Mouse IL-18 ELISA kit and TRIzol reagent were purchased from Invitrogen (Carlsbad, CA, USA). anti-human CD14 MicroBeads and autoMACS Running Buffer-MACS Separation Buffer, and Lymphoprep™ were obtained from Miltenyi Biotec (Bergisch Gladbach, Germany) and AXIS-SHIELD PoC AS (N-0504 Oslo, Norway), respectively. *Dermatophagoides pteronyssinus* allergen extract (DPAE) was bought from Greer Laboratories, Inc. (Lenoir, NC, USA). *Artemisia sieversiana* wild allergen extract (ASWAE) and *Platanus* pollen allergen extract (PPAE) were purchased from Macro Union Pharmaceutical Co. Ltd (Beijing, China). Allergens for skin prick tests were supplied by ALK-Abelló, Inc. (Denmark). Most of the general-purpose chemicals such as salts and buffer components were of analytical grade.

### Subjects and animals

A total of 33 patients with perennial allergic rhinitis (pAR), 9 patients with sAR and 25 healthy control (HC) subjects were recruited in the study. Their general characteristics were summarized in Additional file 1 (Table S1). The diagnosing criteria of pAR and sAR were conformed to the Chinese Society of Allergy Guidelines for Diagnosis and Treatment of Allergic Rhinitis [18]. The informed consent from each volunteer according to the declaration of Helsinki and agreement with the ethical committee of the First Affiliated Hospital of Jinzhou Medical University and with the General Hospital of Shenyang Military Region of PLA was obtained.

Immediately after confirmed diagnosis (acute stage), peripheral blood from each patient with allergic rhinitis was collected. Blood from HC were collected in the outpatient clinic. From each donor, 5 mL was taken into an EDTA containing tube before centrifugation at  $450\times g$  for 10 min. The cells were used for flow cytometric analysis, and plasma was collected and frozen at -80°C for analysis of cytokines. For CD14<sup>+</sup> monocytes isolation study, 180 mL of peripheral blood was taken from each donor.

Five-week-old female BALB/c mice were obtained and maintained as described previously [12]. The animal experiment procedures were authorized by the Animal Care Committee at Jinzhou Medical University.

### **Isolation of CD14<sup>+</sup> monocytes and allergen challenge test**

CD14<sup>+</sup> cells were enriched by density gradient centrifugation and positive selection by using magnetic beads on magnetic cell sorting (MACS) according to the manufacturer's instructions. Final recovery of cells was determined with an improved Neubauer haemocytometer after being stained with trypan blue solution, and recovered cell purities were assessed by flow cytometry with an anti-human CD14 antibody.

To further investigate the direct action of allergen on the expression of IL-18, IL-18BP and IL-18R in monocytes, the isolated primary monocytes at a density of  $1\times 10^6$  per mL were cultured in RPMI 1640 medium containing 3% FBS and 100 U/ml penicillin/streptomycin in a 12-well cell culture plate (Nest, Wuxi, China) in the presence or absence of ASWAE, PPAE, DPAE (all at a concentration of 1.0 µg/mL) or IFNy (as positive control) at 5 ng/mL for 10, 30 and 60 min, respectively at 37°C in a 5% (v/v) CO<sub>2</sub>, water-saturated atmosphere. Brefeldin A at 2 µg/mL was added in wells for detecting the intracellular expression of IL-18 and IL-18BP before stimulation. Cells were then harvested and centrifuged at  $450\times g$  for 10 min at 4°C. Cell pellets containing approximately  $0.5\times 10^6$  and  $1\times 10^6$  cells were resuspended in PBS for flow cytometric analysis, and in TRIzol reagent for RT-PCR, respectively. Cell culture supernatant was collected and frozen at -80°C for further use.

### **Flow cytometric analysis of IL-18, IL-18BP and IL-18R in human peripheral blood monocytes and isolated CD14<sup>+</sup> monocytes**

The procedures for detecting IL-18, IL-18BP and IL-18R expression in human peripheral blood monocytes were mainly adopted from a previous study by Zhang *et al*/[13]. Briefly, whole blood cells were challenged

with or without ASWAE, PPAE, or DPAE (all at a concentration of 1.0 µg/mL) for 1 h, and isolated CD14<sup>+</sup> monocytes were challenged for 10, 30 and 60 min at 37°C.

For cell surface molecules, whole blood cells were preincubated with human Fc receptor blocking solution and a live/dead cell dye (Zombie Green™ Fixable Viability Kit) [19] for 15 min, then stained with PE/Cy7 conjugated anti-human CD14 and APC conjugated anti-human IL-18Ra antibodies. Following red blood cell lysis, cells were analyzed with FACS Verse flow cytometer (BD Biosciences, San Jose, CA, USA). An irrelevant isotype- and concentration-matched antibody of anti-human IL-18Ra was used for fluorescence minus one (FMO) control. Dead cells and doublets were discriminated by SSC-A–live/dead cell dye and FSC-H–FSC-A gating strategies. As for magnetic isolated monocytes, cells were processed as above.

For intracellular molecules, whole blood cells were preincubated with human Fc receptor blocking solution and a live/dead cell dye, subsequently stained with PE/Cy7 conjugated anti-human CD14 as described above. After lysing red blood cells, resuspended leukocytes were fixed and permeabilized, and stained with PE conjugated anti-human IL-18 antibody, and anti-human IL-18BP primary antibody followed by the addition of BV510-conjugated donkey anti-rabbit polyclonal antibody. Finally, cells were processed and analyzed as above. As for isolated primary monocytes, cells were processed as above.

### **Establishment of mouse allergic rhinitis model**

OVA-induced allergic rhinitis mouse model was mainly adopted from a previous study by Mo JH *et al* [20]. Briefly, mice were sensitized on days 0, 7 and 14 by intraperitoneal injection of 25 µg OVA emulsified in 1 mg of alhydrogel. On days 21–27 mice were challenged by intranasal instillation with 500 µg of OVA dissolved in PBS (10 µL/nostril) once daily. For control experiments, healthy mice received vehicle only instead of OVA solution. At 24 h following the last OVA challenge, blood and nasal lavage fluid (NLF) were collected from each mouse. Total cells were determined and collected after centrifugation as described above. The cells were used for flow cytometric analysis, plasma and NLF supernatant was collected and frozen at -80°C until use.

To evaluate allergic symptoms, numbers of sneezing and nasal-rubbing motions during the first 15 min after each OVA challenge were recorded and compared with healthy control mice (HM) by observers blinded to the study. As presented in Additional file 2 (Fig. S1a, b), the numbers of nasal rubbing and sneezing motion in AR mice were substantially higher than that in HM during a 7-day observation period.

### **Flow cytometric analysis of IL-18R in mouse blood monocytes and NLF macrophages**

To detect IL-18R expression in mouse blood monocytes, whole blood cells were preincubated with anti-mouse CD16/32 antibody and a live/dead cell dye (Zombie NIR™ Fixable Viability Kit) [21] for 15 min. Each labeled monoclonal antibody including BV510-conjugated anti-mouse Gr-1, PerCP-conjugated anti-mouse CD11b and APC-conjugated anti-mouse IL-18R was added into tubes for 15 min before red blood cells being lysed. Finally, cells were processed as for human blood samples and analyzed by using flow cytometry.

To detect IL-18R expression in NLF macrophages, cells were incubated with anti-mouse CD16/32 antibodies and a live/dead cell dye, followed by incubation with BV510-conjugated anti-mouse F4/80 and APC conjugated anti-mouse IL-18R antibodies, and analyzed as above.

### Real-time PCR for IL-18, IL-18BP and IL-18R in isolated CD14<sup>+</sup> monocytes

Total RNA was extracted from magnetically sorted blood monocytes as described previously [22]. Briefly, after synthesizing cDNA from total RNA by using RT Master Mix Perfect Real Time, qPCR was performed with SYBR Premix Ex TaqII Kit on the Real-time Thermal Cycler (Thermo Fisher Scientific Oy, Vantaa, Finland). Each reaction contains 12.5 µL of 2×SYBR green Master Mix, 300 nM oligonucleotide primers, and 10 µL of the cDNA. Untreated controls were chosen as the reference samples, and the  $\Delta Ct$  for all experimental samples were subtracted by the  $\Delta Ct$  for the control samples ( $\Delta\Delta Ct$ ). The magnitude change of test gene mRNA was expressed as  $2^{-\Delta\Delta Ct}$ . Each measurement of a sample was conducted in duplicate. The forward and reverse primers for human IL-18, IL-18BP and IL-18R were listed in Additional file 3 (Table S2).

### Measurement of cytokine levels in plasma and NLF supernatant, and calculation of molar concentration ratio of plasma IL-18BP/IL-18

Levels of total IL-18 (tIL-18) and IL-18BP (tIL-18BP) in plasma or NLF supernatant were determined by using ELISA kit according to the manufacturer's instructions. Molar concentration of human IL-18 and IL-18BP was calculated using the equation of mass concentration divided by molecular weight, and free IL-18 (fIL-18) and IL-18BP (fIL-18BP) was calculated on the basis of a 1:1 stoichiometry in the complex of IL-18 and IL-18BP with a dissociation constant of 400 pM [23].

Human bio-plex panel (Bio-Rad Laboratories, California, USA) was employed to detect human plasma levels of IL-1β and TNF-α. The detection ranges for IL-1β and TNF-α were 0.24–3994 pg/mL and 0.57–9270 pg/mL, respectively.

### Statistical analysis

Statistical analyses were performed by using SPSS software (version 21.0, IBM Corporation). Data are displayed as a boxplot, which indicates the median, interquartile range, the largest and smallest values for the number of experiments indicated. Where Kruskal–Wallis analysis indicated significant differences between groups, a pairwise test was used for multiple comparisons between the groups. Correlations were determined by using Pearson's correlation or Spearman rank correlation analysis. For all analyses,  $P < 0.05$  was considered statistically significant.

## Results

### Elevated level of IL-18 in plasma of patients with pAR and sAR

Using ELISA kits, it was observed that levels of tIL-18 (Fig. 1a) and fIL-18 (Fig. 1c) in plasma of patients with pAR and sAR were elevated in comparison with HC subjects. In contrast, there were no significant differences of plasma levels of tIL-18BP (Fig. 1b) and fIL-18BP (Fig. 1d) between patients with pAR or sAR and HC subjects being observed. It was found that the molar concentration ratio of fIL-18BP/fIL-18 for HC subjects (16.5) was markedly greater than that for patients with sAR (9.7) (Fig. 1e), indicating that IL-18 is likely to play a role in sAR. Moreover, significant correlations between fIL-18 and fIL-18BP were observed in patients with pAR, sAR and HC subjects (Fig. 1f).

### **Elevated levels of IL-1 $\beta$ and TNF- $\alpha$ in plasma of patients with sAR**

Since monocytes and macrophages are involved in the pathogenesis of AR possibly by overproducing IL-1 $\beta$  and TNF- $\alpha$  [24-27], we examined levels of IL-1 $\beta$  and TNF- $\alpha$  in plasma of patients with pAR, sAR and HC by using human bio-plex panel kit. As shown in Fig. 2a, plasma level of IL-1 $\beta$  was generally low, nevertheless it appeared that plasma level of IL-1 $\beta$  in pAR and sAR patients were higher than that in HC. The plasma level of TNF- $\alpha$  in sAR patient (8.7 pg/mL), but not the patients with pAR was significantly higher than that in HC subjects (7.2 pg/mL) (Fig. 2b). Moreover, fIL-18, IL-1 $\beta$  and TNF- $\alpha$  were shown to be correlated well between each other in plasma of patients with pAR and sAR (Fig. 2c).

### **Increased expression of IL-18 and IL-18R, and decreased expression of IL-18BP in monocytes of patients with pAR and sAR**

Since fIL-18, IL-1 $\beta$  and TNF- $\alpha$  were correlated well between each other in plasma of patients with pAR and sAR, and tIL-18 and fIL-18 in plasma of patients with pAR and sAR were elevated in comparison with HC subjects, we investigated the expressions of IL-18, its specific receptor IL-18R, and its natural specific neutralizer IL-18BP in peripheral blood monocytes in the present study. The results showed that the proportion of IL-18 $^+$  monocytes was increased by 4.2 and 9.1 fold, and IL-18BP $^+$  monocytes was decreased by 77.5% and 56.0% in pAR and sAR patients, respectively when compared with HC subjects (Fig. 3b, c). PPAE seemed to upregulate IL-18 and IL-18R expression in monocytes of pAR and sAR patients, respectively (Fig. 3b, c).

As for the expression intensity of a single positive cell (mean fluorescence intensity, MFI), pAR and sAR patients appeared to have lower MFI of IL-18BP in monocytes than that in HC (Fig. 3d ii, e), and sAR patients had higher MFI of IL-18R on monocytes than that in HC and pAR patients (Fig. 3d iii, e). Moreover, all allergens tested in this study including ASWAE, PPAE and DPAE enhanced the MFI of IL-18 $^+$  monocyte in HC, and ASWAE increased the MFI of IL-18 $^+$  monocyte in sAR patients (Fig. 3d i, e).

### **Allergens and IFN $\gamma$ induced alteration of IL-18, IL-18BP and IL-18R expression in isolated monocytes**

In order to evaluate the direct effects of allergens on the expression of IL-18, IL-18BP and IL-18R in purified monocytes (purity over 99.7%), we co-cultured DPAE, ASWAE, PPAE or IFN $\gamma$  with purified monocytes in 12-well cell culture plate, and examined expression of IL-18, IL-18BP and IL-18R by flow cytometry. The results showed that proportion of IL-18BP $^+$  monocytes in AR patients was decreased

compared with HC (Fig. 4a, c), and IL-18BP expression appeared to be up-regulated in HC following the stimulation of DPAE for 10 min (Fig. 4a, c). It was also shown that ASWAE, PPAE and IFNy induced the elevated expression of IL-18R in monocytes of AR patients at 10 min following incubation. Moreover, DPAE increased the expression of IL-18R in AR patients at 30 min following stimulation (Fig. 4a, d). However, number of IL-18<sup>+</sup> monocytes in AR patients was decreased compared with that in HC (Fig. 4a, b), which was in contrast with the result seen in Fig. 3b and 3c.

In terms of MFI, DPAE and IFNy seemed to down-regulate MFI of IL-18BP<sup>+</sup> monocyte in AR patients at 60 min following incubation (Fig. 4e i, g). While the enhanced MFI of IL-18R<sup>+</sup> monocyte in patients with AR was observed in comparison with HC (Fig. 4e ii, h), DPAE increased the MFI of IL-18R in HC at 10 min following incubation (Fig. 4e ii, h). However, allergens and IFNy tested in the present study had little effect on IL-18 expression in monocytes (Fig. 4a, b, f).

### Allergen induced IL-18R mRNA expression in primary monocytes

In order to further understand the effects of allergens on the expression of IL-18, IL-18BP and IL-18R in monocytes, we examined expression of IL-18, IL-18BP and IL-18R mRNAs in primary monocytes by using qPCR technique. As seen in Fig. 5, ASWAE, PPAE, DPAE upregulated expression of IL-18R mRNA in monocytes of AR patients by 2.5, 2.5 and 4.6 fold, respectively at 30 min following challenge. At 60 min following challenge, only DPAE-induced expression of IL-18R mRNA in monocytes of AR patients was observed. ASWAE-provoked expression of IL-18R mRNA in monocytes was also found in HC at 30 min following challenge. Allergens tested had little effect on expression of IL-18 and IL-18BP mRNAs in monocytes of AR and HC subjects following 10, 30 and 60 min challenge periods (data not shown).

### Increased level of IL-18 in both plasma and NLF of AR mice

To understand further the role of IL-18 in AR, influence of OVA challenge on IL-18 and IL-18BP production in AR mice was examined. The results showed that IL-18 (Fig. 6a, b), but not IL-18BP (data not shown) levels were elevated in both plasma and NLF of AR mice.

### Down-regulated expression of IL-18R in blood monocytes and up-regulated expression of IL-18R in NLF macrophages of AR mice

To confirm the role of IL-18R in AR, we examined IL-18R expression in both blood monocytes and NLF macrophages of AR mice. Compared with HM group, while the number of monocytes (Fig. 7a, b) and MFI of IL-18R<sup>+</sup> monocyte (data not shown) in blood leukocytes had little change, the percentage of IL-18R<sup>+</sup> monocytes was reduced by 58.3% in AR mice (Fig. 7a, c). In contrast, the numbers of F4/80<sup>+</sup> macrophages and IL-18R<sup>+</sup> macrophages were increased approximately 1.2 (Fig. 7d, e) and 1.4 fold (Fig. 7d, f), respectively in AR mice.

## Discussion

IL-18 is a pro-inflammatory cytokine that induces IFN- $\gamma$  production, which is closely related to the pathophysiologic mechanism of allergic respiratory disorders [28]. In the present study, we showed that free plasma IL-18 was elevated in pAR and sAR patients, sAR patients had a decreased IL-18BP/IL-18 ratio (9.7), and free IL-18 correlated well with free IL-18BP in the plasma of both pAR patients and sAR patients, indicating that the imbalance between IL-18 and IL-18BP is likely to be crucial to the development of AR as a molar excess of 10 of IL-18BP over IL-18 is required to decrease a pathological level of 400 pg/ml of IL-18 to a level of a HC subject (40 pg/ml) [11]. Since IL18BP has neutralizing capacity of IL-18 [10] and excessive free IL18 can cause inflammatory conditions [12, 29], our data suggest that IL-18 may participate in the development of AR as a causative factor.

In the present study, we also observed that elevated levels fIL-18, IL-1 $\beta$  and TNF- $\alpha$  were correlated well between each other in plasma of patients with pAR and sAR. Given the fact that IL-1 $\beta$  and TNF- $\alpha$  are mainly produced by monocytes and macrophages [30, 31], we anticipate that the elevated levels of fIL-18, IL-1 $\beta$  and TNF- $\alpha$  are at least partially originated from monocytes. Our observation that the proportion of IL-18 $^+$  monocytes was increased in pAR and sAR patients may support the above anticipation, we hence believe that monocyte-derived IL-18 is likely to play a role in AR. The decreased IL-18BP $^+$  monocytes were found in AR peripheral blood may help to understand excessive fIL-18 in AR plasma as reduced IL-18BP production can eliminate IL-18/IL-18BP complex, and consequently free more IL-18 in the plasma. The enhanced MFI of IL-18R on monocytes of sAR patients suggest that IL-18 may act on monocytes through its receptor, which could implicate a paracrine mechanism that monocytes secrete IL-18, and IL-18 act on adjacent monocytes via IL-18R.

The results in the present study that PPAE upregulates IL-18 and IL-18R expression in monocytes of pAR and sAR patients, and ASWAE enhances the MFI of IL-18 $^+$  monocyte in sAR patients suggest that airborne allergens can directly affect IL-18 and IL-18R expression in monocytes even though direct contact of allergens with blood monocytes hardly occurs in the body. Unexpectedly, the MFI of IL-18 $^+$  monocyte in HC can be enhanced by all allergens tested in this study including ASWAE, PPAE and DPAE. Since IL-18 plays regulatory roles in both innate and adaptive immunity [32], elevated serum IL-18 during natural pollen exposure is closely associated with bronchial hyperresponsiveness in seasonal AR patients [8], and monocytes are one of the major sources of IL-18, the enhanced expression of IL-18 on monocytes may help to promote sensitization of HC to airborne allergens. The finding that synergy of IL-5 and IL-18 in eosinophil mediated pathogenesis of allergic diseases [33] may also support the view that IL-18 promote allergy.

Using primary monocytes, the elevated expression of IL-18R in monocytes of AR patients induced by allergens ASWAE, PPAE and DPAE was confirmed at both protein and mRNA levels, suggesting that allergen-induced upregulation of expression of IL-18R is most likely a direct event. Although the proportion of IL-18BP $^+$  monocytes in AR patients was decreased compared with HC, and DPAE seemed to down-regulate MFI of IL-18BP $^+$  monocyte in AR patients, allergens tested had little effect on expression of IL-18BP mRNAs in monocytes of AR and HC subjects, suggesting that reduced IL-18BP expression in

monocytes most likely occurred at protein synthesis process such as elongation, transport or modification stages.

On the other hand, compared with HC blood, less proportion of IL-18<sup>+</sup> populations were found in primary monocytes from peripheral blood of AR patients. This is an unexpected result considering our previous observation that the proportion of IL-18<sup>+</sup> monocytes was increased in peripheral blood of pAR and sAR patients. It is difficult to explain these conflict results without performing more detailed investigation, but the isolation procedure and individual difference between patients may take into account.

The results that IL-18 levels were elevated in both plasma and NLF of AR mice following OVA challenge support our observation that IL-18 level was increased in patients with AR. Since expression of IL-18R in blood monocytes appeared to be down-regulated and expression of IL-18R in NLF macrophages was up-regulated in AR mice, increased IL-18 may contribute to AR through macrophages or IL-18R expressing cells other than monocytes.

## Conclusions

In conclusion, we demonstrated for the first time that enhanced fIL-18 in AR plasma, and upregulated expression of IL-18 and IL-18R expression in monocytes of AR patients, which implicate strongly that IL-18 may serve as a causative factor for AR. Regulation of expression of IL-18, IL-18BP and IL-18R in monocytes by specific allergens suggests allergens can directly act on monocytes, thereafter modify IL-18, IL-18BP and IL-18R expression. These observations imply that monocyte-derived IL-18 is likely to contribute to the pathogenesis of AR, and therefore IL-18 could be therapeutic target for AR.

## Abbreviations

IL-18: Interleukin -18; fIL-18: free IL-18; tIL-18: total IL-18; IL-18BP: IL-18 binding protein; fIL-18BP: free IL-18BP; tIL-18BP: total IL-18BP; IL-18R: IL-18 receptor; AR: allergic rhinitis; pAR: perennial allergic rhinitis; sAR: seasonal allergic rhinitis; HC: healthy control; HM: healthy mice; PPAE: *Platanus* pollen allergen extract; DPAE: *Dermatophagoides pteronyssinus* allergen extract; ASWAE: *Artemisia sieversiana* wild pollen allergen extract; NLF: nasal lavage fluid; IFNg: interferon-g; FMO: fluorescence minus one; MFI: mean fluorescence intensity; PBMC: peripheral blood mononuclear cells.

## Declarations

### Acknowledgements

Not applicable.

### Authors' contributions

JL Wang and FQ Gu performed most experiments and drafted a large part of the first version of the manuscript. MM Zhan, L Wang, RM Yang and YL Hu participated in magnetic cell sorting and flow cytometry. HY Zhang carried out ELISA and Bio-plex experiment, and drew the Fig.s. D Chen, H Xie and RN Chai recruited volunteers, collected their general information and conducted clinical study. N Zhao carried out PCR experiment and data interpretation. Z Li contributed to the literature review. SH He designed and supervised the study, analyzed the data and wrote the second and final draft of the manuscript. All authors commented on previous manuscript, read and approved the final manuscript.

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## Availability of data and materials

All quantitative data generated during this study are included in this published article. Raw data support the findings of this study will be available from the corresponding author upon reasonable request.

## Ethics approval and consent to participate

All participants were included in this study after signing a written informed consent. Ethics approval of healthy donors and patients with AR was granted by the ethical committee of the First Affiliated Hospital of Jinzhou Medical University and the General Hospital of Shenyang Military Region of PLA. The procedures followed were in accordance with the ethical guidelines of the Declaration of Helsinki. Animal ethics was approved by the Animal Care Committee at Jinzhou Medical University.

## Consent for publication

Not applicable.

## Competing interests

The authors declare they have no competing interest regarding the publication of this article.

## Author details

<sup>1</sup> Department of Immunology, College of Basic Medical Sciences, China Medical University, No. 77 Puhe Road, Shenyang North New Area, Shenyang 110122, People's Republic of China; <sup>2</sup> Allergy and Clinical Immunology Research Centre, the First Affiliated Hospital of Jinzhou Medical University, Jinzhou, Liaoning 121001, China; <sup>3</sup> Translational Medicine Institute, Shenyang Medical College, Shenyang,

Liaoning 110034, China; <sup>4</sup>The PLA Center of Respiratory and Allergic Disease Diagnosing Management, General Hospital of Shenyang Military Area Command, Shenyang 110840, China.

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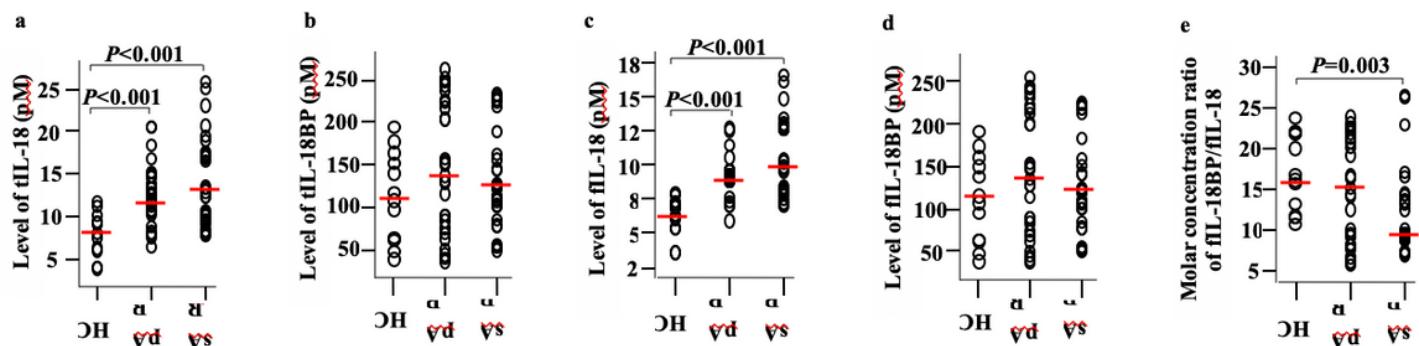
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## Supplemental Legend

**Supplement Fig. 1.** Nasal symptom scores of mice. Mouse rubbing (**a**) and sneezing (**b**) numbers were recorded for 15 min following OVA or vehicle challenge. Data are displayed as a boxplot for OVA-induced allergic rhinitis (AR) mice ( $n = 7$ ) and vehicle treated healthy mice (HM,  $n = 7$ ), which indicates the median, interquartile range, the largest and smallest values for the number of subjects indicated.  $P < 0.05$  was taken as statistically significant.

## Figures



**f** Pearson's correlation coefficients between plasma fIL-18 and fIL-18BP in AR patients and HC

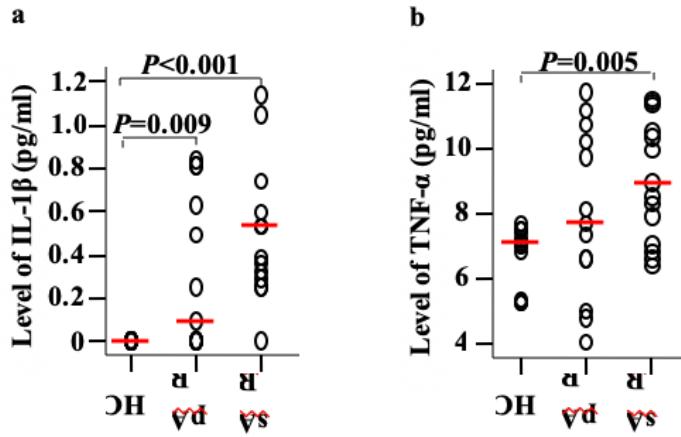
Compound	Correlation between plasma fIL-18 and fIL-18BP ( $r$ values)					
	pAR		sAR		HC	
	fIL-18	fIL-18BP	fIL-18	fIL-18BP	fIL-18	fIL-18BP
fIL-18	1	0.920*	1	0.979*	1	0.993*
fIL-18BP	0.920*	1	0.979*	1	0.993*	1

\* $P < 0.05$ .

## Figure 1

Levels of total and free plasma IL-18 and IL-18BP in patients with AR. Scatter plots of levels of total IL-18 (tIL-18, a) and total IL-18BP (tIL-18, b), free IL-18 (fIL-18, c) and free IL-18BP (fIL-18, d) in plasma of

patients with perennial allergic rhinitis (pAR) and seasonal allergic rhinitis (sAR), and healthy control (HC) subjects. (e) shows the molar concentration ratios of fIL-18BP/fIL-18. Each symbol represents the value from one subject. The median value of each defined group of subjects is indicated as a horizontal line. The Pearson's correlation coefficient between plasma levels of fIL-18 and fIL-18BP is shown in (f).  $P < 0.05$  was taken as statistically significant.



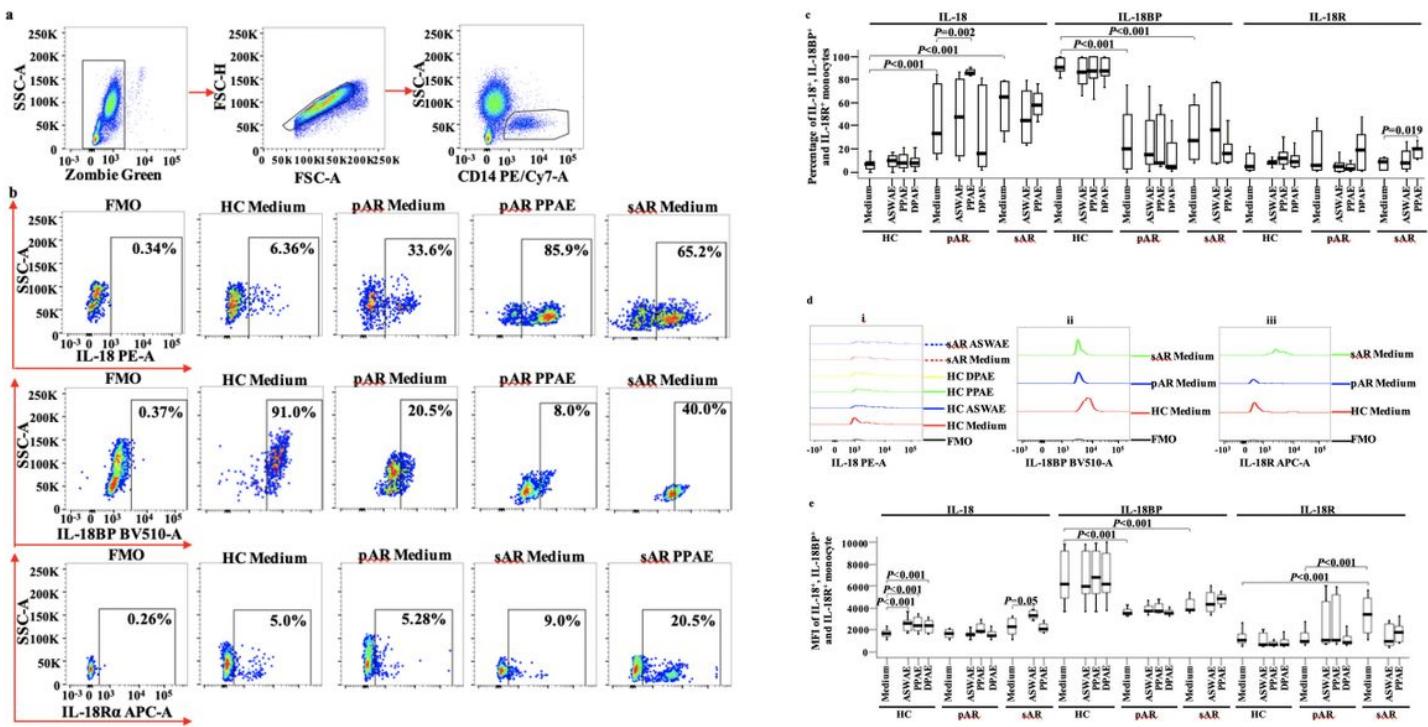
**c** Rank correlation (Spearman's  $\rho$  correlation coefficient) among plasma fIL-18, IL-1 $\beta$  and TNF- $\alpha$  in pAR and sAR patients.

Compound	Correlation among plasma fIL-18, IL-1 $\beta$ and TNF- $\alpha$ ( $r$ values)						
	pAR			sAR			
	fIL-18	IL-1 $\beta$	TNF- $\alpha$		fIL-18	IL-1 $\beta$	TNF- $\alpha$
fIL-18	1	0.865*	0.965*		1	0.975*	0.998*
IL-1 $\beta$	0.865*	1	0.921*		0.975*	1	0.985*
TNF- $\alpha$	0.965*	0.921*	1		0.998*	0.985*	1

\* $P < 0.05$ .

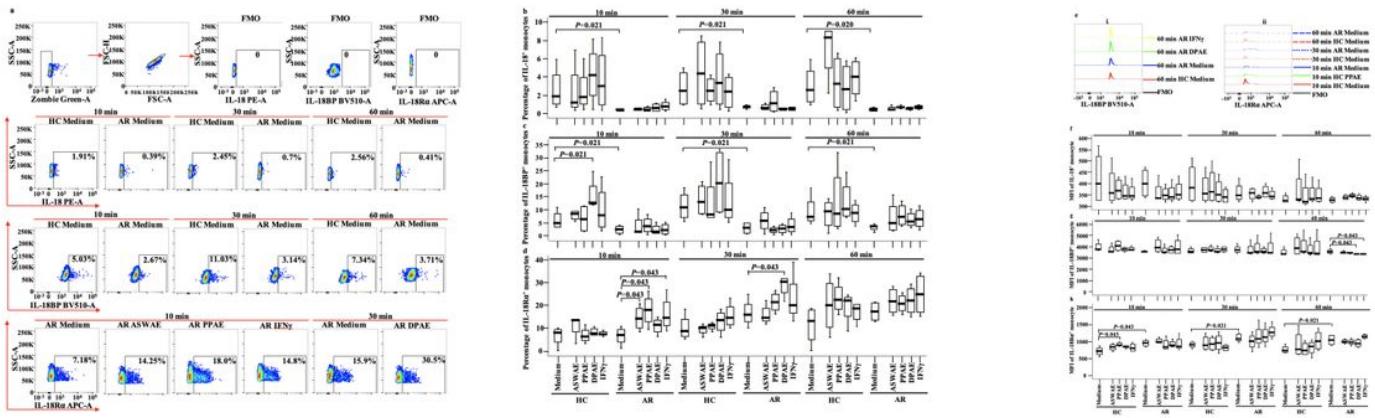
## Figure 2

Plasma levels of IL-1 $\beta$  and TNF- $\alpha$  in AR patients and HC (Bio-plex). Scatter plots of levels of IL-1 $\beta$  (a) and TNF- $\alpha$  (b) in plasma of perennial allergic rhinitis (pAR) and seasonal allergic rhinitis (sAR) patients and healthy control (HC) subjects. Each symbol represents the value from one subject. The median value of each defined group of subjects is indicated as a horizontal line. The Spearman's  $\rho$  correlation coefficient between the plasma levels of fIL-18, IL-1 $\beta$  and TNF- $\alpha$  in pAR and sAR patients is shown in (c).  $P < 0.05$  was taken as statistically significant.



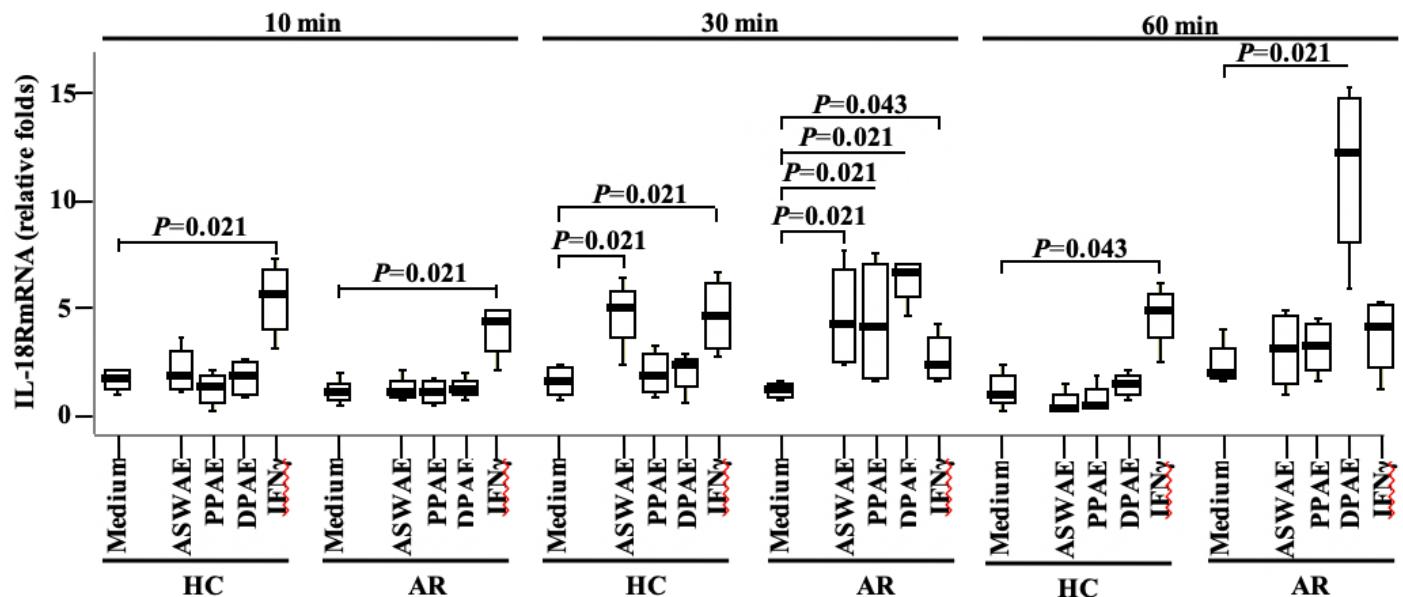
**Figure 3**

IL-18, IL-18BP and IL-18R expressing cell populations in CD14+ monocytes of human peripheral blood. Expression of IL-18, IL-18BP and IL-18R in peripheral blood CD14+ monocytes of patients with perennial allergic rhinitis (pAR) and seasonal allergic rhinitis (sAR), and healthy control (HC) subjects in the presence or absence of Dermatophagoides pteronyssinus allergen extract (DPAE), Artemisia sieversiana wild allergen extract (ASWAE) and Platanus pollen allergen extract (PPAE). (a) represents a gating strategy of CD14+ monocytes in leukocytes; (b) is representative figures of proportions of IL-18+, IL-18BP+ and IL-18R+ cells in CD14+ monocytes; (c) demonstrates percentages of IL-18, IL-18BP and IL-18R expressing monocytes in CD14+ monocytes; (d) shows representative flow cytometric figures of mean fluorescence intensity (MFI) of IL-18+ (i), IL-18BP+ (ii) and IL-18R+ (iii) CD14+ monocyte. (e) reveals MFI levels of IL-18, IL-18BP and IL-18R expression in CD14+ monocyte. Data are displayed as a boxplot for pAR patients ( $n = 33$ ), sAR patients ( $n = 9$ ), and HC ( $n = 25$ ), which indicates the median, interquartile range, the largest and smallest values for the number of volunteers indicated.  $P < 0.05$  was taken as statistically significant. FMO = fluorescence minus one control.



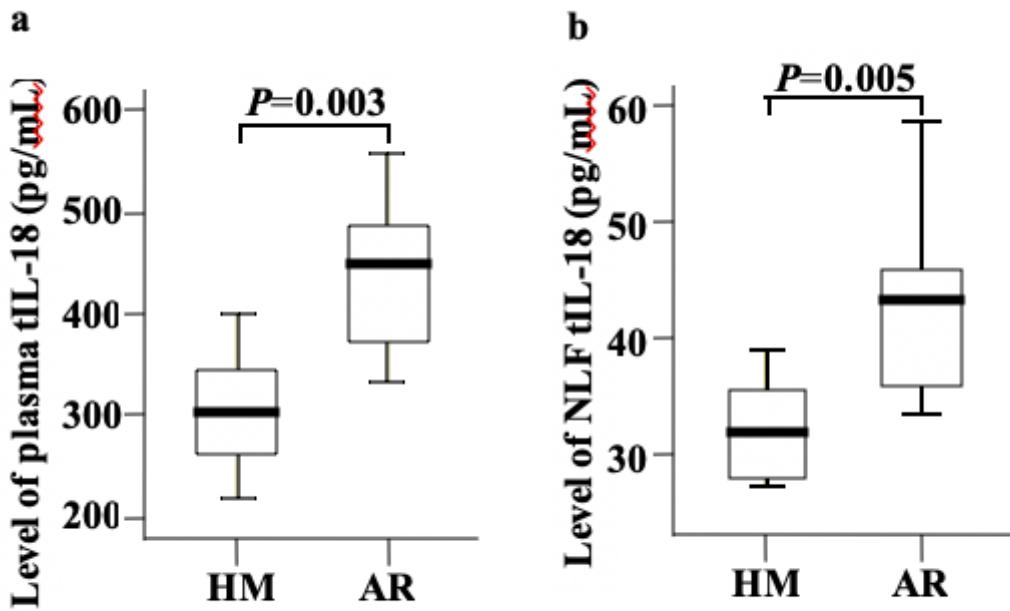
**Figure 4**

Expression of IL-18, IL-18BP and IL-18R in isolated CD14+ monocytes of human peripheral blood. Expression of IL-18, IL-18BP and IL-18R in isolated monocytes of allergic rhinitis (AR) patients and healthy control subjects (HC) in the presence or absence of Dermatophagoides pteronyssinus allergen extract (DPAE), Artemisia sieversiana wild allergen extract (ASWAE) and Platanus pollen allergen extract (PPAE). (a) is a gating strategy of expression of IL-18, IL-18BP and IL-18R in isolated monocytes; (b, c, d) demonstrate percentages of IL-18, IL-18BP and IL-18R expressing monocytes, respectively; (e) shows representative figures of mean fluorescence intensity (MFI) of IL-18BP+ (i) and IL-18R+ (ii) monocyte. (f, g, h) reveal MFI of IL-18, IL-18BP and IL-18R expression in monocyte, respectively. Data are displayed as a boxplot for AR patients ( $n = 6$ ) and HC ( $n = 6$ ), which indicates the median, interquartile range, the largest and smallest values for the number of volunteers indicated. FMO = fluorescence minus one control.  $P < 0.05$  was taken as statistically significant.



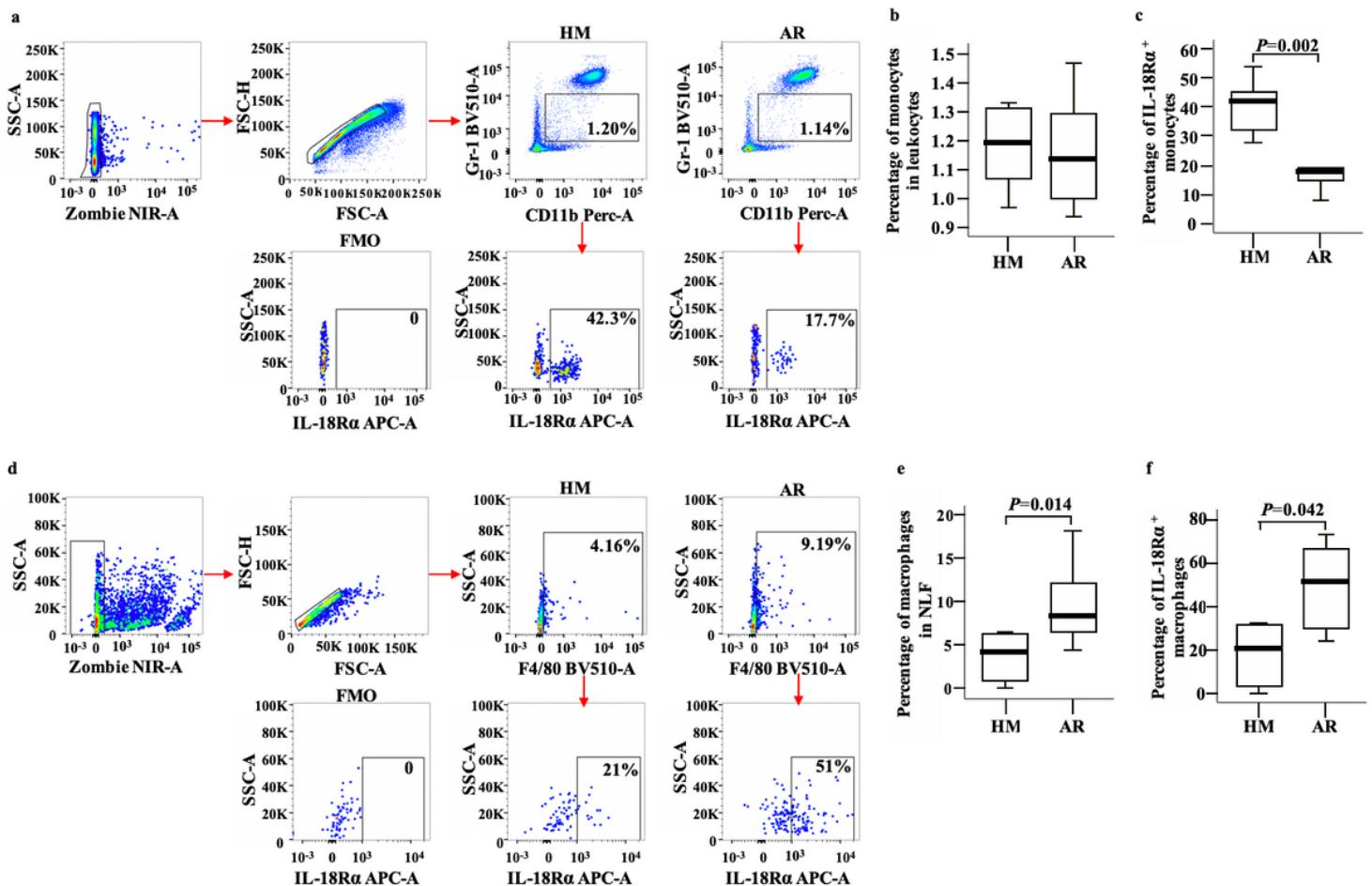
**Figure 5**

Induction of upregulated expression of IL-18R mRNA in sorted monocytes by allergens and IFNy. Quantitative real-time PCR (qPCR) analysis of expression of IL-18 receptor (IL-18R) mRNA in isolated monocytes of patients with allergic rhinitis (AR) and healthy control (HC) volunteers. Cells were incubated in the presence or absence of Artemisia sieversiana wild allergen extract (ASWAE), Platanus pollen allergen extract (PPAE) and Dermatophagoides allergen extract (DPAE) or IFNy. Expression of IL-18R mRNA was analyzed by qPCR. The data displayed as a boxplot for AR patients ( $n = 6$ ) and HC volunteers ( $n = 6$ ), which indicates the median, interquartile range, the largest and smallest values for the number of volunteers indicated.  $P < 0.05$  was taken as statistically significant.



**Figure 6**

Increased level of IL-18 in plasma and nasal lavage fluid of OVA induced AR mice. Levels of IL-18 in mouse plasma (a) and NLF (b). Following a seven-day OVA challenger or vehicle control treatment, plasma and NLF supernatant of OVA-induced allergic rhinitis (AR) mice and healthy mice (HM) were taken, and analyzed by using sandwich ELISA kits. Data are displayed as a boxplot for AR mice ( $n = 7$ ) and HM ( $n = 7$ ), which indicates the median, interquartile range, the largest and smallest values for the number of animals indicated.  $P < 0.05$  was taken as significant.



**Figure 7**

Expression of IL-18R in blood monocytes and NLF macrophages from OVA induced allergic rhinitis mice. Expression of IL-18 receptor (R) in blood monocytes and nasal lavage fluid (NLF) macrophages of OVA-induced allergic rhinitis (AR) mice or vehicle treated healthy mice (HM). (a) shows a gating strategy of CD11b+ Gr-1low monocyte expression in mouse leukocytes, and IL-18R expression in monocytes; (b, c) demonstrate percentages of monocytes in leukocytes, and proportions of IL-18R expressing monocytes, respectively; (d) represents a gating strategy of F4/80+ macrophage expression in mouse NLF, and IL-18R expression in macrophages of mouse NLF. (e, f) reveal percentages of macrophages in NLF, and proportions of IL-18R expressing NLF macrophages, respectively. Data are displayed as a boxplot for AR mice ( $n = 7$ ) and HM ( $n = 7$ ), which indicates the median, interquartile range, the largest and smallest values for the number of subjects indicated.  $P < 0.05$  was taken as statistically significant. FMO = fluorescence minus one control.

## Supplementary Files

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