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Altered expression of IL-18 binding protein and IL-18 receptor in basophils and mast cells of asthma patients

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Abstract

IL-18 is likely to contribute to asthma. However, little is known regarding the role of IL-18 binding protein (BP) and IL-18 receptor (R) in asthma. Because the action of IL-18 in the body is regulated by IL-18BP and mast cells and basophils are key cell types involved in asthma, we investigated the expression of IL-18, IL-18BP and IL-18R in basophils and mast cells using flow cytometry and a mouse asthma model. We found that among basophils, approximately 53 and 51% were IL-18⁺, 85 and 81% were IL-18BP⁺ basophils, and 19.8 and 8.6% were IL-18R⁺ in healthy control (HC) and asthmatic blood, respectively. The allergens tested had little effect on the expression of IL-18 and related factors. Only 3.5, 14.3 and 2.4% of dispersed mast cells expressed IL-18, IL-18BP and IL-18R, respectively, in asthmatic sputum. In a mouse asthma model, OVA-sensitized mice exhibited decreased IL-18BP⁺ but increased IL-18R⁺ basophils in their blood. IL-18 increased the number of basophils but eliminated IL-18BP⁺ basophils in mouse blood. IL-18 increased the number of mast cells and IL-18R⁺ mast cells in the lung as well as increased the mast cell numbers and IL-18BP⁺ mast cells in the bronchoalveolar lavage fluid (BALF) of OVA-sensitized mice. Thus, basophils and mast cells may be involved in asthma pathogenesis via an IL-18 associated mechanism.

Keywords: Basophil, IL-18, IL-18 binding protein, IL-18 receptor, Mast cell

Introduction

IL-18 is a pleiotropic proinflammatory cytokine that plays a key role in both innate and acquired immunity. IL-18 variants are significantly associated with asthma severity [1], and IL-18 protein and IL-18R are upregulated in the lungs of patients with asthma [2], implicating IL-18 in asthma pathogenesis. Furthermore, low levels of IL-18 were observed in the sputum of asthmatics [3] and in the sputum supernatant of patients with severe refractory asthma [4], which correlates with the number of macrophages and inversely correlates with the percentage of neutrophils, but does not correlate with eosinophil cationic protein (ECP) and eosinophils [4]. To clarify the potential role of IL-18 in asthma, we examined the expression of IL-18, IL-18BP and IL-18R in blood basophils and sputum mast cells in the present study.

IL-18BP is an endogenous antagonist with high neutralizing capacity that inhibits the action of IL-18 by preventing its interaction with cell surface receptors [5]. 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 the level of an HC subject (40 pg/ml) [6]. It is possible that an imbalance between IL-18 and IL-18BP might account for increased IL-18 activity in asthma. We therefore chose to investigate the influence of IL-18 on the expression of IL-18BP and IL-18R in blood basophils and lung mast cells.

It has been reported that *Alternaria* extract induces the rapid release of IL-18 from cultured normal human bronchial epithelial cells and directly initiates Th2 differentiation of naïve CD4⁺ T cells via a unique NF-κB-dependent pathway [7]. Furthermore, IL-18 has been linked to the induction of IL-13 production in mast cells

and basophils [8], suggesting that allergens may trigger airway reactions via IL-18 and that IL-18 may affect the function of mast cells and basophils.

Because mast cells and basophils are key cell types involved in asthma [9] that produce an array of cytokines [10], they could be a major source of IL-18. We therefore examined the potential contribution of mast cells and basophils to asthma via IL-18, IL-18BP and IL-18R-associated mechanisms. The aim of the current study is to further investigate the role of IL-18-related mechanisms in the pathogenesis of asthma.

Materials and Methods

Reagents

The following reagents were purchased from Biolegend (San Diego, USA):

PerCP/Cy5.5-conjugated mouse anti-human CCR3, Brilliant Violet (BV)421-conjugated mouse anti-human CD123, APC/Cy7-conjugated mouse anti-human HLA-DR, PE/Cy7-conjugated mouse anti-human CD34, PerCP/Cy5.5-conjugated mouse anti-human Fc ϵ RI α , PerCP/Cy5.5-conjugated rat anti-mouse CD49b, PE-conjugated rat anti-mouse Fc ϵ RI α , PerCP/Cy5.5-conjugated rat anti-mouse CD117, BV510-conjugated donkey anti-rabbit IgG polyclonal Ab, human Fc receptor blocking solution, and anti-mouse CD16/32. Recombinant mouse IL-18BP and FITC-conjugated rabbit anti-mouse IL-18BP were obtained from Cloud Clone (Houston, USA). APC-conjugated mouse anti-human IL-18R α , PE-conjugated mouse anti-human IL-18, APC-conjugated rat anti-mouse IL-18R, and recombinant

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mouse IL-18 were supplied by R&D Systems (Minneapolis, USA). Aluminum hydroxide [Al(OH)₃] gel adjuvant was purchased from Brenntag Biosector (Frederikssund, Denmark). Rabbit anti-human IL-18BP primary antibody, goat anti-mouse IL-18BP, and biotin-conjugated goat anti-mouse IL-18BP were obtained from Abcam (Cambridge, UK). BSA (fraction V), MEM containing 25 mM HEPES, ovalbumin (OVA), and Trypan blue dye were purchased from Sigma-Aldrich (St Louis, MO, USA). Cytofix/CytopermTM Fixation/Permeabilization Kit and BV421-conjugated mouse anti-human CD117 were obtained from BD Biosciences Pharmingen (Belford, MA, USA). 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 31 patients with asthma and 14 HC subjects were recruited in the study. Their general characteristics and diagnostic criteria were previously described [11]. Informed consent was obtained from each volunteer according to the declaration of Helsinki in agreement with the ethical committee of the General Hospital of Shenyang Military Region of PLA, with the Second Affiliated Hospital of Shenyang Medical College or with the First Affiliated Hospital of Jinzhou Medical University. The procedure for collecting and treating peripheral blood specimens was described previously [11]. For certain patients, sputum was collected immediately after admission (during the acute exacerbation stage).

BALB/c mice (6-7 w, 18-22 g) were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) under Certificate No 11400700118761 and were bred and reared under strict ethical conditions approved by the Animal Care Committee at Jinzhou Medical University [12].

Sputum collection and processing

Sputum induction and processing was performed according to a method described previously [13]. A minimum of 5 ml sputum was collected from each patient. After mixing with a 4-fold volume of 0.1% dithiothreitol for 15 min, the suspended sputum was centrifuged. The supernatant was stored at -80°C until use, and the cell pellet was resuspended in PBS for flow cytometry analysis as described below.

Flow cytometry analysis of IL-18, IL-18BP and IL-18R expression by human basophils and mast cells

The detection of IL-18, IL-18BP and IL-18R expression by human blood basophilic granulocytes (CD123⁺ HLA-DR⁻ CCR3⁺ cells) was performed as described previously [12]. Cells were incubated in the presence of 2 µg/ml brefeldin A for 1 h at 37°C. Each labeled monoclonal antibody, including PerCP/Cy5.5-conjugated anti-human CCR3, BV421-conjugated anti-human CD123, APC/Cy7-conjugated anti-human HLA-DR, and APC-conjugated anti-human IL-18R α , was added into the tube. After fixation and permeabilization by the Cytofix/CytopermTM Fixation/Permeabilization Kit, the cells were incubated with PE-conjugated anti-human IL-18 and anti-human

IL-18BP primary antibodies before the addition of BV510-conjugated donkey anti-rabbit polyclonal antibody for 30 min. Finally, the cells were resuspended in fluorescence-activated cell sorting (FACS)-Flow solution and were analyzed with a FACS Verse flow cytometer (BD Biosciences, San Jose, CA). A total of 10,000 events in a live cell gate were analyzed for each sample. The data were analyzed with FlowJo software version 7.0 (Treestar, Ashland, USA). Dead cells and doublets were excluded from the analysis by live/dead cell dyes.

To detect the expression of IL-18, IL-18BP and IL-18R in sputum mast cells isolated from asthmatics, dispersed cells were incubated with human Fc receptor blocking solution and a live/dead cell dye (Zombie Green™ Fixable Viability Kit) [14] for 15 min before the addition of PE/Cy7-conjugated anti-human CD34, PerCP/Cy5.5-conjugated anti-human FcεRIα, BV421-conjugated mouse anti-human CD117 and APC-conjugated anti-human IL-18Rα antibodies to the tube. Cells were then permeabilized, processed following human blood sample protocols, and analyzed by flow cytometry.

Mouse sensitization and challenge

Mice were sensitized and challenged with an OVA solution following a procedure described previously [11]. Normal saline (1 ml) containing 1 mM EDTA was infused into the unilateral lung for 2 min before the animals were sacrificed and their bronchoalveolar lavage fluid (BALF) collected. Blood samples were collected by

enucleation of the eyeball and were centrifuged at 450 g for 10 min. The cells were used for flow cytometric analysis, and plasma was collected and frozen at -80°C until further use.

Dispersion of mouse lung cells

Lung tissues were excised and digested into single cells as described previously [15]. Briefly, lung tissues were digested with collagenase and hyaluronidase. Dispersed cells were incubated in DMEM in the presence of DNase and 5% fetal calf serum before flow cytometric analysis.

Flow cytometric analysis of IL-18, IL-18BP and IL-18R expression by mouse basophils and mast cells

To detect the expression of IL-18BP and IL-18R by basophils of mouse peripheral blood leukocytes, resuspended cells were incubated with live/dead cell dye (Zombie Aqua™ Fixable Viability Kit) [16] and anti-mouse CD16/32 as described above before the addition of APC/Cy7-conjugated anti-mouse Ly6C, PE-conjugated anti-mouse FcεRIα, PerCP/Cy5.5-conjugated anti-mouse CD49b, and APC-conjugated anti-mouse IL-18R antibodies to the tube for 15 min. The resuspended cells were then fixed and permeabilized using a Cytofix/Cytoperm™ Fixation/Permeabilization kit, followed by the addition of FITC-conjugated anti-mouse IL-18BP antibody for 30 min. Finally, the cells were processed and analyzed as described above.

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To detect the expression of IL-18BP and IL-18R by mast cells of mouse BALF and lung tissues, the cells were incubated with anti-mouse CD16/32 for 15 min before the addition of PE-conjugated anti-mouse FcεRIα, PerCP/Cy5.5-conjugated anti-mouse CD117 and APC-conjugated anti-mouse IL-18R antibodies to the tube. After incubating at room temperature for 15 min, the cells were fixed and FITC-conjugated anti-mouse IL-18BP antibody was added. The flow cytometer analysis procedure used was as same as for human cells.

Determination of IL-18BP level in mouse plasma

The level of IL-18BP in mouse plasma was detected by sandwich ELISA. A polyclonal anti-mouse IL-18BP antibody was used as the capture antibody, and a biotinylated anti-mouse IL-18BP antibody was employed as the detection antibody. A recombinant mouse IL-18BP was used as a standard. The detection range of the standard curve was 7.8-125 ng/ml.

Statistical analysis

Statistical analyses were performed using SPSS software (Version 17.0, IBM Corporation). The plasma levels of IL-18BP as well as data for the expression of IL-18, IL-18BP and IL-18R in basophils and mast cells are displayed as boxplots indicating the median, interquartile range, and largest and smallest values for the number of experiments indicated. Where Kruskal-Wallis analysis indicated significant differences between groups for pre-planned comparisons of interest, the paired

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Mann-Whitney U-test was employed. For all analyses, $P < 0.05$ was taken as significant.

Results

Enhanced number of basophils in peripheral blood of patients with atopic asthma

Increased plasma levels of IL-18 [17] and expression of IL-18R by inflammatory cells [18] in asthma patients have been reported. However, the source of increased plasma IL-18 in asthma remains to be determined, and the expression levels of IL-18BP and IL-18R in the basophils of asthma patients are unknown. We therefore examined the expression levels of IL-18, IL-18BP and IL-18R in basophils isolated from the blood of asthmatics. Our results showed that the proportion of basophils (0.45%) in asthmatic blood was much greater than that observed in the blood of HCs (0.14%).

We found that approximately 53 and 51% of basophils in HC and asthmatic blood, respectively, expressed IL-18, and more than 85 and 81% of basophils in HC and asthmatic blood, respectively, expressed IL-18BP. However, only 19.8 and 8.6% of basophils in HC and asthmatic blood, respectively, expressed IL-18R (Figure 1B, 1C), representing a 56.4% reduction in IL-18R⁺ basophils in asthmatic blood compared with HC blood. The allergens tested had little effect on IL-18, IL-18BP and IL-18R expression in basophils (data not shown). In terms of MFI, basophils expressed a high density of IL-18BP and much lower densities of IL-18 and IL-18R.

Furthermore, asthmatic basophils appeared to express less IL-18BP than HC basophils (Figure 1D, 1E).

Expression of IL-18, IL-18BP and IL-18R by sputum mast cells obtained from asthma patients

In contrast to plasma [17], the level of IL-18 in asthmatic sputum seemed to be lower than that in HC sputum [3]. Because mast cells are one of the key cell types involved in asthma [9] and produce an array of cytokines [10], they could be a major source of IL-18. Reduced expression of IL-18 by mast cells may help to explain the reduction in IL-18 level in asthmatic sputum. We therefore examined the expression of IL-18, IL-18BP and IL-18R in the induced sputum of patients with asthma. The results showed that CD34⁺ FcεRIα⁺ CD117⁺ mast cells consisted of only 0.39% dispersed nucleated cells (Figure 2A, 2B), and only 3.5, 14.3 and 2.4% of these cells expressed IL-18 (Figure 2C), IL-18BP (Figure 2D) and IL-18R (Figure 2E), respectively, in asthmatic sputum.

Altered IL-18BP and IL-18R expression in basophils following sensitization and IL-18 treatment

To further understand the action of IL-18 in asthma, we examined the effect of IL-18 on IL-18BP and IL-18R expression in blood basophils obtained from OVA-sensitized mice. We found that sensitization enhanced the number of basophils present in mouse blood (Figure 3A, 3B). Compared with non-sensitized mice, sensitized mice

had a marked decrease in the proportion of IL-18BP⁺ basophils (Figure 3A, 3C), but exhibited a dramatic increase in the number of IL-18R⁺ basophils (Figure 3A, 3D) in their blood. Sensitization also enhanced the MFI of IL-18R (Figure 3Eb, 3G), but reduced IL-18BP in basophils (Figure 3Ea, 3F). IL-18 treatment increased the number of basophils (Figure 3A, 3B), but reduced the proportion of IL-18BP⁺ basophils in mouse blood (Figure 3A, 3C). IL-18 had little effect on the MFI of IL-18BP⁺ (Figure 3Ea, 3F) and IL-18R⁺ blood basophils (Figure 3Eb, 3G) in sensitized mice.

Altered expression of IL-18BP and IL-18R in mast cells of BALF by sensitization and IL-18

It is difficult to obtain permission to collect bronchoalveolar lavage fluid (BALF) for experimental purposes from patients with asthma. We therefore investigated the influence of IL-18 on the expression of IL-18BP and IL-18R by BALF mast cells of sensitized mice. We found that sensitization and IL-18 increased the number of mast cells in BALF (Figure 4A, 4B) and that IL-18 enhanced the number of IL-18BP⁺ mast cells (Figure 4A, 4C). IL-18 also upregulated the expression of IL-18R by mast cells (Figure 4Eb, 4G) isolated from sensitized mice.

Sensitization and IL-18 treatment alter the expression of IL-18BP and IL-18R in lung tissue mast cells

IL-18 and IL-18R are strongly expressed and the numbers of eosinophils and lymphocytes are elevated in the lungs of fatal asthmatics [2]. However, the

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expression of IL-18BP and IL-18R in asthmatic mast cells has not been characterized. Therefore, we investigated the influence of sensitization and IL-18 on the expression of IL-18BP and IL-18R by mast cells isolated from lung tissue. We found that sensitization did not alter the number of mast cells (Figure 5A, 5B), the proportion of IL-18BP⁺ mast cells (Figure 5A, 5C) or the percentage of IL-18R⁺ mast cells (Figure 5A, 5D) in the lung tissue of mice. However, IL-18 enhanced the number of mast cells (Figure 5A, 5B) and the percentage of IL-18R⁺ mast cells (Figure 5A, 5D) in the lung of OVA-sensitized mice. IL-18 also elicited MFI of IL-18R on mast cells (Figure 5Eb, 5G) isolated from OVA-sensitized mice.

Enhanced levels of IL-18BP in mouse plasma

To examine the influence of sensitization and IL-18 on IL-18BP production, we investigated the effects of OVA and IL-18 on IL-18BP plasma level in mice. We found that OVA sensitization decreased plasma IL-18BP level in comparison with non-sensitized mice. IL-18 injection increased the plasma IL-18BP levels of non-sensitized and OVA-sensitized mice by 48.0% and 16.1%, respectively (Figure 6).

Discussion

The current study clearly demonstrates that the number of basophils increases in the peripheral blood of patients with asthma and in the blood of OVA-sensitized mice, suggesting that basophils are likely to participate in asthma pathogenesis. Because the involvement of basophils in asthma has long been recognized [19], our finding

could be important for understanding asthma pathogenesis. We found that greater than 50% and 80% of human peripheral blood basophils express IL-18 and IL-18BP, respectively, regardless of whether the cells are of HC or asthmatic patient origin, suggesting that the contribution of basophils to human asthma pathogenesis of asthma might be IL-18-independent.

In contrast, in a mouse asthma model, a marked decrease in the proportion of IL-18BP⁺ basophils can decrease the ratio of IL-18BP/IL-18 [6], thereby increasing the level of free IL-18 in plasma and dramatically increasing the number of IL-18R⁺ basophils. These findings indicate that IL-18 might be involved in the pathogenesis of mouse asthma. However, extrinsic IL-18 appeared to exert dual actions on IL-18BP. On one hand, it reduced the proportion of IL-18BP⁺ basophils in OVA-sensitized mouse blood by approximately 47.8%; on the other hand, it caused a 16.1% increase in the level of plasma IL-18BP. These findings suggest that the IL-18-induced increase in plasma IL-18BP level is unlikely to be mediated by basophils. Because the expression of IL-18, IL-18BP and IL-18R by asthmatic basophils was previously uncharacterized, our observations provide novel insight into the relationship between IL-18, basophils and asthma pathology.

Contradictions between the human and mouse models were observed in the present study. For example, more than 80% of human blood basophils, but only 22.7% of mouse blood basophils, express IL-18BP in asthma models. Furthermore, almost all mouse basophils express IL-18R, but only 8.6% of basophils in human asthmatic blood expressed IL-18R. These findings add more information to the controversy over the function of murine and human basophils in allergic

inflammation [20], and suggest once again the limitation of animal models in studying human diseases. Obviously, further work is required to address these issues.

We also found only 3.5, 14.3 and 2.4% of mast cells in asthmatic sputum expressed IL-18, IL-18BP and IL-18R, respectively. The low proportion of IL-18-expressing mast cells and the fact that only 0.39% of mast cells in sputum were nucleated suggests that mast cells are unlikely to be the primary source of IL-18 in asthmatic sputum, which may help to explain the low level of IL-18 present in the sputum of asthmatics [3].

Extrinsic IL-18 elicited an increase in mast cell number in the BALF of OVA-sensitized mice of approximately 6%, suggesting that IL-18 might play a role in asthma via mast cells. Although we lack data regarding the mechanism of IL-18-mediated mast cell migration, the finding that IL-18 attracts plasmacytoid dendritic cells (DC2s) through IL-18R expression [21] suggests that IL-18-induced mast cell accumulation acts via its ability to up-regulate the expression of IL-18R on mast cells. Because mast cells can release proinflammatory mediators such as prostaglandin D₂, leukotriene C₄, platelet activation factor [9], and substance P [22]; the proteinases tryptase, chymase and carboxypeptidase; and proinflammatory cytokines such as eotaxin, IL-6 and TNF (Tumor necrosis factor) [23], IL-18 is likely to be involved in the pathogenesis of asthma via mast cells. Unlike sputum mast cells, up to 30% and 60% of mouse BALF mast cells express IL-18BP and IL-18R,

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respectively, in response to IL-18, providing further evidence that an IL-18-related mechanism is likely to contribute to mouse asthmatic model.

It has been reported that IL-18 and IL-18R are strongly expressed and that the number of eosinophils and lymphocytes is elevated in the lungs of fatal asthmatics [2]. However, the expression of IL-18BP and IL-18R in asthmatic mast cells remains uncharacterized. We found that while sensitization did not alter the number of mast cells, the proportion of IL-18BP⁺ mast cells or the percentage of IL-18R⁺ mast cells in the lung tissue of mice, IL-18 increased the number of mast cells and the percentage of IL-18R⁺ mast cells in the lungs of OVA-sensitized mice. Because IL-18 induces the release of IFN- γ , IL-13 and eotaxin in the lungs of OVA-sensitized and challenged transgenic mice along and increases the number of IL-13-producing CD4⁺ T cells and airway hyperresponsiveness [24], we believe that IL-18 may play a role in asthma.

In conclusion, a large proportion blood basophils from patients with asthma express IL-18 and IL-18BP, a dramatic increase in IL-18R⁺ basophils is observed in OVA-sensitized mouse blood, and extrinsic IL-18 enhances mast cell as well as IL-18R⁺ mast cell numbers in the BALF and lung tissue of OVA-sensitized mice. Together, these findings implicate mast cells and basophils in the pathogenesis of asthma via an IL-18 related mechanism.

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Conflicts of Interest

The authors declare that there is no competing interest regarding the publication of this article.

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Figure Legends

Figure 1 Expression of IL-18, IL-18 binding protein (BP) and IL-18 receptor (R) in peripheral blood basophils of asthmatics and healthy control (HC) subjects. (A) represents a gating strategy of basophils out of leukocytes. (B) is a gating strategy of expression of IL-18, IL-18BP and IL-18R in basophils. (C) shows proportions of IL-18⁺, IL-18BP⁺ and IL-18R⁺ basophils. (D) is representative flowcytometric figures of mean fluorescent intensity (MFI) of IL-18⁺ (a), IL-18BP⁺ (b) and IL-18R⁺ (c) basophil. (E) shows MFI of IL-18⁺, IL-18BP⁺ and IL-18R⁺ basophil. Data are displayed as a boxplot for asthmatics (n = 31), and HC subjects (n = 14), which indicates the median, interquartile range, the largest and smallest values for the number of subjects indicated. FMO = fluorescence minus one. *P* < 0.05 was taken as significant (paired Mann-Whitney *U*-test).

Figure 2 Identification of IL-18, IL-18 binding protein (BP) and IL-18 receptor (R) expressing mast cells in asthmatic sputum. (A) represents a gating strategy of CD34⁺ FcεRIα⁺ CD117⁺ mast cells out of nucleated cells in sputum. (B) is the expression levels of mast cells in asthmatic sputum. (C-E) shows proportions of IL-18⁺, IL-18BP⁺ and IL-18R⁺ mast cells. Data are displayed as a boxplot, which indicates the median, interquartile range, the largest and smallest values for 31 patients with asthma. FMO

= fluorescence minus one. $P < 0.05$ was taken as significant (paired Mann-Whitney U -test).

Figure 3 Expressions of IL-18 binding protein (BP) and IL-18 receptor (R) in basophils of sensitized or healthy mouse blood in the presence or absence of IL-18. (A) represents a gating strategy of $\text{Ly6C}^- \text{CD49b}^+ \text{Fc}\epsilon\text{RI}\alpha^+$ basophil expression in mouse blood leukocytes, and gating strategies for IL-18BP and IL-18R expression in basophils. (B-D) demonstrate changes in percentage of basophils out of leukocytes, and proportions of IL-18BP and IL-18R expressing basophils in healthy mice (HM) and sensitized mice (SM), respectively in response to IL-18 (10 ng/ml) or normal saline (NS). (E) shows representative flowcytometric figures of mean fluorescence intensity (MFI) of IL-18BP⁺ (a) and IL-18R⁺ (b) basophil. (F) and (G) show changes in MFI of IL-18BP and IL-18R expression in basophil, respectively in response to IL-18 or NS. Data are displayed as a boxplot for sensitized (AM, $n = 7$), and healthy (HM, $n = 6-7$) mice, which indicates the median, interquartile range, the largest and smallest values for the number of animals indicated. AF = all fluorescence, FMO = fluorescence minus one. $P < 0.05$ was taken as significant (paired Mann-Whitney U -test).

Figure 4 Expressions of IL-18 binding protein (BP) and IL-18 receptor (R) in mast cells of bronchoalveolar lavage fluid (BALF) of sensitized or non-sensitized mice. (A) represents a gating strategy of $\text{Fc}\epsilon\text{RI}\alpha^+ \text{CD117}^+$ mast cell expression in nucleated cells

of mouse BALF, and gating strategies for IL-18BP and IL-18R expression in mast cells of mouse BALF. (B-D) demonstrate changes in percentage of mast cells out of nucleated cells, and proportions of IL-18BP and IL-18R expressing mast cells in healthy mice (HM) and sensitized mice (SM), respectively in response to IL-18 (10 ng/ml) or normal saline (NS). (E) shows representative flowcytometric figures of mean fluorescent intensity (MFI) of IL-18BP⁺ (a) and IL-18R⁺ (b) mast cell. (F) and (G) show changes in MFI of IL-18BP and IL-18R expression in mast cell, respectively in response to IL-18 or NS. Data are displayed as a boxplot for sensitized (AM, n = 7), and healthy (HM, n = 6-7) mice, which indicates the median, interquartile range, the largest and smallest values for the number of animals indicated. FMO = fluorescence minus one. $P < 0.05$ was taken as significant (paired Mann-Whitney *U*-test).

Figure 5 Expressions of IL-18 binding protein (BP) and IL-18 receptor (R) in mast cells of lung. (A) represents a gating strategy of FcεRIα⁺ CD117⁺ mast cell expression in nucleated cells of mouse lung, and gating strategies for IL-18BP and IL-18R expression in mast cells of mouse lung. (B-D) demonstrate changes in percentage of mast cells out of nucleated cells, and proportions of IL-18BP and IL-18R expressing mast cells in healthy mice (HM) and sensitized mice (SM), respectively in response to IL-18 (10 ng/ml) or normal saline (NS). (E) shows representative flowcytometric figures of mean fluorescence intensity (MFI) of IL-18BP⁺ (a) and IL-18R⁺ (b) mast cell. (F) and (G) show changes in MFI of IL-18BP and IL-18R expression in mast cell, respectively in response to IL-18 or NS. Data are displayed as a boxplot for sensitized (AM, n = 7), and healthy (HM, n = 6-7) mice, which indicates the median,

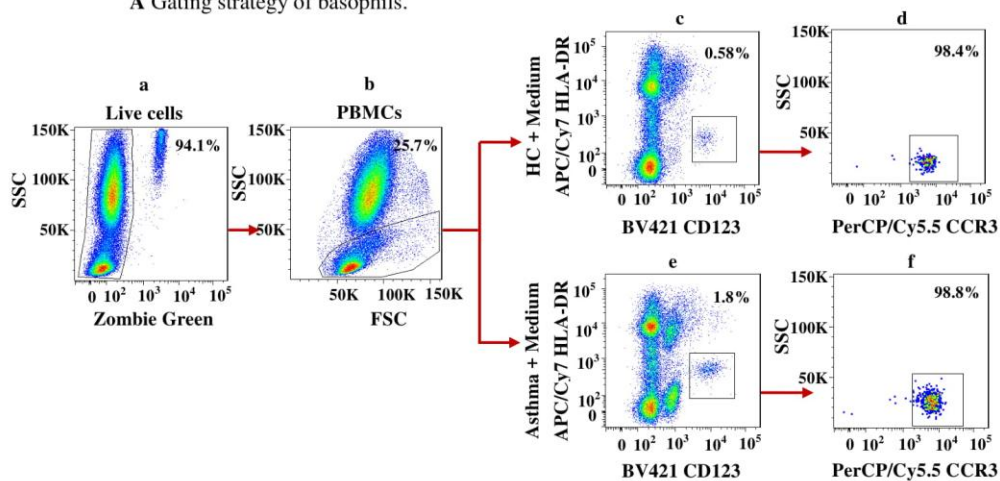
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interquartile range, the largest and smallest values for the number of animals indicated. FMO = fluorescence minus one. $P < 0.05$ was taken as significant (paired Mann-Whitney U -test).

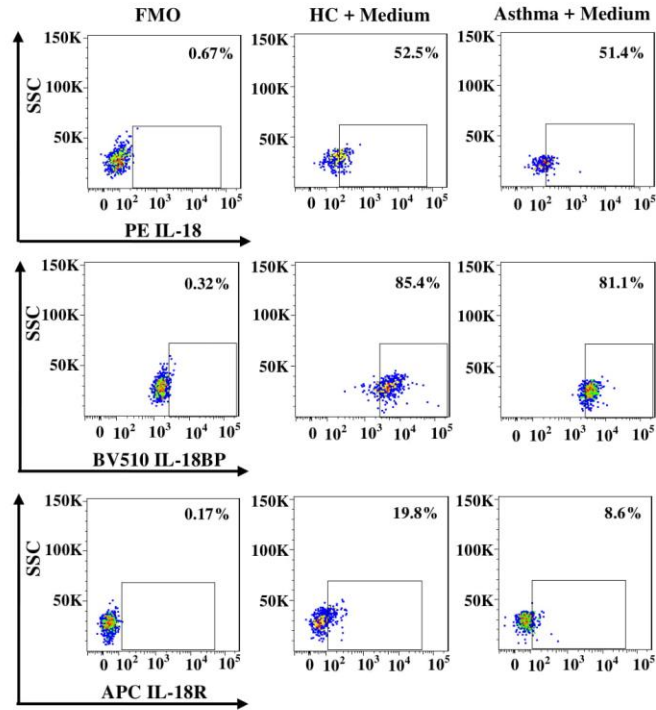
Figure 6 Levels of IL-18BP in plasma of mice. Following injection of normal saline (NS) and IL-18, plasma of healthy mice (HM) and sensitized mice (SM) was taken, and analyzed by using a sandwich ELISA. Data are displayed as a boxplot for sensitized (AM, $n = 7$), and healthy (HM, $n = 6-7$) mice, which indicates the median, interquartile range, the largest and smallest values for the number of animals indicated. $P < 0.05$ was taken as significant (paired Mann-Whitney U -test).

Figure 1 Expression of IL-18, IL-18BP and IL-18R in peripheral blood basophils of asthmatics and healthy controls.

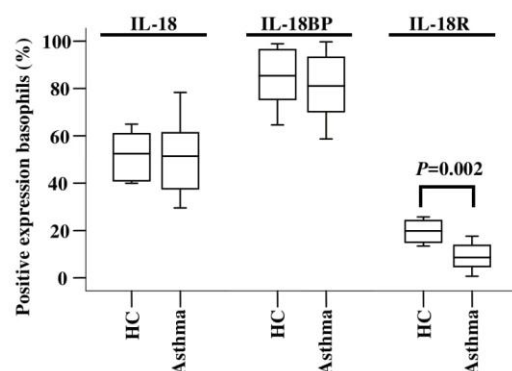
A Gating strategy of basophils.



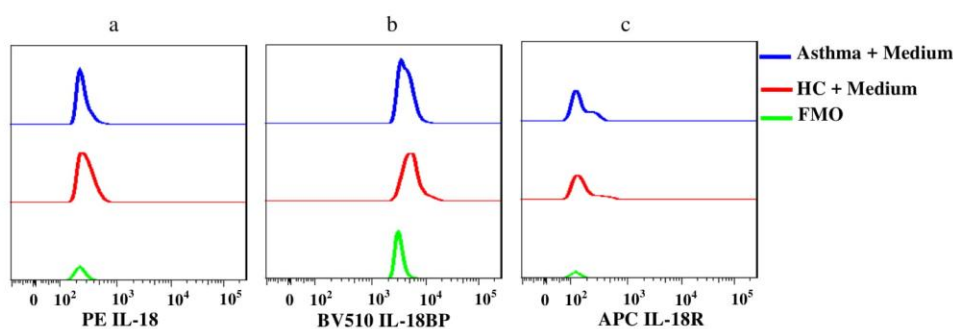
B Gating strategy of IL-18, IL-18BP and IL-18R expressing basophils.



C Percentage expression of IL-18, IL-18BP and IL-18R in basophils



D Representative flowcytometric figures of MFI of IL-18⁺, IL-18BP⁺ and IL-18R⁺ basophil.



E MFI of IL-18⁺, IL-18BP⁺ and IL-18R⁺ basophil

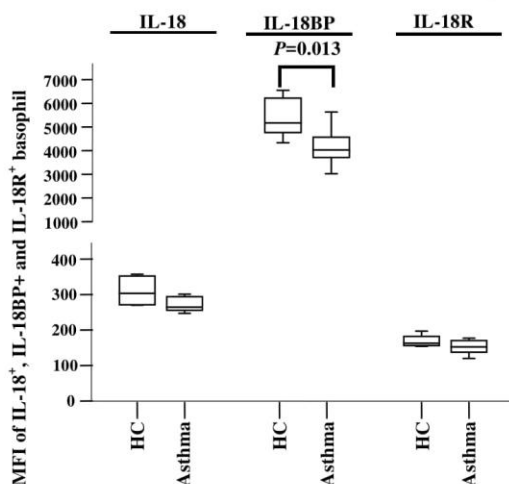
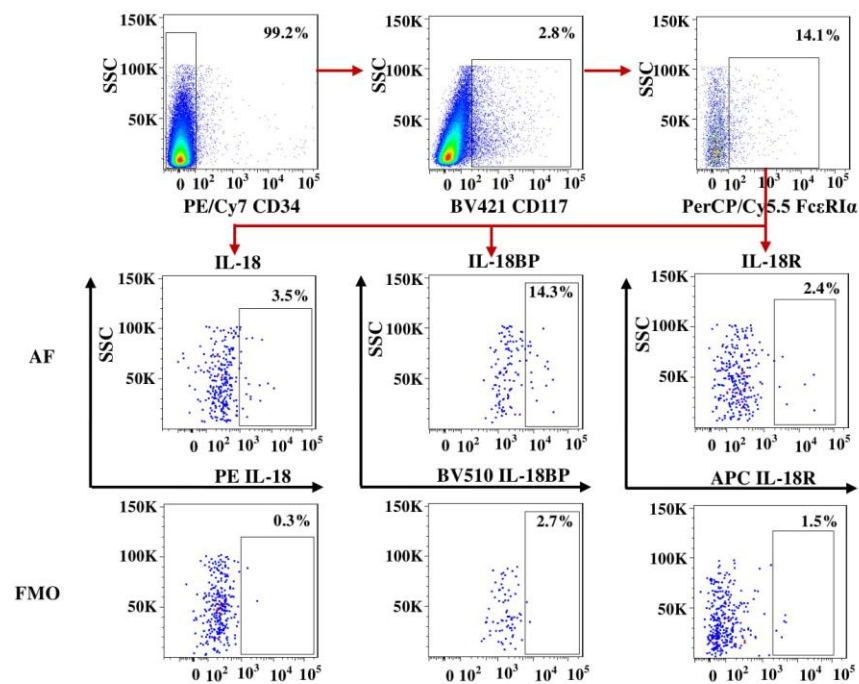


Figure 2 Expression levels of IL-18, IL-18BP and IL-18R in mast cells of asthmatic sputum.

A Gating strategy of mast cells in asthmatic sputum, and IL-18, IL-18BP and IL-18R expressing mast cells.



Percentage of mast cells in asthmatic sputum, and expressions of IL-18, IL-18BP and IL-18R in mast cells.

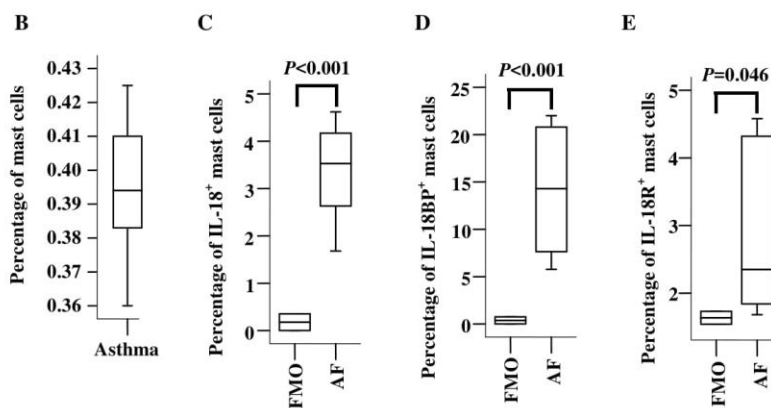
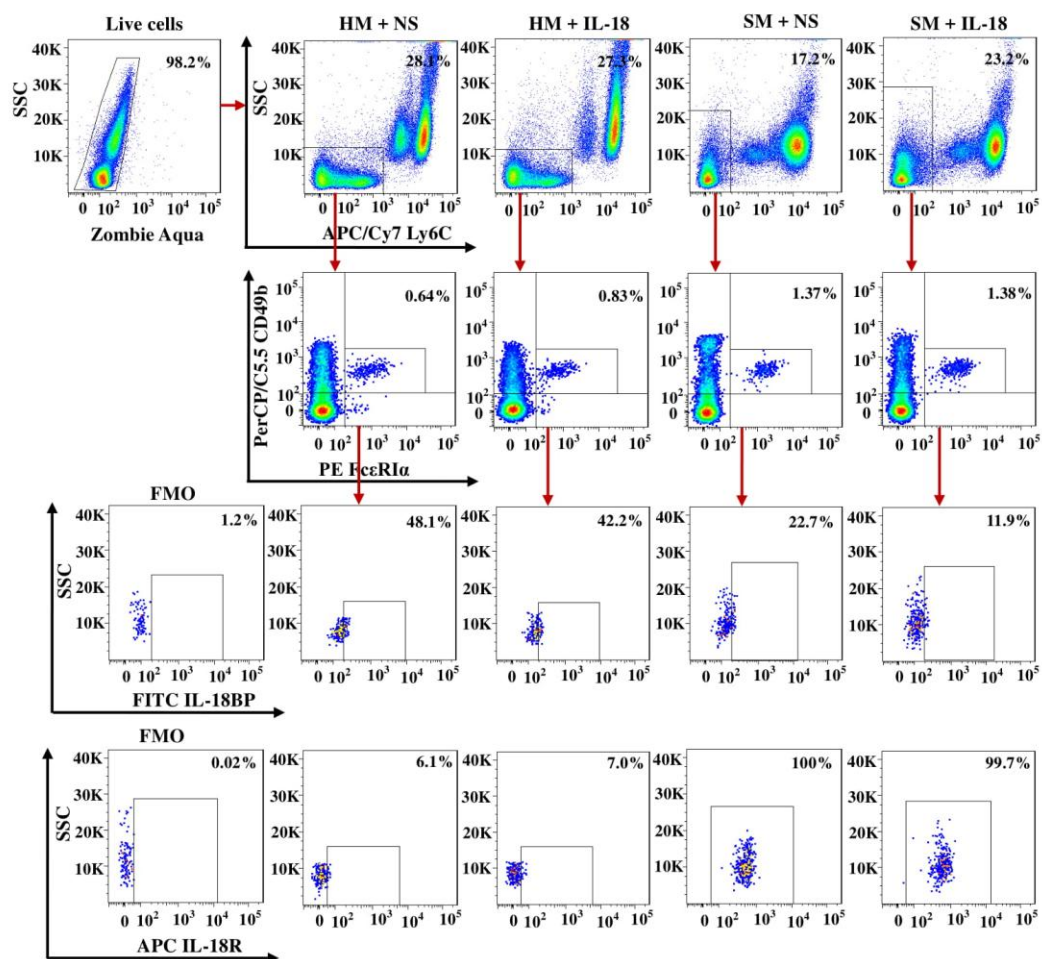
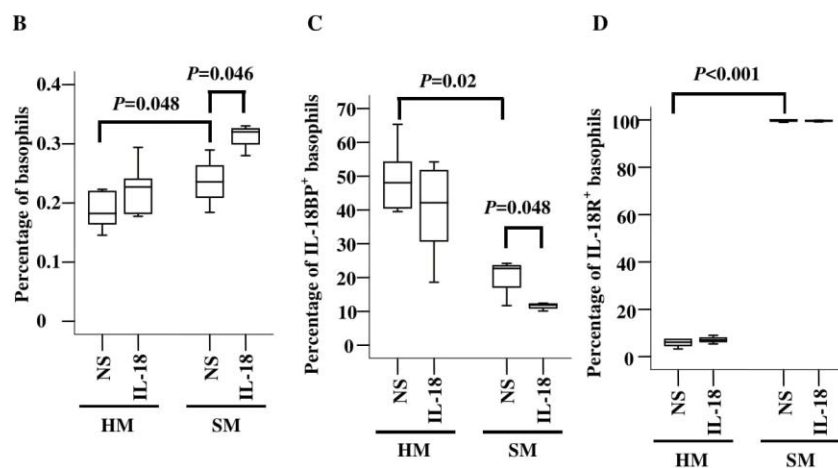


Figure 3 Altered expression of IL-18BP and IL-18R in sensitized and non-sensitized mouse basophils induced by IL-18.

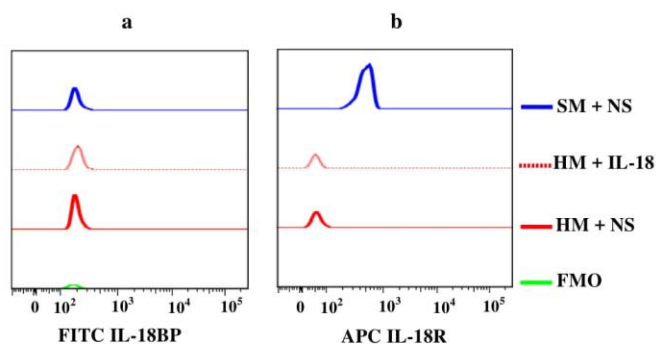
A Gating strategy of basophils in mouse blood leucocytes, and IL-18BP and IL-18R expressing basophils.



Percentage of basophils in mouse blood leucocytes, and expressions of IL-18BP and IL-18R in basophils.



E Representative flowcytometric figures of MFI of IL-18BP⁺ and IL-18R⁺ basophil.



MFI of IL-18BP⁺ and IL-18R⁺ basophil

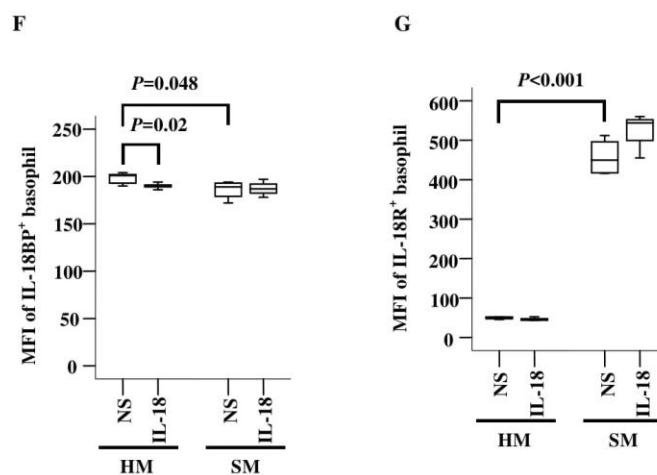
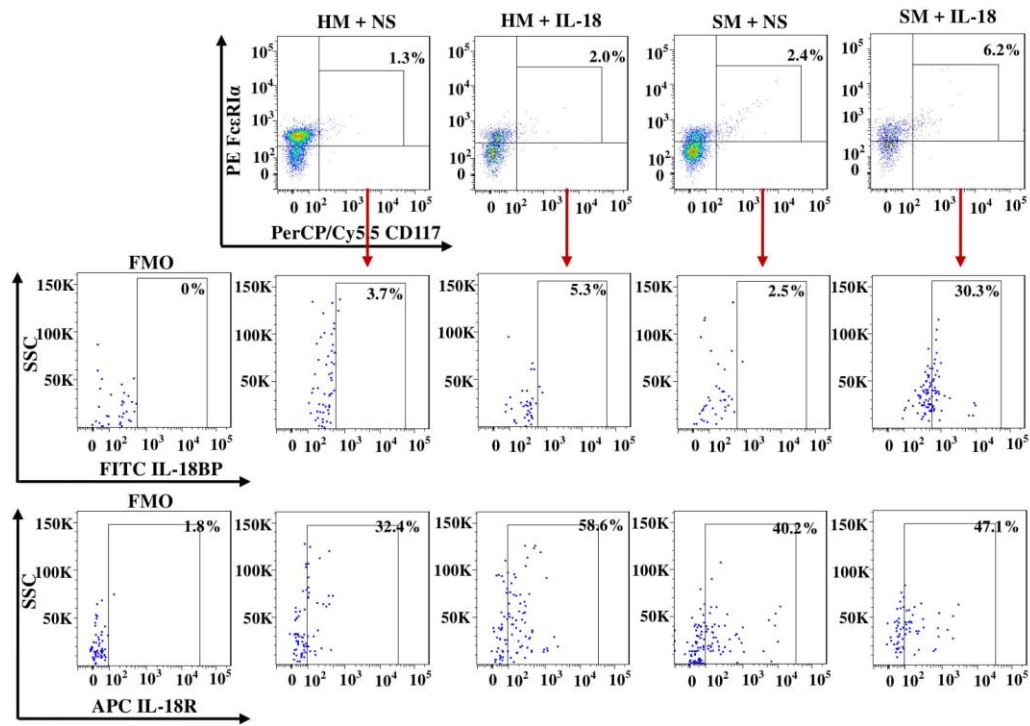
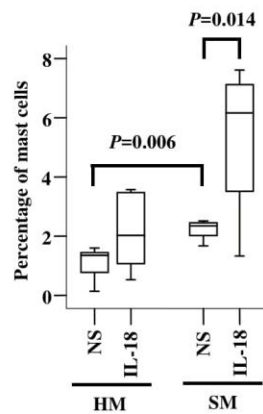


Figure 4 Altered expressions of IL-18BP and IL-18R in BALF mast cells from sensitized and non-sensitized mouse induced by IL-18.

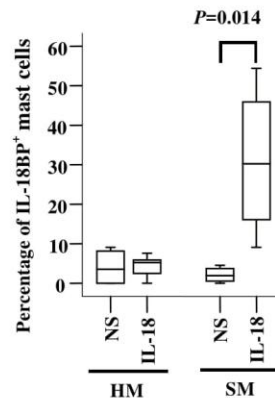
A Gating strategy of mast cells in mouse BALF, and IL-18BP and IL-18R expressing mast cells.



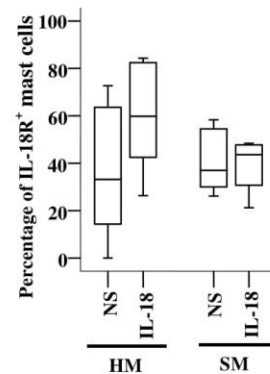
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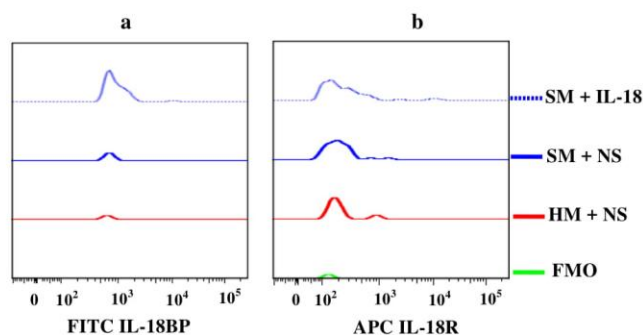
C



D



E Representative flowcytometric figures of MFI of IL-18BP⁺ and IL-18R⁺ mast cell.



MFI of IL-18BP and IL-18R in mast cells in sensitized and non-sensitized mouse mast cells induced by IL-18.

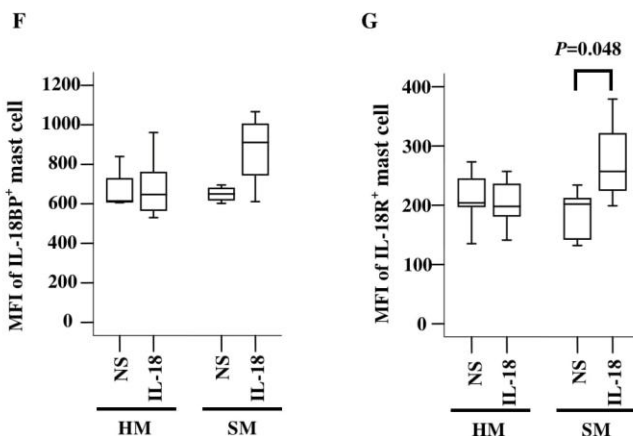
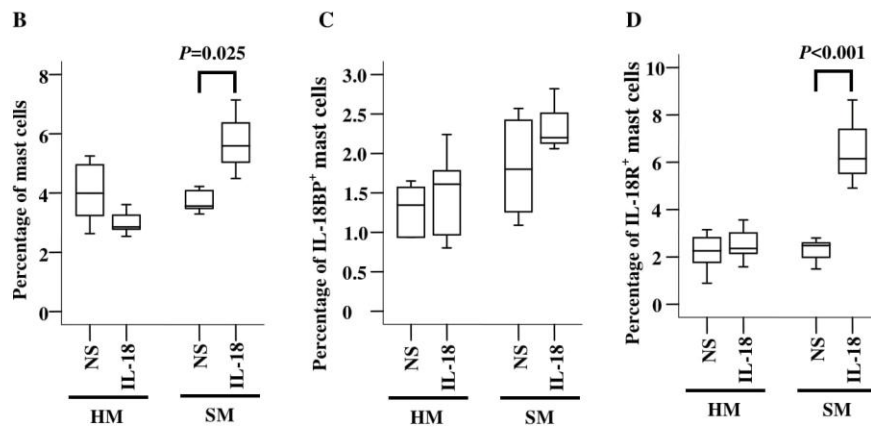
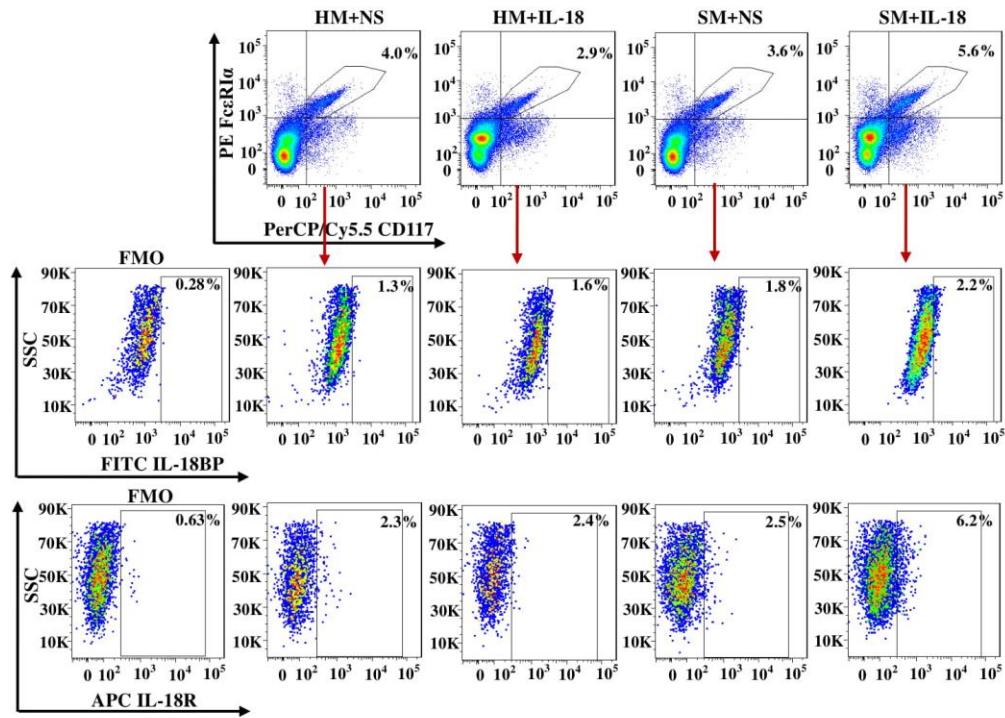
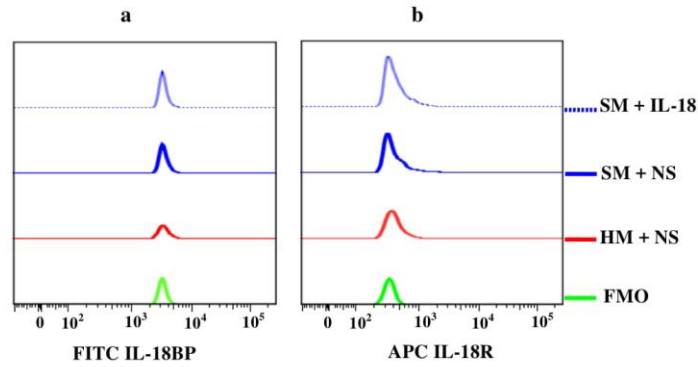


Figure 5 Altered expressions of IL-18BP and IL-18R in lung mast cells from sensitized and non-sensitized mouse induced by IL-18.

A Gating strategy of mast cells in mouse lung, and IL-18BP and IL-18R expressing mast cells.



E Representative flowcytometric figures of MFI of IL-18BP⁺ and IL-18R⁺ mast cell.



MFI of IL-18BP and IL-18R in mast cells in sensitized and non-sensitized mouse mast cells induced by IL-18

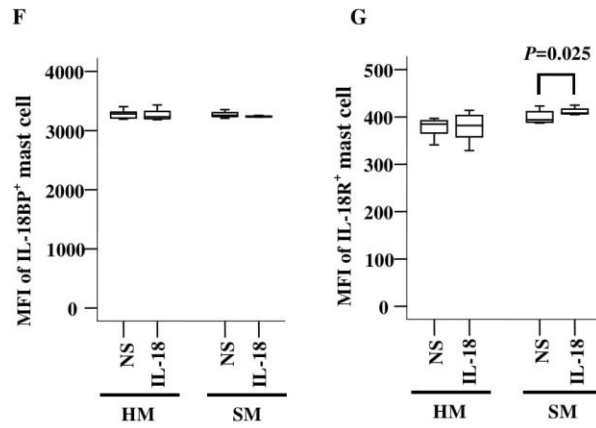


Figure 6 Levels of IL-18BP in mouse plasma of asthma model and **HM**.

