Altered Expression of IL-18, IL-18 Binding Protein and IL-18 Receptor in Blood Monocytes of Patients with Allergic Rhinitis

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

Results: Plasma IL-18 and IL-1β in AR patients was higher than those in healthy control subjects. Free (f)IL-18 had a high correlation with IL-18BP, IL-1β and TNF-α 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 isolated 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: These suggest that 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)-γ-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 major cell type 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.

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. 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 being observed. It was found that the molar concentration ratio of fIL-18BP/fIL-18 for HC (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 (Fig. 1F).

Elevated levels of IL-1β and TNF-α in plasma of patients with sAR

Since monocytes and macrophages are involved in the pathogenesis of AR possibly by overproducing IL-1β and TNF-α [18–21], we examined levels of IL-1β and TNF-α in plasma of patients with pAR, sAR and HC using human bio-plex panel kit. As shown in Fig. 2A, plasma level of IL-1β was generally low, nevertheless it appeared that plasma level of IL-1β in pAR and sAR patients were higher than that in HC. The plasma level of TNF-α in sAR patient (8.7 pg/mL), but not the patients with pAR was significantly higher than that in HC (7.2 pg/mL) (Fig. 2B). Moreover, fIL-18, IL-1β and TNF-α 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-1β and TNF-α 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, 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 $IL\text{-}18^{+}$ monocytes was increased by 4.2 and 9.1 fold, and $IL\text{-}18BP^{+}$ monocytes was decreased by 77.5% and 56.0% in pAR and sAR patients, respectively when compared with HC (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 IFNγ 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 up to 99.7%), we co-cultured DPAE, ASWAE, PPAE or IFNγ 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 IFNγ 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^{+} 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 IFNγ seemed to down-regulate MFI of IL-18BP^{+} monocyte in AR patients at 60 min following incubation (Fig. 4E i, G). While the enhanced MFI of IL-18R^{+} 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 IFNγ 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 IL-18, IL-18BP and IL-18R expression in monocytes, we examined expression of IL-18, IL-18BP and IL-18R mRNAs in primary monocytes 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 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 macrophgaes 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$^+$ monocyte (data not shown) in blood leukocytes had little change, the percentage of IL-18R$^+$ monocytes was reduced by 58.3% in AR mice (Fig. 7A, C). In contrast, the numbers of F4/80$^+$ macrophages and IL-18R$^+$ 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-γ production, which is closely related to the pathophysiologic mechanism of allergic respiratory disorders [22]. 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 (40 pg/ml) [11]. Since IL-18BP has neutralizing capacity of IL-18 [10] and excessive free IL-18 can cause inflammatory conditions [12, 23], 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-1β and TNF-α were correlated well between each other in plasma of patients with pAR and sAR. Given the fact that IL-1β and TNF-α are mainly produced by monocytes and macrophages [24, 25], we anticipate that the elevated levels of fIL-18, IL-1β and TNF-α 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 [26], elevated serum IL-18 during natural pollen exposure is closely associated with bronchial hyperresponsiveness in seasonal AR patients [8], and monocytes are one major source 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 [27] 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, 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$^+$ 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$^+$ 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.

Conclusion

In summary, we demonstrated for the first time that enhanced fIL-18 in AR plasma, and upregulated 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.

Methods

Reagents

The following reagents were purchased from Biolegend (San Diego, CA, USA): human RBC 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 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-18Rα monoclonal antibody, PE-conjugated mouse anti-human IL-18 monoclonal antibody, APC-conjugated rat anti-mouse IL-18Rα 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). IFNγ 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. Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit was bought from BD Biosciences (Beldford, 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

General characteristics of 33 patients with perennial allergic rhinitis (pAR), 9 patients with sAR and 25 healthy control subjects (HC) recruited in this study were summarized in Supplement table 1. The diagnosing criteria of pAR and sAR were conformed to the Chinese Society of Allergy Guidelines for Diagnosis and Treatment of Allergic Rhinitis [28].

The experiment was approved by the ethical committees of the First Affiliated Hospital of Jinzhou Medical University and the General Hospital of Northern Military Area. 5 mL of peripheral blood samples were drawn into K_2EDTA containing tubes after obtaining written informed consent from each participant, and centrifuged at 450 × *g* for 10 min. The cells and plasma were collected separately for flow cytometric and ELISA analysis. For $CD14^+$ monocytes isolation study, approximately 180 mL of peripheral blood was taken from each donor.

Five-week-old female BALB/c mice, weighting 18–22 g, were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China), and maintained in specific pathogen-free facilities with free access to standard rodent chow and water, at a constant temperature 23–28°C and relative humidity of 60–75% of the First Affiliated Hospital of Jinzhou Medical University maintained as described previously [12]. The animal experiment procedures were authorized by the Animal Care Committee at Jinzhou Medical University.

Isolation Of Cd14 Monocytes And Allergen Challenge Test

$CD14^+$ cells were enriched by density gradient centrifugation and positive selection on magnetic cell sorting (MACS) according to the manufacturer's instructions. Purities of recovered $CD14^+$ cells were evaluated by flow cytometry with PE/Cy7-conjugated 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 monocytes at a density of 1×10^6 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 IFNγ (as positive control) at 5 ng/mL for 10, 30 and 60 min, respectively at 37 °C in a 5% (v/v) CO_2, 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 × *g* for 10 min at 4 °C. Cell pellets containing approximately 0.5×10^6 and 1×10^6 cells were resuspended in PBS for flow cytometric analysis and in TRIzol reagent for RT-PCR, respectively.

Flow cytometric analysis of IL-18, IL-18BP and IL-18R in human peripheral blood monocytes and isolated $CD14^+$ 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.

For cell surface molecules, whole blood cells were preincubated with human Fc receptor blocking solution and Zombie Green dye [29], then stained with PE/Cy7 conjugated anti-human CD14 and APC conjugated anti-human IL-18Rα antibodies. Following erythrocyte lysis, leukocytes were analyzed with FACSVerse flow cytometer (BD Biosciences, San Jose, CA, USA). An irrelevant isotype- and concentration-matched antibody of anti-human IL-18Rα 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 isolated monocytes, cells were processed as above.

For intracellular molecules, resultant leukocytes prepared as above were fixed and permeabilized, and stained with anti-human IL-18BP and PE conjugated anti-human IL-18 antibodies, which was followed by

the addition of BV510-conjugated donkey anti-rabbit polyclonal antibody. These procedures were applicable to isolated monocytes.

Establishment Of Mouse Ar Model

A total of 14 mice were randomly divided into control and AR groups. OVA-induced AR mouse model was mainly adopted from a previous study by Mo JH *et al* [30]. 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, control mice received vehicle only instead of OVA solution. At 24 h following the last OVA challenge, mice were sacrificed by eyeball enucleation, blood and nasal lavage fluid (NLF) samples were thereafter collected. Total cells were determined and centrifuged as above, amongst cells were used for flow cytometric analysis, plasma and NLF supernatant was aliquoted and frozen at -80°C for ELISA assay.

To evaluate allergic symptoms, numbers of sneezing and nasal-rubbing motions of all survived mice during the first 15 min after each challenge were recorded, and compared with healthy control mice (HM) by observers blinded to the study. As presented in Supplement Fig. 1A and Fig. 1B, the numbers of nasal rubbing and sneezing motion in AR mice (n = 7) were substantially higher than that in HM (n = 7) during a 7-day observation period.

Flow cytometric analysis of IL-18R expression 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 Zombie NIR dye [31]. 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 before lysing erythrocytes. Finally, cells were processed as for human blood samples and analyzed using flow cytometry.

To detect IL-18R expression in NLF macrophages, cells were first incubated with anti-mouse CD16/32 antibodies and Zombie NIR dye, subsequently incubated 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 $CD14^+$ monocytes

cDNA generated from total RNA of isolated human blood $CD14^+$ cells was used as templates, and qPCR assay was performed as described by Zhang *et al* [32] using specific primers of IL-18, IL-18BP and IL-18R as listed in Supplement table 2. Each measurement of a sample was conducted in duplicate.

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, and free IL-18 (fIL-18) and IL-18BP (fIL-18BP) were calculated as described by Novick D *et al* [33].

Human bio-plex panel (Bio-Rad Laboratories, California, USA) was employed to detect 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 SPSS software (version 21.0, IBM Corporation). Data were displayed as a boxplot, which indicates the median, interquartile range, the largest and smallest values for indicated experiments. Where Kruskal–Wallis analysis indicated significant differences between groups, a pairwise test was used for multiple comparisons between the groups. Correlations were determined using Pearson's correlation or Spearman rank correlation analysis. For all analyses, $P < 0.05$ was considered statistically significant.

Abbreviations

fIL-18
free IL-18
tIL-18
total IL-18
fIL-18BP
free IL-18BP
tIL-18BP
total IL-18BP
AR
allergic rhinitis
pAR
perennial allergic rhinitis
sAR
seasonal allergic rhinitis
HC
healthy control subjects
HM
healthy mice
PPAE
Platanus pollen allergen extract
DPAE
Dermatophagoides pteronyssinus allergen extract

ASWAE
Artemisia sieversiana wild pollen allergen extrac
NLF
nasal lavage fluid
FMO
fluorescence minus one
MFI
mean fluorescence intensity
PBMC
peripheral blood mononuclear cells

Declarations

Ethics approval and consent to participate

The research was in compliance with the Declaration of Helsinki and was approved by the ethical committees of the First Affiliated Hospital of Jinzhou Medical University and the General Hospital of Northern Military Area. Animal ethics was approved by the Animal Care Committee at Jinzhou Medical University.

Written informed consent was obtained from all individual participants included in this study.

Consent for publication

Not applicable.

Availability of data and materials

The materials and data generated or analyzed during this study are included in this article and its supplementary information files. Raw data support the findings of this study will be available from the corresponding author upon reasonable request.

Competing interests

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

All the authors including Junling Wang, Fangqiu Gu, Zhi Li, Mengmeng Zhan, Ling Wang, Huiyun Zhang, Dong Chen, Hua Xie, Ruonan Chai, Nan Zhao, Ruiming Yang, Yalin Hu and Shaoheng He have approved the manuscript and agree with submission to your journal.

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Authors' contributions

JL Wang and FQ Gu performed most experiments and drafted the original 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 generated the figures. D Chen, H Xie and RN Chai recruited volunteers and collected their general information. N Zhao carried out PCR experiment and data interpretation. Z Li contributed to animal study and literature review. SH He designed and supervised the study, analyzed the data and wrote the final version of the manuscript. All authors commented on previous manuscript, read and approved the final manuscript.

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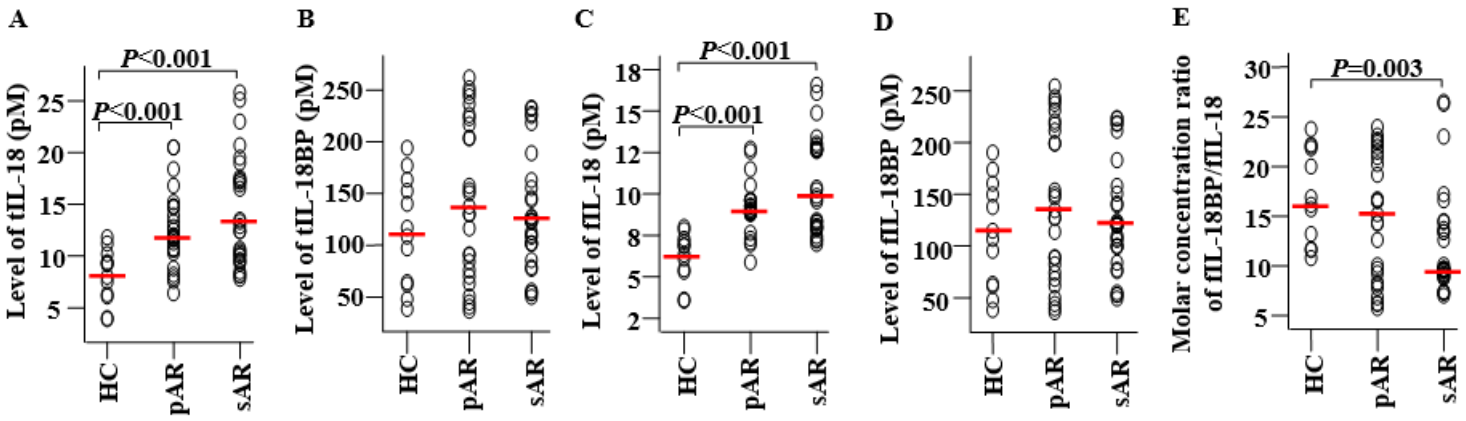
Figures



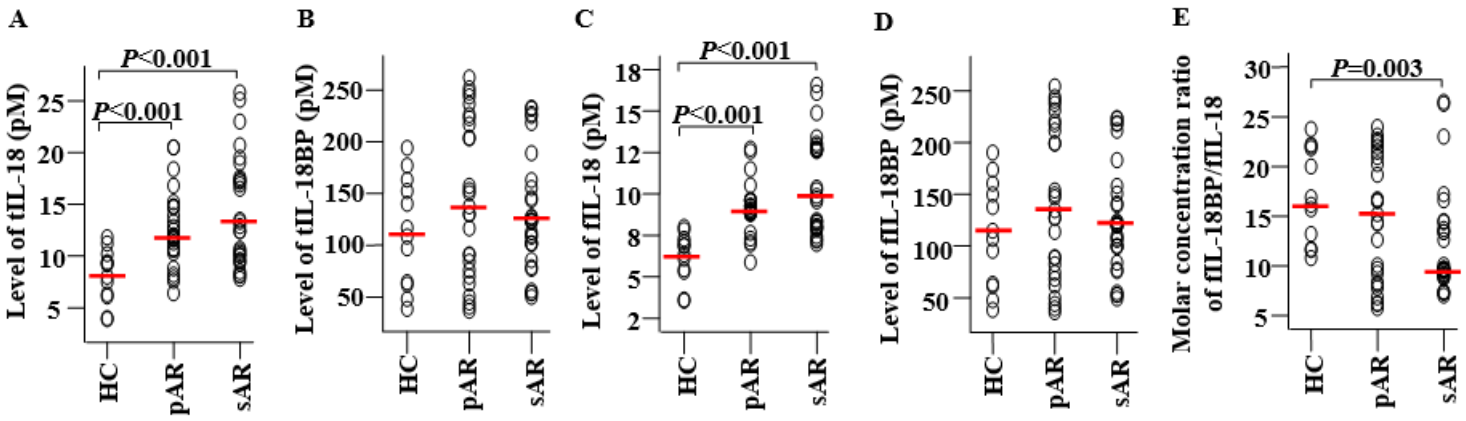
F Pearson's correlation coefficients between plasma fIL-18 and fIL-18BP in AR patients and HC

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

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 subjects (HC). (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.



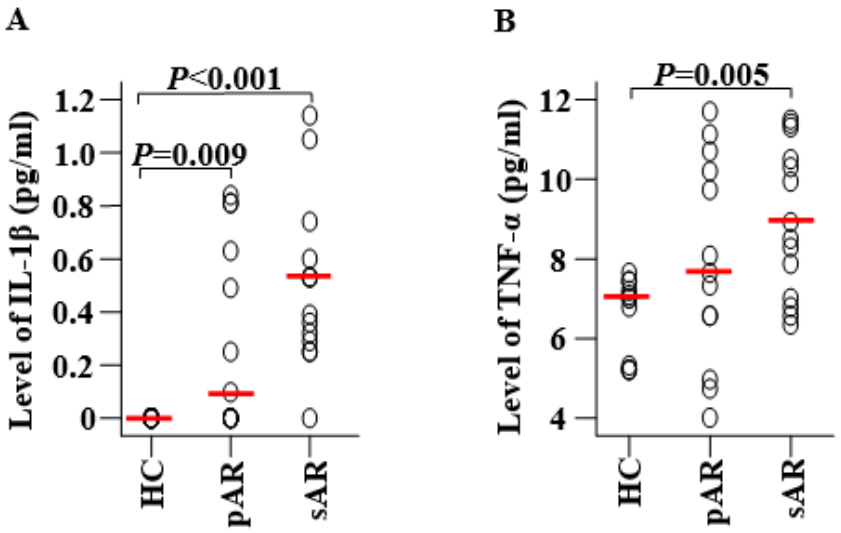
F Pearson's correlation coefficients between plasma fIL-18 and fIL-18BP in AR patients and HC

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

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 subjects (HC). (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.



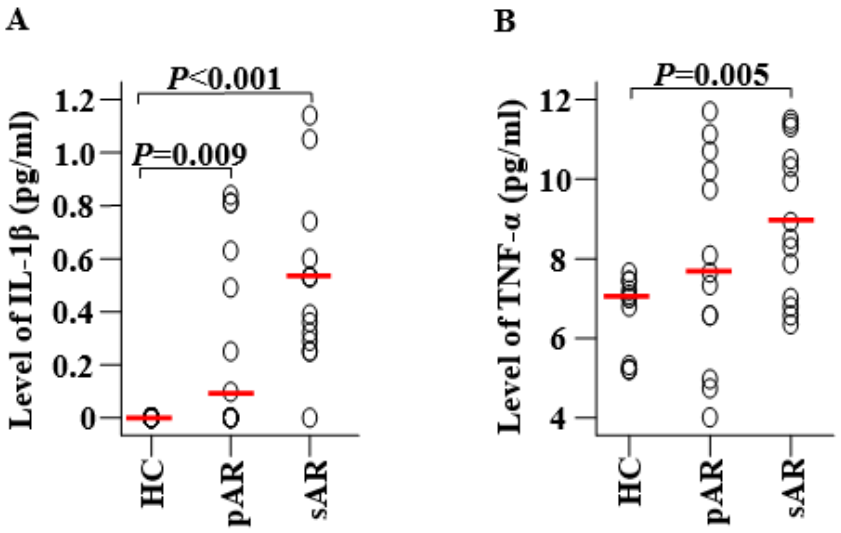
C Rank correlation (Spearman's ρ correlation coefficient) among plasma fIL-18, IL-1β and TNF-α in pAR and sAR patients.

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

*$P < 0.05$.

Figure 2

Scatter plots of levels of IL-1β (A) and TNF-α (B) in plasma of perennial allergic rhinitis (pAR) and seasonal allergic rhinitis (sAR) patients and healthy control subjects (HC). 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 ρ correlation coefficient between the plasma levels of fIL-18, IL-1β and TNF-α in pAR and sAR patients is shown in (C). P < 0.05 was taken as statistically significant.



C Rank correlation (Spearman's ρ correlation coefficient) among plasma fIL-18, IL-1β and TNF-α in pAR and sAR patients.

Correlation among plasma fIL-18, IL-1β and TNF-α (*r* values)

Compound	pAR			sAR		
	fIL-18	IL-1β	TNF-α	fIL-18	IL-1β	TNF-α
fIL-18	1	0.865*	0.965*	1	0.975*	0.998*
IL-1β	0.865*	1	0.921*	0.975*	1	0.985*
TNF-α	0.965*	0.921*	1	0.998*	0.985*	1

*$P < 0.05$.

Figure 2

Scatter plots of levels of IL-1β (A) and TNF-α (B) in plasma of perennial allergic rhinitis (pAR) and seasonal allergic rhinitis (sAR) patients and healthy control subjects (HC). 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 ρ correlation coefficient between the plasma levels of fIL-18, IL-1β and TNF-α in pAR and sAR patients is shown in (C). P < 0.05 was taken as statistically significant.

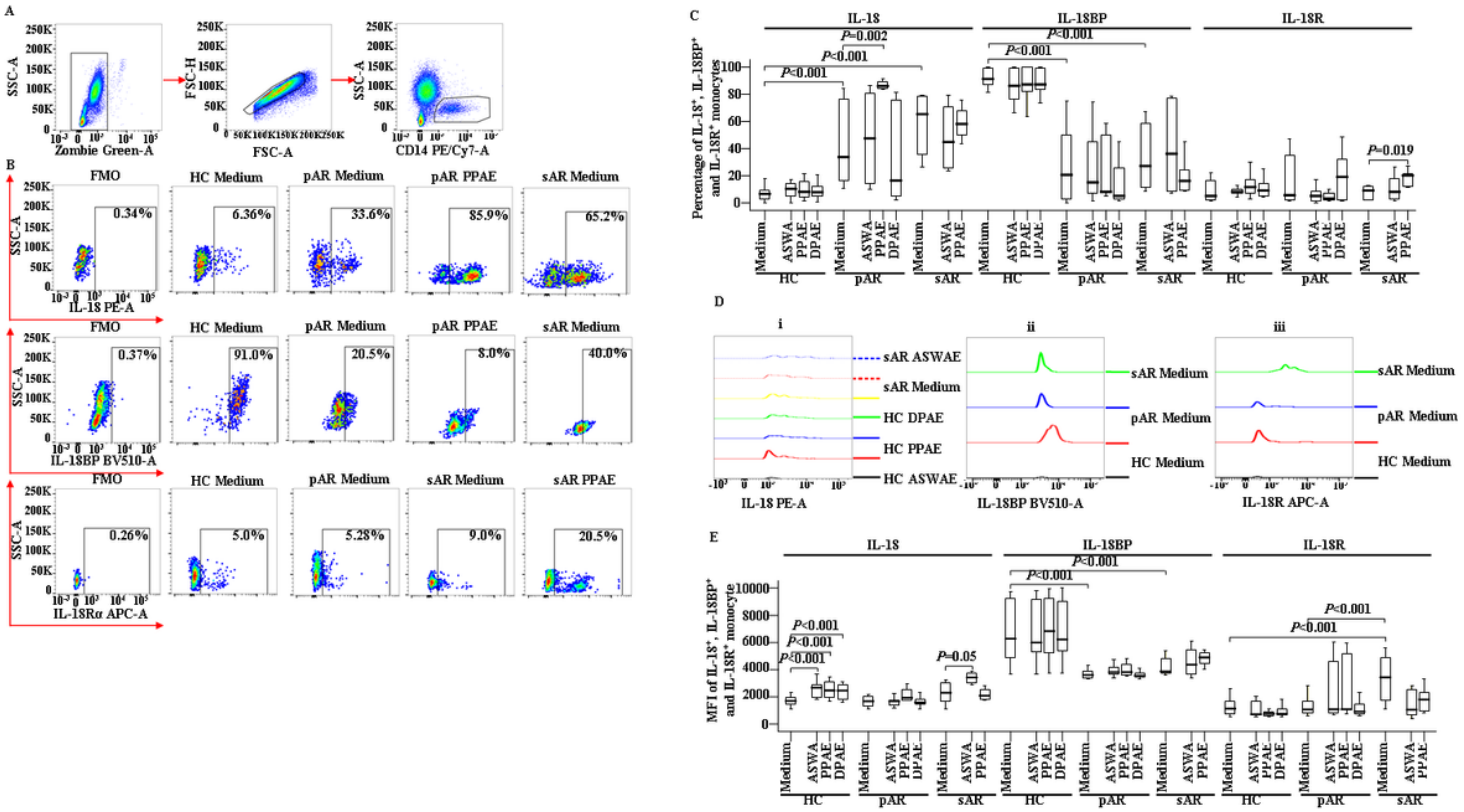
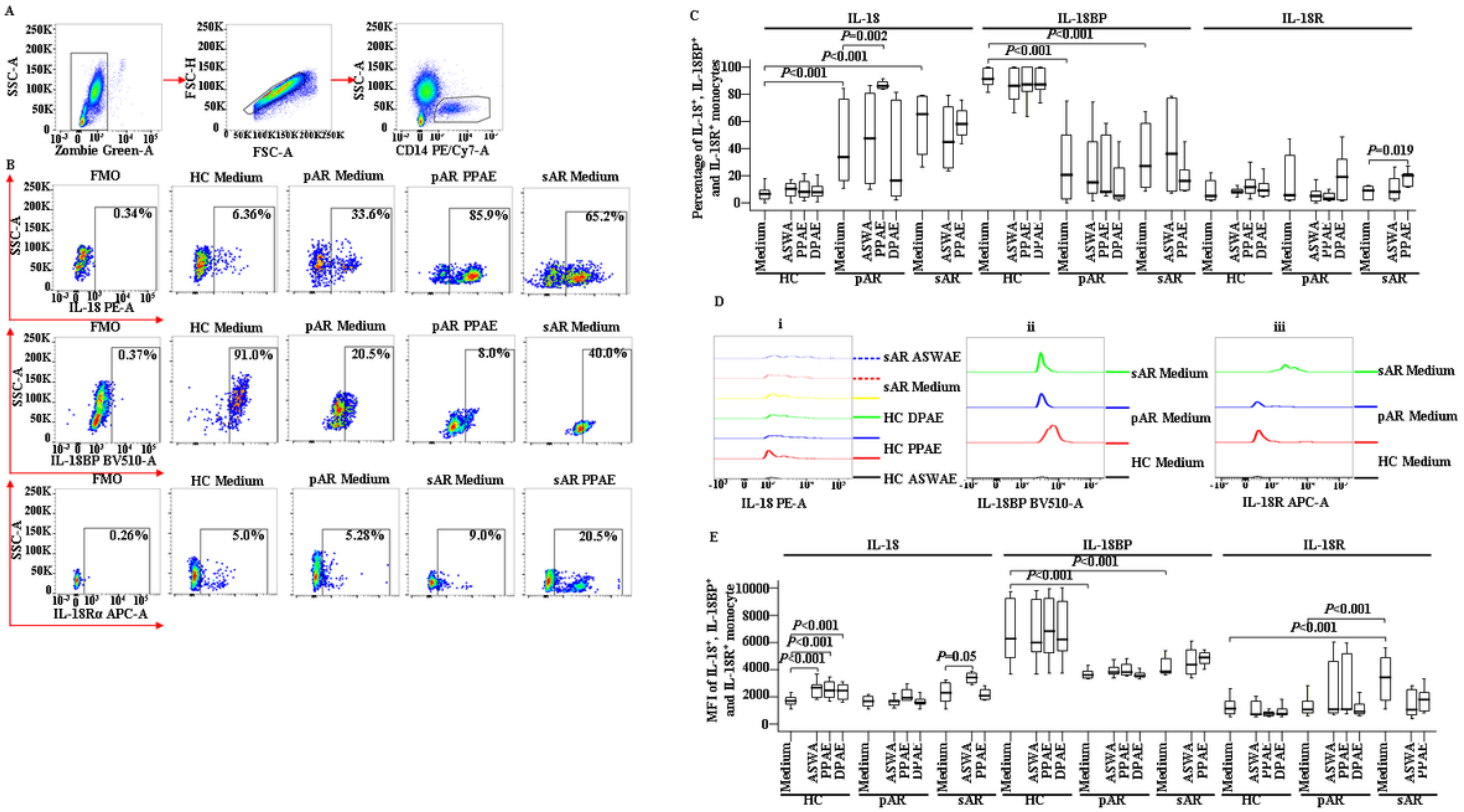


Figure 3

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 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) represents a gating strategy of CD14+ monocytes in leukocytes; (B) is representitive 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.



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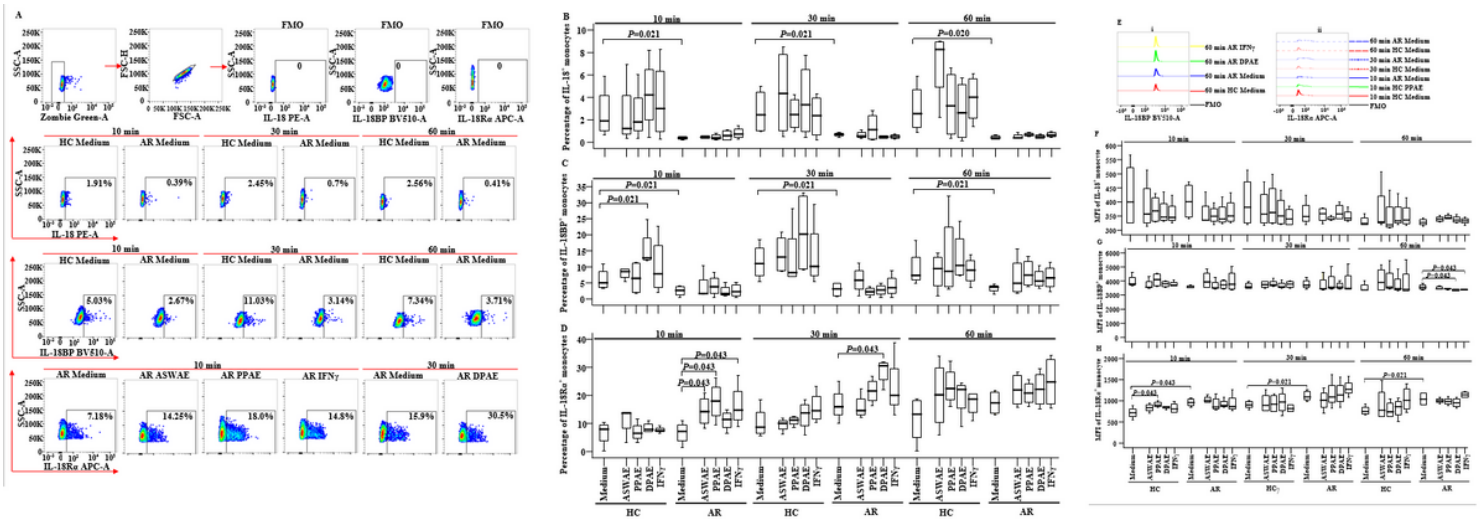


Figure 4

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.

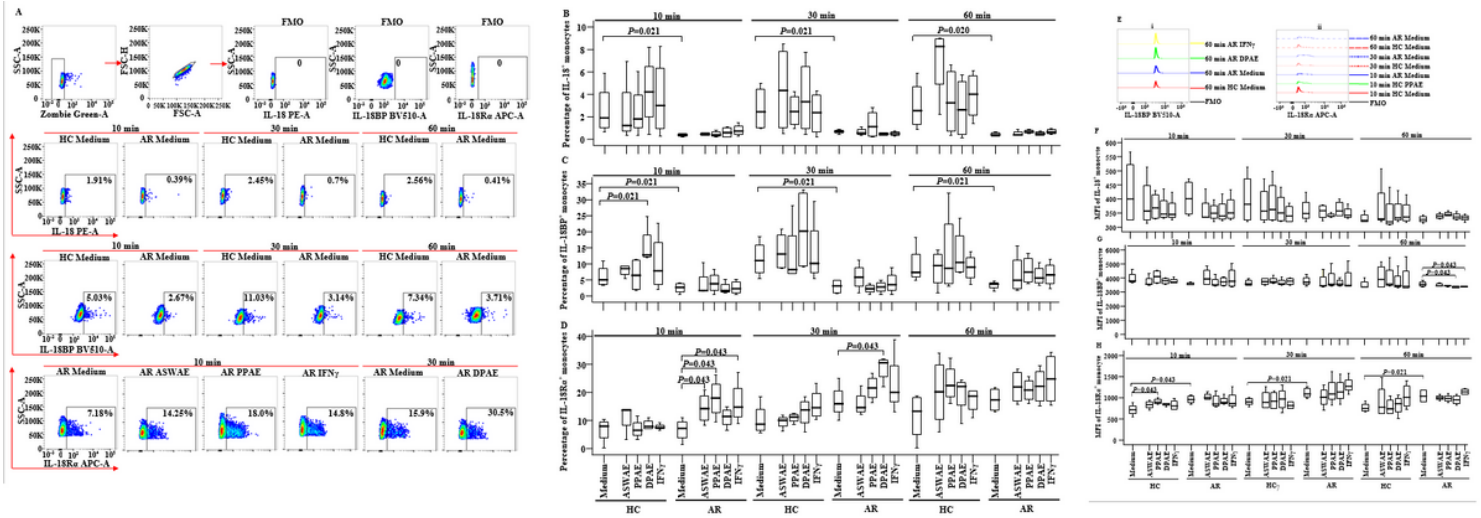


Figure 4

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.

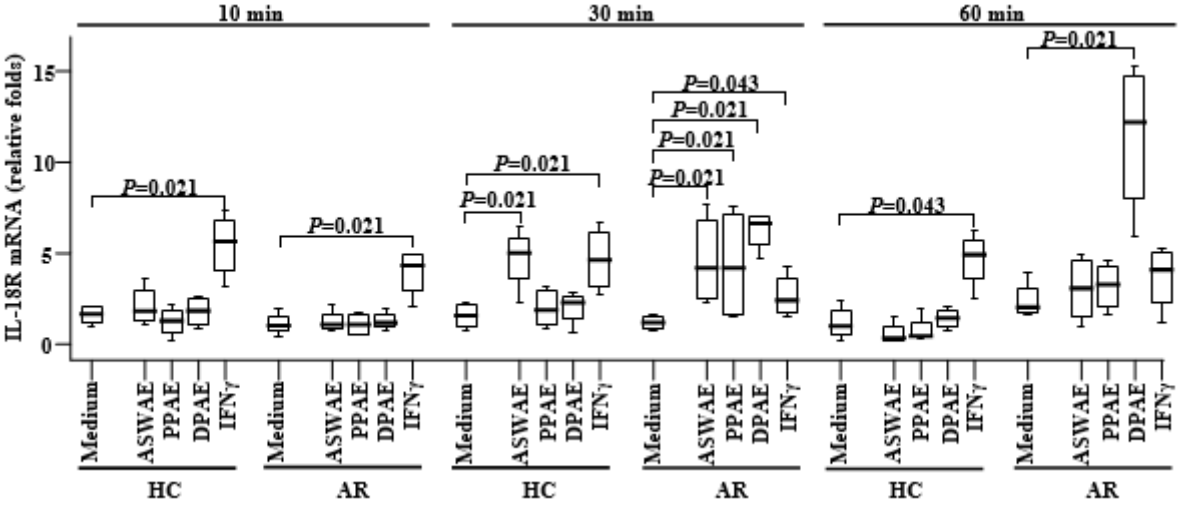


Figure 5

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 subjects (HC). 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 IFNγ. Expression of IL-18R mRNA was analyzed by qPCR. The data 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. P < 0.05 was taken as statistically significant.

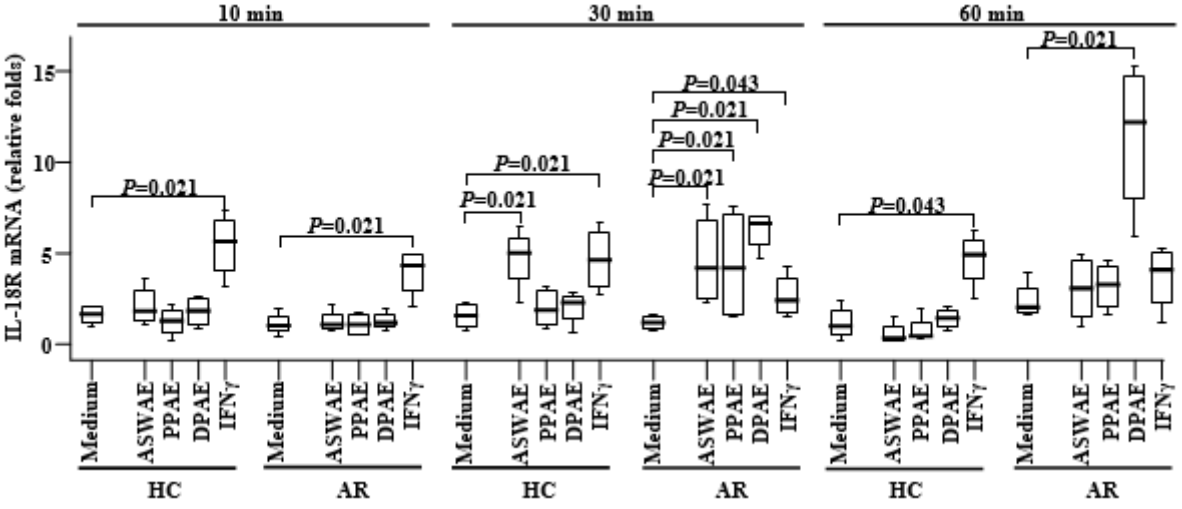


Figure 5

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 subjects (HC). 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 IFNγ. Expression of IL-18R mRNA was analyzed by qPCR. The data 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. P < 0.05 was taken as statistically significant.

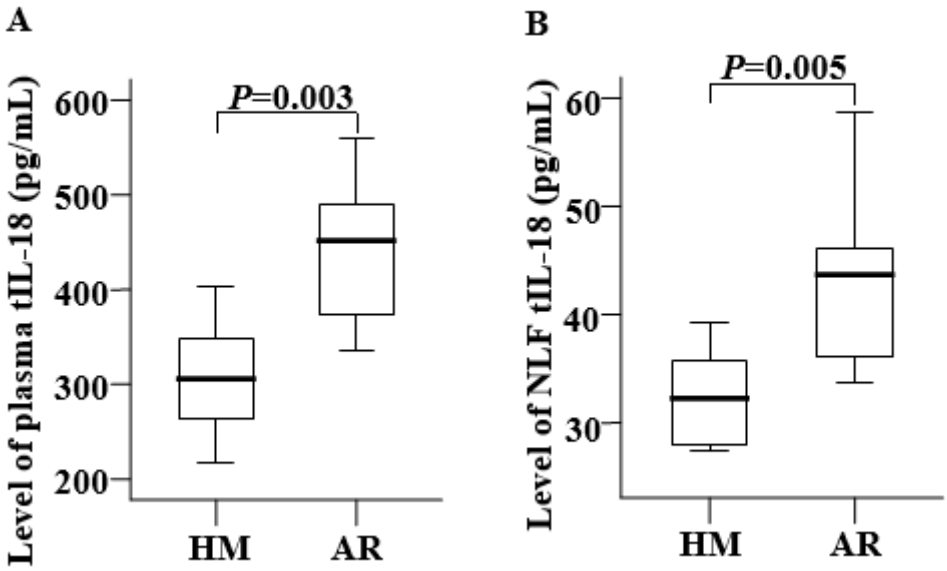
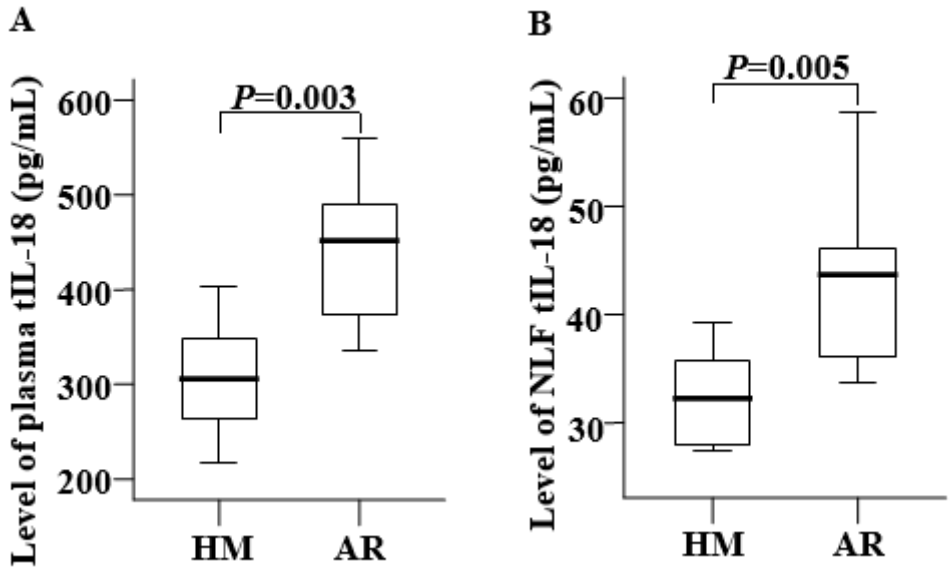


Figure 6

Levels of IL-18 in mouse plasma (A) and NLF (B). Following a seven-day OVA challergen 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.



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Levels of IL-18 in mouse plasma (A) and NLF (B). Following a seven-day OVA challergen 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.

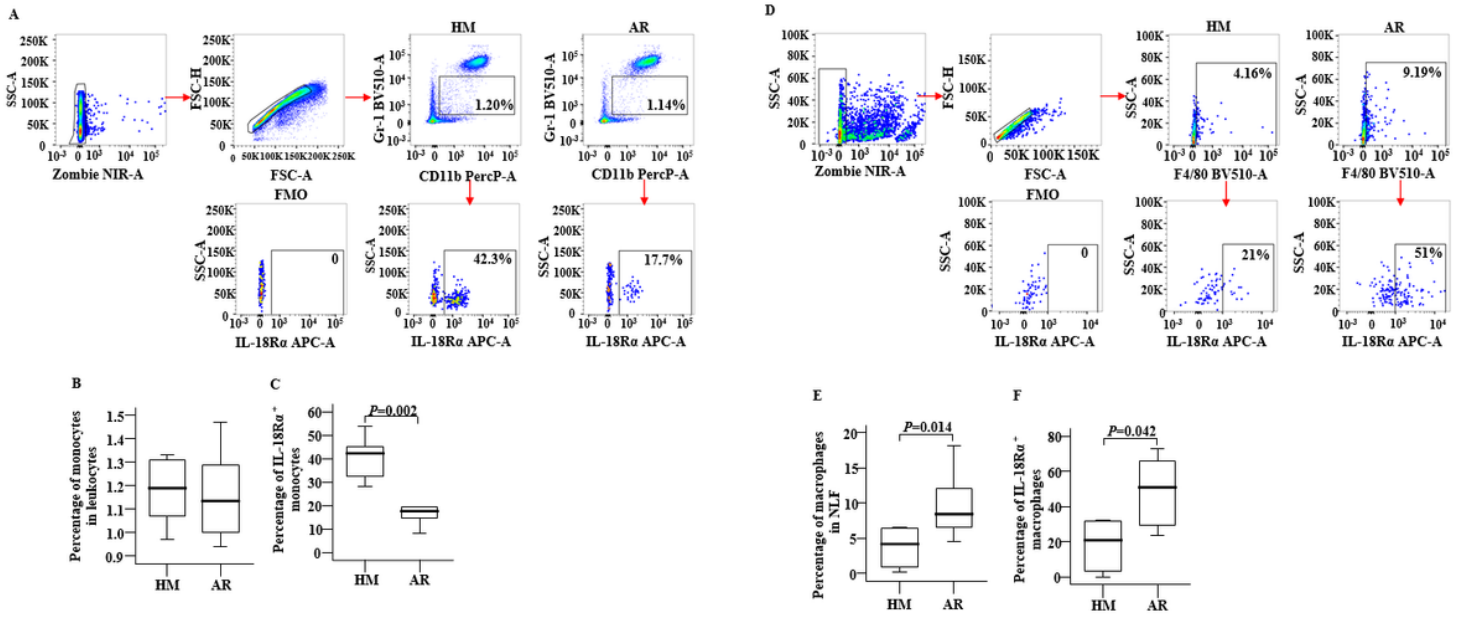

Figure 7

Expression of IL-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.

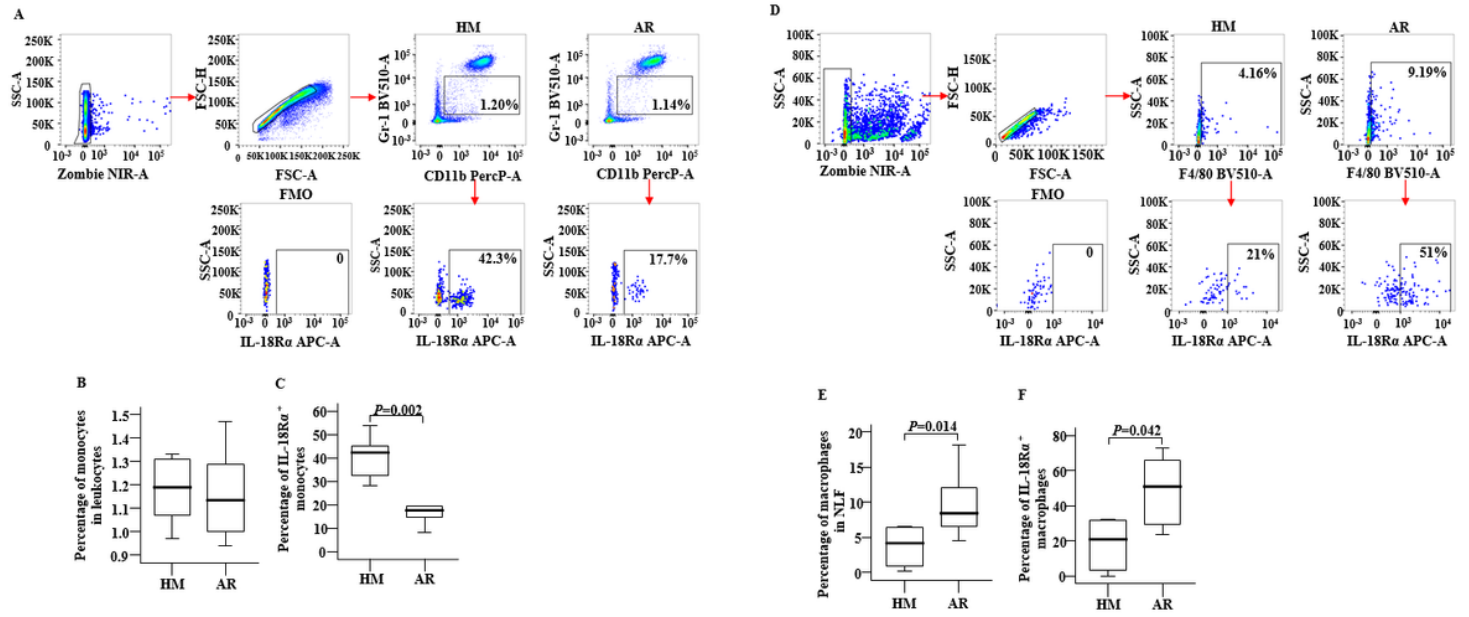

Figure 7

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

This is a list of supplementary files associated with this preprint. Click to download.

- ARRIVEchecklist.docx
- ARRIVEchecklist.docx

- SupplementalMaterialIL18IL18RinMonocytesofAR.doc
- SupplementalMaterialIL18IL18RinMonocytesofAR.doc