ABSTRACT

Purpose: To explore expression of SP and NK1R in basophils of allergic asthma (AA), allergic rhinitis (AR) and AR combined with AA (ARA), and influence of allergens and immunoglobulin E (IgE) mediated mechanisms on SP and NK1R expression.

Methods: Expression of SP and NK1R was detected by flow cytometry, NK1R mRNA expression was detected by real time quantitative polymerase chain reaction (qPCR), and mouse AR and AA models were employed for in vivo study.

Results: SP\(^+\) and NK1R\(^+\) cells increased in CCR3\(^+\) and CD123\(^+\)HLA-DR\(^-\)granulocytes of AA. PPE elevated proportions of SP\(^+\) cells in CCR3\(^+\) and CD123\(^+\)HLA-DR\(^-\)granulocytes, whereas ASWE and HDME augmented SP\(^+\) cells in CD123\(^+\)HLA-DR\(^-\)granulocytes of AR and ARA patients. ASWE, HDME and PPE increased proportions of NK1R\(^+\) cells in CCR3\(^+\) PBMC and CD123\(^+\)HLA-DR\(^-\)granulocytes of AR patients. OVA, Der p1, IL-33, IL-37, IgE and SP enhanced NK1R expression on KU812 cells. NK1R expressing basophils were increased in blood of OVA sensitized and challenged AR and AA mice. Fc\(\varepsilon\)RI-KO AA mice seemed to have less NK1R\(^+\) basophils than WT AA mice in their blood.

Conclusion: CCR3\(^+\) and CD123\(^+\)HLA-DR\(^-\) cells are likely involved in AA and AR via SP and NK1R. IgE-related mechanism may participate in upregulation of NK1R expression.

Keywords: Substance P; neurokinin 1 receptor; allergy, asthma, rhinitis, IgE, allergen
Often, asthma and AR are comorbid conditions, with AR being a major risk factor for the occurrence of asthma. However, the pathophysiological mechanisms of these diseases remain obscure.

In recent years, CCR3+ and CD123HLA-DR− cells in peripheral blood peripheral blood mononuclear cells (PBMCs) have been recognized as basophils, which are primary effector cells of allergy, including AA and AR. Prior studies have shown that AA patients exhibit phenotypically distinct basophil populations in the peripheral blood, some of which respond robustly to IgE-mediated activation. Basophils have been identified in the nasal washes of patients with AR, and are thought to be the dominant source of histamine in late-phase responses to allergen challenge in patients. Studies have identified that basophils are highly enriched in the post-mortem lung tissue of patients who have died of asthma as well as in bronchial biopsies of patients with asthma.

The involvement of substance P (SP) in basophil degranulation and accumulation has been highlighted. SP proved to be a potent chemoattractant for human basophils in vitro acting via its specific receptor neurokinin-1 receptor (NK1R) in basophils. Increased amounts of SP are found in sputum and BAL fluid of asthmatics as well as in nasal secretions of AR patients. SP causes many of the typical changes observed in asthmatic airways, including bronchoconstriction, increased mucus secretion, and plasma leakage. Some studies have shown upregulated NK1R expression in asthmatic lung compared to normal controls. These findings suggest a role of SP and NK1R in allergic inflammatory processes. However, little is known about the expression of SP and NK1R in basophils in patients with AR and AA.

It has been reported that the house dust mite (HDM) protease Dermatophagoide pteronyssinus (Der p) 1 can promote the production of interleukin (IL)-4, IL-5 and IL-13 from human basophils. In a murine model of HDM-induced airway inflammation, basophils were found to play a direct role in promoting optimal Th2 cytokine responses. However, the influence of allergens on the expression of SP and NK1R in basophils has not been investigated.

Therefore, the aim of the study is to investigate the expression of SP and NK1R in basophils of allergic airway diseases, and the influence of allergens and IgE-mediated mechanisms on SP and NK1R expression in basophils. We found that IgE-mediated mechanisms can affect NK1R expression.

**MATERIALS AND METHODS**

**Reagent**

The following reagents were purchased from Biolegend (San Diego, CA, USA): human Fc receptor blocking solution, red blood cell lysis buffer, Zombie Aqua Fixable Viability kit, BV421-conjugated mouse anti-human CCR3 antibody, APC-conjugated anti-human CCR3 antibody, PE-conjugated mouse anti-human CD123 antibody, FITC-conjugated anti-human CD123 antibody, PerCP-conjugated mouse anti-human HLA-DR antibody, APC/Cy7-conjugated anti-human HLA-DRα antibody, APC-conjugated rabbit anti-mouse CD49b antibody, and PE/Cy7-conjugated rabbit anti-mouse FcRRIα antibody. APC-conjugated mouse anti-human NK1R antibody and its isotype control antibody APC-conjugated mouse IgG3 were obtained from R&D Systems (Minneapolis, MN, USA). FITC-conjugated rabbit anti-human SP antibody and its isotype control antibody FITC-conjugated rabbit IgG were...
obtained from LifeSpan BioSciences (Seattle, WA, USA). AF488-conjugated rabbit anti-mouse NKIR antibody and its isotype control antibody AF488-conjugated rabbit IgG, PE-conjugated rabbit anti-mouse NKIR antibody and its isotype control antibody PE-conjugated rabbit IgG were obtained from Novus (Centennial, CO, USA). Cytofix/Cytoperm™Fixation/Permeabilization kit was obtained from BD Biosciences Pharmingen (Bedford, MA, USA). Fetal bovine serum (FBS, HyClone, Logan, UT, USA) and RPMI 1640 were from Gibco BRL (Grand Island, NY, USA). Ovalbumin (OVA, grade V), DNase I and Trypan blue dye were purchased from Sigma-Aldrich (St Louis, MO, USA). Artemisia sieversiana wild allergen extract (ASWE), HDM extract (HDME) or Platanus pollen allergen extract (PPE) were purchased from Macro Union Pharmaceutical Co. Ltd. (Beijing, China). Human IL-3, human IL-31, human IL-33, and human IL-37 were obtained from Peprotech (London, UK). Human IgE, IgA, IgD, IgM, IgG and anti-IgE antibody were obtained from Abcam (London, UK). ovalbumin Der p 1 was synthesized by Beijing Protein Innovation Co., Ltd. (Beijing, China). Oligonucleotide primers for real time quantitative polymerase chain reaction (qPCR) were synthesized by Invitrogen Biotechnology Co. (Shanghai, China). Trizol reagent was obtained from Invitrogen (Carlsbad, CA, USA). cDNA was synthesized by using a PrimeScript RT reagent kit. The resultant cDNA was subjected to qPCR, that was performed with a LightCycler using a SuperScript III Platinum SYBR Green 2-step qPCR kit. RNA was extracted by using a TaKaRa Mini BEST Universal RNA Extraction kit. Quantification of mRNA expression level was performed by applying TaKaRa SYBR Premix EX Tag kit (TaKaRa, Beijing, China). Most of the general chemicals, such as salts and buffer components were of analytical grade.

Patients and samples
A total of 38 AR, 30 AA, 16 AR combined with AA (ARA) and 21 healthy control (HC) subjects were recruited in the study. The diagnosing criterion of AA was conformed to the Global Initiative for Asthma, and diagnosis for AR in this experiment are in line with the 2015 AR Clinical Practice Guidelines issued by the American Academy of Otorhinolaryngology Head and Neck Surgery. ARA was diagnosed based on the AR and its impact on asthma. Samples were obtained at the First Affiliated Hospital of Jinzhou Medical University, China. 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 (approved number: KY201405) was obtained. The general characteristics of the patients and control subjects are summarized in Table 1. The blood from each patient with AR, AA, ARA and HC subject was taken in outpatient clinics. From each individual, 10 mL of peripheral blood was taken into an EDTA containing tube before centrifugation at 450 g for 10 minutes. The cells were used for flow cytometric analysis, and plasma was collected and frozen at −80°C until use.

| Table 1. General characteristics of the patients with AR, AA, ARA and HC subjects |
|---------------------------------|-----------------|-----------------|-----------------|
| Age (yr)                        | 26 (20–48)      | 35 (16–60)      | 49 (18–62)      |
| Median age at onset (yr)        | 26 (4–47)       | 40 (3–56)       | 28 (8–50)       |
| Female/Male                     | 11/6            | 16/22           | 21/8            |
| No. of positive skin prick for Arctemisia spp. | 19 | 14 | 11 |
| No. of positive skin prick for dust mite | 16 | 6 | 7 |
| No. of positive skin prick for platanus pollen | 15 | 8 | 5 |

AR, allergic rhinitis; AA, allergic asthma; ARA, allergic rhinitis combined with allergic asthma; HC, healthy control.
Animals

BALB/c mice (6–8 weeks) were obtained from Vital River Laboratory Animal Technology Co. Ltd (Beijing, China). They were housed in the Animal Experimental Center of Jinzhou Medical University in a specific pathogen-free environment with free access to standard rodent chow and water at a constant temperature of 23°C–28°C and relative humidity of 60%–75%. The animal experiment procedures were approved by the Animal Care Committee at the First Affiliated Hospital of Jinzhou Medical University.

C57-wild type (WT) mice and C57-FcεRI knock out (KO) mice (6–8 weeks) were obtained from Gem Pharmatech Co, Ltd (Jiangsu, China). FcεRIα-KO mouse model had been established by targeted disruption of the gene encoding the FcεRI-α chain, exon 3 and exon 4 (also available at http://www.informatics.jax.org/allele/summary?markerId=MGI:95494&alleleType=Targeted). In our study, the exon 1-5 of FcεRIα gene was knocked out and validated by PCR of genomic DNA.

Mouse AR model sensitized by OVA was mainly adopted from a previous study. Briefly, OVA sensitized and OVA challenged (OVA-OVA) mice, and OVA-sensitized and normal saline (NS)-challenged (OVA-NS) mice were sensitized on days 0, 7, 14 and 21 with an intranasal injection of 50 μg of OVA and 1.5 mg of Al(OH)3 suspended in NS to a total volume of 0.5 mL. Control mice (NS-sensitized and NS-challenged, NS-NS) received only the equal volume (0.5 mL) of NS on the same days. On day 28, OVA-OVA mice were challenged with nasal drops of either 20 μL of 1% OVA for 7 days, and OVA-NS mice and NS-NS mice were challenged with 20 μL of NS for 7 days, 10 μL on each side of the nasal cavity. On the last day, 3 hours after nasal drip being completed, each animal was sacrificed and its blood was collected for analysis.

For mouse AA model sensitized by OVA, which was largely adopted from previous study. OVA-OVA mice and OVA-NS mice were actively sensitized on days 0 and 7 with intra-peritoneal injection of 10 μg of OVA and 1 mg of Al(OH)3 suspended in NS to a total volume of 0.5 mL. NS-NS mice received only the equal volume (0.5 mL) of NS on the same days. On days 14 to 21, OVA-OVA mice were exposed daily to aerosolized 10 mg/mL of OVA over a 30-minute period, OVA-NS mice and NS-NS mice were exposed daily to NS over a 30-minute period. On the last day, 24 hours after aerosol inhalation being completed, animal was sacrificed and its blood was collected for analysis.

Flow cytometry analysis of the expressions of SP and NK1R in CCR3+ and CD123+HLA-DR− blood granulocytes and PBMCs

The procedure was mainly adopted from previously published work. Briefly, blood cells were challenged with or without allergens ASWE, HDME or PPE (all at concentrations of 0.1 and 1.0 μg/mL) for 60 minutes at 37°C, and 2 μg/mL of brefeldin A was also added into the tube. Cells were then incubated with human Fc receptor blocking solution for 15 minutes, and each labelled monoclonal antibody including BV421-conjugated mouse anti-human CCR3 antibody, PE-conjugated mouse anti-human CD123 antibody and PerCP-conjugated mouse anti-human HLA-DR antibody were added into the tube at room temperature for 15 minutes in the dark. Following ligation of red blood cells, white blood cells were fixed and permeabilized by using the Cytofix/Cytoperm™ Fixation/Permeabilization Kit according to the manufacturer’s instructions. APC-conjugated mouse anti-human NKIR antibody and FITC-conjugated rabbit anti-human SP antibody were then added to the testing tube, and APC-conjugated mouse IgG3 and FITC-conjugated rabbit IgG were used as isotype controls. Cells were incubated at 4°C for 30 minutes in the dark. Finally, cells were resuspended in fluorescence activated cell sorting
(FACS)-flow solution and analyzed with FACS Verse flow cytometer (BD Biosciences, San Jose, CA, USA). A total of 10,000 events in live cell gate were analyzed for each sample. Data were analyzed with FlowJo software version 7.0 (Treestar, Ashland, OR, USA). Dead cells and doublets were excluded from analysis by live/dead cell dyes. Granulocytes and PBMCs in human blood leukocytes were defined according to high side scatter (SSC)-A (SSC-Ahigh forward scatter [FSC]-A) and low SSC-A (SSC-Alow FSC-A) by flow cytometry, respectively.25

**Flow cytometric cell sorting**

Fresh peripheral blood samples were first incubated with human Fc receptor-blocking solution for 10 minutes and then stained with FITC-conjugated anti-human CD123, APC-conjugated anti-human CCR3 and APC/Cy7-conjugated anti-human HLA-DR α Abs. Target cells in granulocytes and PBMC were sorted into individual tubes containing RPMI 1640 medium supplemented with 3% FBS on a 3-laser SH800 sorter (Sony Biotechnology, San Jose, CA, USA) in purity mode.

**Preparation of cytopsin slides and cell count**

Cytocentrifuge slides were prepared with Shanton cytospin 4 (Thermo Fisher Scientific, Waltham, MA, USA) and stained with Wright Giemsa stain according to the manufacturer's instructions. Based on the morphology of leukocyte, differential cell counts under the microscope were performed with a minimum count of 100 cells. The results were expressed as percentage of each cell type out of total cells counted per preparation.

**Flow cytometry analysis of the expressions of NK1R on mouse blood basophils**

To detect expression of NK1R in mouse blood basophils, cells were incubated with TruStain fcX™ (anti-mouse CD16/32) and a live/dead cell dye (Zombie NIR™ Fixable Viability Kit) for 15 minutes, and each labelled monoclonal antibody including BV510-conjugated rabbit anti-mouse CD49b and PE/Cy7-conjugated rabbit anti-mouse FcεRIα was added into the tubes. This was followed by adding PE-conjugated rabbit anti-mouse NK1R antibody into the tube and incubated at room temperature for 15 minutes in the dark. Cells were resuspended in FACS-flow solution and analyzed with a FACS Verse flow cytometer for mouse blood cells.

**Cell line and culture**

The KU812 human basophil line was purchased from American Type Culture Collection. Cells were cultured in an RPMI 1640 medium supplemented with 10% heat-inactivated FBS and 100 units·mL⁻¹ penicillin/streptomycin in 75 cm² tissue culture flasks (Falcon, Mexico City, Mexico) at 37°C in a 5% (v/v) CO₂, water-saturated atmosphere.

**Flow cytometric analysis of NK1R expression on KU812 cells**

The challenging procedure for KU812 cells was mainly adopted from a method previously described by Zhong et al.24 for P815 cells. Briefly, cultured KU812 cells at a density of 0.5 × 10⁶ cells/mL were incubated with various concentrations of OVA and Der p 1, IL-33, IL-37 and SP, and ASWE (3.0 μg/mL), HDME (3.0 μg/mL), IL-3 (3.0 ng/mL), IL-31 (10.0 ng/mL), thymic stromal lymphopoietin (TSLP, 10.0 ng/mL), regulated upon activation, normal T-cell expressed and secreted (RANTES, 0.1 μg/mL), tryptase (1.0 μg/mL), histamine (1.0 μg/mL), olopatadine hydrochloride (Olo, 10.0 μg/mL), prostaglandin D2 (PGD2, 0.1 μg/mL), BAY-u-3405 Ramatroban (BAY, 0.35 μg/mL), leukotriene C4 (LTC4, 300 nM), montelukast sodium (Mon, 900 nM), human IgE (1.0 μg/mL), anti-IgE antibody (1.0 μg/mL), human IgA (1.0 μg/mL), human IgD (1.0 μg/mL), human IgM (1.0 μg/mL), human IgG (1.0 μg/mL), calcium ionophore (CI, 1.0 μM) or phosphate buffered saline (PBS, pH 7.4) for 2 or 16 hours.
at 37°C. Cells were then harvested and centrifuged at 300 g for 8 minutes at 4°C before the culture supernatant being collected and frozen at -80°C. Cell pellets were resuspended for flow cytometry and quantitative real time (q)PCR analysis, respectively. The challenge tests were repeated 4 times. For those blocking compounds, they were preincubation with the corresponding reagents for 30 minutes at room temperature before adding to cells.

To examine NK1R expression, KU812 cells were incubated with human Fc receptor blocking solution and Zombie NIR dye for 15 minutes and then stained with APC-conjugated mouse anti-human NK1R antibody. Following washing with PBS, cells were analyzed by flow cytometry as described above.

**qPCR analysis of expression of NK1R mRNA in KU812 cells**
cDNA generated from total RNA of KU812 cells was used as templates, and qPCR assay was performed as described by Zhang et al.\(^\text{26}\) by using specific primers of NK1R and β-actin as listed in Table 2. Briefly, qPCR was performed with the SYBR Premix Ex Taq II Kit on a Real-time Thermal Cycler (Thermo Fisher Scientific). Each reaction contains 10 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 ΔCt for all experimental samples were subtracted by the ΔCt for the control samples (ΔΔCt). The magnitude change of test gene mRNA was expressed as 2^−ΔΔCt. Each measurement of a sample was conducted in duplicate.

**Statistical analysis**
Statistical analyses were performed by using SPSS version 13.0 software (SPSS, Inc., Chicago, IL, USA). Human peripheral blood basophil data are displayed as a scatter plot, where Kruskal-Walles analysis indicated significant differences between groups, for the pre-planned comparisons of interest, the paired Mann-Whitney U test was employed. Data from KU812 cell lines were presented as mean ± standard error of mean and analyzed by Student’s t-test. Data for expression of NK1R in basophils of mouse blood were displayed as boxplots, which indicate the median, interquartile range, the largest and smallest values for the number of experiments indicated. For all analyses, \(P < 0.05\) was considered statistically significant.

**RESULTS**

**Upregulated expression of SP in CCR3\(^+\) and CD123\(^+\)HLA-DR\(^-\) granulocytes of patients with AR, AA, ARA and HC subjects**
The involvement of SP in basophil degranulation and accumulation has been highlighted.\(^\text{13}\) However, little is known about expression of SP in basophils of allergic airway diseases. We therefore investigated the issue in the present study. Since several gating strategies have been proposed to determine the phenotype of basophils, and it is rather difficult to determine the best strategy, we therefore select 2 gating strategies, CCR3\(^+\) and CD123\(^+\)HLA-DR\(^-\) cells (Fig. 1A).\(^\text{27}\) The results showed that the percentages of SP\(^+\) cells out of CCR3\(^+\) (Fig. 1B and C) and CD123\(^+\)HLA-DR\(^-\) granulocytes (Fig. 1D and E) of AA were increased by 1.4- and 1.33-fold, respectively. Mean fluorescent intensity (MFI) of SP in CCR3\(^+\) granulocytes of AA enhanced by 24.8% (Fig. 1G).

**Table 2.** Specific primers of NK1R

| Name  | Primer sequence   |
|-------|-------------------|
| NK1R-F | ccc cca aca gga cac taa ga |
| NK1R-R | ctg gaa aac arg gaa acc tc |

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Fig. 1. Flow cytometric analysis of the expression of SP in peripheral blood granulocytes and PBMC. (A) represents a gating strategy of CCR3+ and CD123+HLA-DR− cells in peripheral blood granulocytes and PBMCs. (B, C) Proportions of SP+ cells in CCR3+ granulocytes from AR, AA, ARA and HC subjects. (B) representative graphs of the percentages of SP+ cells in CCR3+ granulocytes, and (C) the percentages of SP+ cells in CCR3+ granulocytes. (D, E) proportions of SP+ cells in CD123+HLA-DR− granulocytes. (D) representative graphs of the percentages of SP+ cells in CD123+HLA-DR− granulocytes, and (E) the percentages of SP+ cells in CD123+HLA-DR− granulocytes. (F, G) MFI of SP expression in CCR3+ granulocytes. (F) representative graphs and (G) the percentages of SP+ cells in CCR3+ and CD123+HLA-DR− granulocytes or MFI of SP in CCR3+ granulocytes, respectively. Each symbol represents the value from one subject. The median value is indicated by a horizontal line. *P < 0.05 was taken as statistically significant. Cells were stimulated with or without HDME, ASWE or PPE, all at 0.1 and 1 μg/mL respectively, for 1 hour at 37°C.

SP, substance P; FMO, fluorescence minus one; PBMC, peripheral blood mononuclear cell; AR, allergic rhinitis; AA, allergic asthma; ARA, allergic rhinitis combined with allergic asthma; HC, healthy control; MFI, mean fluorescent intensity; HDME, house dust mite extract; ASWE, Artemisia sieversiana wild allergen extract; PPE, Platanus pollen allergen extract; SSC, side scatter; FSC, forward scatter.

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representative graphs of the percentages of SP
CD123
extract; PPE, SP, substance P; FMO, fluorescence minus one; PBMC, peripheral blood mononuclear cell; AR, allergic rhinitis; AA, allergic asthma; ARA, allergic rhinitis
respectively, for 1 hour at 37°C.

Flow cytometric analysis of the expression of SP in peripheral blood granulocytes and PBMC. (A) represents a gating strategy of CCR3
and
CD123
Platanus
−
SSC-A
150K
HLA-DR
BV/five.LP/one.LP./zero.LP-Live Cell Dye
granulocytes. (D) representative graphs of the percentages of SP
2,000
4,000
CCR/three.LP+ granulocytes
Percentage of SP+ cells in
−
−
−
−
granulocytes or MFI of SP in CCR3
100K
granulocytes. (D) representative graphs of the percentages of SP
2,000
4,000
CCR/three.LP+ granulocytes
Percentage of SP+ cells in
−
−
−
−
granulocytes of HCs
ARA
and CD123
HLA-DR
−
granulocytes of patients with AR and ARA
(continued to the next page)

Fig. 1. (Continued) Flow cytometric analysis of the expression of SP in peripheral blood granulocytes and PBMC. (A) represents a gating strategy of CCR3
and
CD123
HLA-DR
−
cells in peripheral blood granulocytes and PBMCs. (B, C) Proportions of SP
cells in CCR3
granulocytes from AR, AA, ARA and HC subjects. (B) representative graphs of the percentages of SP
cells in CCR3
granulocytes, and (C) The percentages of SP
 cells in CCR3
granulocytes. (D, E) proportions of SP
cells in CD123
HLA-DR
−
granulocytes, and (F) the percentages of SP
cells in CD123
HLA-DR
−
granulocytes of patients with AR and ARA
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Allergen extract PPE increased the proportions of SP
 cells in CCR3
granulocytes of HCs
and AR patients (Fig. 1C), and in CD123
HLA-DR
−
granulocytes of HCs, and patients with AR and ARA (Fig. 1E). Similarly, ASWE augmented the proportions of SP
 cells in CD123
HLA-DR
−
granulocytes of HCs, AR and ARA patients (Fig. 1E). However, HDME only enhanced the proportions of SP
 cells in CD123
HLA-DR
−
granulocytes of patients with AR and ARA (Fig. 1E).

On the other hand, the percentages of SP
 cells out of CCR3
and CD123
HLA-DR
−
PBMC and MFI of SP expression on CCR3
and CD123
HLA-DR
−
PBMCs of AR, AA and ARA patients had little changes in comparison to HC subjects (data not shown).
Identification of isolated CCR3\(^+\) and CD123\(^+\)HLA-DR\(^-\) cells in peripheral blood granulocytes and PBMC

To visualize CCR3\(^+\) and CD123\(^+\)HLA-DR\(^-\) cells under a microscope, we isolated them from human peripheral blood by using flow-cytometric cell sorting technique. The results showed that more than 97.7% CCR3\(^+\) (Fig. 2A and B) and 99% CD123\(^+\)HLA-DR\(^-\) cells (Fig. 2C and D) in PBMCs of HC subjects were basophils. On the other hand, up to 13% basophils, 85% eosinophils and 2% basophils.
neutrophils in CCR3+ granulocytes of HC subjects were observed (Fig 2E and F). In terms of CD123+HLA-DR− granulocytes of HC subjects, approximately 50% basophils, 4% eosinophils and 46% neutrophils were found (Fig 2G and H).

Enhanced expression of NK1R on CCR3+ and CD123+HLA-DR− peripheral blood cells of patients with AR, AA, ARA and HC subjects

Activation of NK1R can mediate SP-induced migration of basophils and consequently amplify the proinflammatory response. However, little is known of NK1R expression on blood basophils of allergic airway diseases. In the present study, the percentages of NK1R+ cells out of CCR3+ granulocytes of AR increased by 1.02-fold (Fig 3B). In comparison, the percentages of NK1R+ cells in CCR3+ (Fig 3B) and CD123+HLA-DR− granulocytes (Fig 3D) of AA and ARA were increased by 1.79- and 2.23-fold and 3.46- and 3.42-fold, respectively.

On the other hand, it was observed that the percentages of NK1R+ cells out of CCR3+ (Fig 3F) and CD123+HLA-DR− (Fig 3H) of ARA patients increased by 1.31- and 1.09-fold in comparison...
with HC subjects. Whilst, the percentage of NK1R+cells in CCR3+PBMCs of AR patients increased by 78% in comparison to HCs subjects (Fig. 3F).

MFI of NK1R expression on CD123+HLA-DR− granulocytes of AR and ARA patients were enhanced by 78% and 71% in comparison to HC subjects. MFI of NK1R expression on CCR3+ (Fig. 4E) and CD123+HLA-DR− PBMCs (Fig. 4F) of AR were augmented by 1.46- and 0.12-fold. Similarly, MFI of NK1R expression on CD123+HLA-DR− PBMC of AA (Fig. 4F) were increased by 34%.

Allergen extracts ASWE and PPE increased the proportions of NK1R+ cells in CCR3 and CD123+HLA-DR− granulocytes (Fig. 3B) of HC subjects, and the percentages of NK1R+ cells in CCR3+ PBMCs of AR patients (Fig. 3F). ASWE and PPE also enhanced the proportions of NK1R+ cells in CD123+HLA-DR− PBMCs (Fig. 4F) of AR were augmented by 1.46- and 0.12-fold. Similarly, HDME augmented the proportions of NK1R+ cells in CD123+HLA-DR− granulocytes of HC subjects (Fig. 3D), and in CCR3+ PBMC of AR patients (Fig. 3F). It was observed that ASWE at 0.1 and 1.0 μg/mL raised MFI of NK1R expression on CCR3+ PBMCs of HC subjects by 1.18-fold and 83.73%, respectively (Fig. 4C). PPE enhanced MFI of NK1R expression on CCR3+ PBMC of HC subjects and AR patients by up to 1.26- and 1.32-fold (Fig. 4C).

**Positive correlations between SP+ and NK1R+ cells of CCR3+ and CD123+HLA-DR− granulocytes**

In order to learn more about the relationships between expressions of SP and NK1R in CCR3+ and CD123+HLA-DR− granulocytes of AR, AA, ARA patients and HC subjects, Pearson’s correlation test was employed. As shown in Fig. 4, correlation coefficient ranges were defined as follows: $R < 0.3$ as weak correlation; $0.3 \leq R \leq 0.7$ as moderate correlation;
Flow cytometric analysis of the expression of NKIR on peripheral blood granulocytes and PBMCs. (A, B) Proportions of NKIR⁺ cells on CCR3⁺ granulocytes from patients with AR, AA, ARA and HC subjects. (C, D) Proportions of NKIR⁺ cells on CD123-HLA-DR⁺ granulocytes. (E, F) Proportions of NKIR⁺ cells on CCR3⁺ PBMCs. (G, H) Proportions of NKIR⁺ cells on CD123-HLA-DR⁺ PBMCs. (A, C, E, G) Representative graphs and (B, D, F, H) the percentages of NKIR⁺ cells in CCR3⁺ and CD123-HLA-DR⁺ granulocytes or PBMCs, respectively. Each symbol represents the value from one subject. The median value is indicated by a horizontal line. P < 0.05 was taken as statistically significant. Cells were stimulated with or without HDME, ASWE or PPE, all at 0.1 and 1 μg/mL, respectively, for 1 hour at 37°C. FMO, fluorescence minus one; NKIR, neurokinin-1 receptor; PBMC, peripheral blood mononuclear cell; AR, allergic rhinitis; AA, allergic asthma; ARA, allergic rhinitis combined with allergic asthma; HC, healthy control; HDME, house dust mite extract; ASWE, Artemisia sieversiana wild allergen extract; PPE, Platanus pollen allergen extract; SSC, side scatter.

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and R > 0.7 as strong correlation. There were moderate correlations between percentages of SP⁺CCR3⁺ and NKIR⁺CCR3⁺ granulocytes of HC subjects; strong correlations between percentages of SP⁺CCR3⁺ and NKIR⁺CCR3⁺ granulocytes of AR and AA patients (Fig. 5A). Moderate correlations between percentages of SP⁺CD123⁺HLA-DR⁺ and NKIR⁺CD123⁺HLA-DR⁺ granulocytes of HC subjects and AR patients; strong correlations between percentages of SP⁺CD123⁺HLA-DR⁺ and NKIR⁺CD123⁺HLA-DR⁺ granulocytes of AA and ARA patients were also observed (Fig. 5B).
Flow cytometric analysis of the expression of NK1R in peripheral blood granulocytes and PBMCs. (A, B) Proportions of NK1R⁺ cells on CCR3⁺ granulocytes from patients with AR, AA, ARA and HC subjects. (C, D) Proportions of NK1R⁺ cells on CD123⁺ HLA-DR⁻ granulocytes. (E, F) Proportions of NK1R⁺ cells on CCR3⁺ PBMCs. (G, H) Proportions of NK1R⁺ cells on CD123⁺ HLA-DR⁻ PBMCs. (A, C, E, G) Representative graphs and (B, D, F, H) the percentages of NK1R⁺ cells in CCR3⁺ and CD123⁺ HLA-DR⁻ granulocytes or PBMCs, respectively. Each symbol represents the value from one subject. The median value is indicated by a horizontal line. *P < 0.05 was taken as statistically significant. Cells were stimulated with or without HDME, ASWE or PPE, all at 0.1 and 1 µg/mL, respectively, for 1 hour at 37°C.

FMO, fluorescence minus one; NK1R, neurokinin-1 receptor; PBMC, peripheral blood mononuclear cell; AR, allergic rhinitis; AA, allergic asthma; ARA, allergic rhinitis combined with allergic asthma; HC, healthy control; HDME, house dust mite extract; ASWE, Artemisia sieversiana wild allergen extract; PPE, Platanus pollen allergen extract; SSC, side scatter.

Upregulated expression of NK1R in KU812 cells induced by allergens, cytokines, mediators and lgs
In order to understand the potential mechanisms of the elevated expression of NK1R in basophils, we investigated actions of allergens including Der p 1, OVA, ASWE, HDME, proinflammatory cytokines including IL-3, IL-31, IL-33, IL-37, TSLP, RANTES, and...
Fig. 3. (Continued) Flow cytometric analysis of the expression of NKIR in peripheral blood granulocytes and PBMCs. (A, B) Proportions of NKIR+ cells on CCR3+ granulocytes from patients with AR, AA, ARA and HC subjects. (C, D) Proportions of NKIR+ cells on CD123+ HLA-DR+ granulocytes. (E, F) Proportions of NKIR+ cells on CCR3+ PBMCs. (A, C, E, G) representative graphs and (B, D, F, H) the percentages of NKIR+ cells out of CD123+ HLA-DR− granulocytes or PBMCs, respectively. Each symbol represents the value from one subject. The median value is indicated by a horizontal line. \( P < 0.05 \) was taken as statistically significant. Cells were stimulated with or without HDME, ASWE or PPE, all at 0.1 and 1 \( \mu g/mL \), respectively, for 1 hour at 37°C. FMO, fluorescence minus one; NKIR, neurokinin-1 receptor; PBMC, peripheral blood mononuclear cell; AR, allergic rhinitis; AA, allergic asthma; ARA, allergic rhinitis combined with allergic asthma; HC, healthy control; HDME, house dust mite extract; ASWE, Platanus incisa wild allergen extract; PPE, Platanus pollen allergen extract; SSC, side scatter.

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proinflammatory mediators and some of their blockers including SP, tryptase, CI, His, Olo, Olo+His, PGD2, BAY, BAY+PGD2, LTC4, Mon, Mon+LTC4 and IgS including IgE, IgA, IgD, IgM and IgG on KU812 cells. The results showed that compared to the medium alone group, OVA (at the concentrations of 0.03, 0.3, 1.0 and 3.0 \( \mu g/mL \)), Der p 1 (at the concentrations of 0.03, 0.3, 1.0 and 3.0 \( \mu g/mL \)), IL-33 (at the concentrations of 1.0, 3.0 and 10 ng/mL), IL-37 (at the concentrations of 1.0, 3.0 and 10 ng/mL) and SP (at the concentrations of 3.0, 10 and 30

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Fig. 3. (Continued) Flow cytometric analysis of the expression of NKIR in peripheral blood granulocytes and PBMCs. (A, B) Proportions of NKIR+ cells on CCR3+ granulocytes from patients with AR, AA, ARA and HC subjects. (C, D) Proportions of NKIR+ cells on CD123+HLA-DR+ granulocytes. (E, F) Proportions of NKIR+ cells on CCR3+ PBMCs. (G, H) proportions of NKIR+ cells on CD123+HLA-DR+ PBMCs. (A, C, E, G) representative graphs and (B, D, F, H) the percentages of NKIR+ cells in CCR3+ and CD123+HLA-DR+ granulocytes or PBMCs, respectively. Each symbol represents the value from one subject. The median value is indicated by a horizontal line. *P < 0.05 was taken as statistically significant. Cells were stimulated with or without HDME, ASWE or PPE, all at 0.1 and 1 μg/mL, respectively, for 1 hour at 37°C.

FMO, fluorescence minus one; NKIR, neurokinin-1 receptor; PBMC, peripheral blood mononuclear cell; AR, allergic rhinitis; AA, allergic asthma; ARA, allergic rhinitis combined with allergic asthma; HC, healthy control; HDME, house dust mite extract; ASWE, Artemisia sieversiana wild allergen extract; PPE, Platanus pollen allergen extract; SSC, side scatter.

ng/mL) enhanced percentages of NKIR+ KU812 cells and MFI of NKIR expression on KU812 cells at 2 hours (Fig. 6A and C) and 16 hours (Fig. 6B and D) following incubation. IgE at 1.0 μg/mL appeared to increase MFI of NKIR expression (Fig. 6C) and NKIR mRNA expression (Fig. 6E) in KU812 cells at 2 hours following incubation, which was blocked by co-incubation with anti-IgE antibody for 30 minutes. IgG at 1.0 μg/mL seemed to enhance MFI of NKIR expression in KU812 cells at 2 hours following incubation (Fig. 6C). IgD and IgM both at 1.0 μg/mL augmented MFI of NKIR expression in KU812 cells at 16 hours following incubation (Fig. 6D). IL-33, IL-37, IL-3, IL-31, SP, PGD2 and IgA, but not OVA and Der p 1 at the concentrations tested elevated NKIR mRNA expression in KU812 cells at 2 hours following incubation (Fig. 6E).
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Fig. 4. Flow cytometric analysis of MFI of NKIR in granulocytes and PBMCs. (A) representative graph of MFI of NKIR on CD123^HLA-DR^ granulocytes. (B) showed MFIs of NKIR on CD123^HLA-DR^ granulocytes from patients with AR, AA, ARA and HC subjects, respectively. (C, D) representative graphs of MFIs of NKIR in (C) CCR3^ PBMC and (D) CD123^HLA-DR^ PBMC, respectively. Each symbol represents the value from one subject. The median value is indicated by a horizontal line. P < 0.05 was taken as statistically significant. Cells were stimulated with or without HDME, ASWE or PPE, all at 0.1 and 1 μg/mL respectively, for 1 hour at 37°C. FMO, fluorescence minus one; MFI, mean fluorescent intensity; NK1R, neurokinin-1 receptor; PBMC, peripheral blood mononuclear cell; AR, allergic rhinitis; AA, allergic asthma; ARA, allergic rhinitis combined with allergic asthma; HC, healthy control; HDME, house dust mite extract; ASWE, Artemisia sieversiana wild allergen extract; PPE, Platanus pollen allergen extract.

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Increased NK1R^ blood basophils of allergic mice

In order to confirm that allergen can induce upregulation of expression of NK1R in basophils, we investigated the expression of NK1R in basophils of AR and AA mice sensitized and challenged by OVA. The results showed that the percentages of NK1R expressing basophils were increased in blood of OVA-sensitized as well as OVA sensitized and challenged AR mice (Fig. 7C). However, in AA mice, only OVA sensitized and challenged mice showed increased

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Allergen extract; PPE, allergic asthma; ARA, allergic rhinitis combined with allergic asthma; HC, healthy control; HDME, house dust mite extract; ASWE, FMO, fluorescence minus one; MFI, mean fluorescent intensity; NK1R, neurokinin-1 receptor; PBMC, peripheral blood mononuclear cell; AR, allergic rhinitis; AA, stimulated with or without HDME, ASWE or PPE, all at 0.1 and 1 μg/mL respectively, for 1 hour at 37°C. FMO, fluorescence minus one; MFI, mean fluorescent intensity; NK1R, neurokinin-1 receptor; PBMC, peripheral blood mononuclear cell; AR, allergic rhinitis; AA, allergic asthma; ARA, allergic rhinitis combined with allergic asthma; HC, healthy control; HDME, house dust mite extract; ASWE, Artemisia sieversiana wild allergen extract; PPE, Platanaus pollen allergen extract.

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![Flow cytometric analysis of MFI of NK1R in granulocytes and PBMCs.](https://e-aair.org)

**Fig. 4.** (Continued) Flow cytometric analysis of MFI of NK1R in granulocytes and PBMCs. (A) representative graph of MFI of NK1R on CD123+HLA-DR− granulocytes. (B) showed MFIs of NK1R on CD123+HLA-DR− granulocytes from patients with AR, AA, ARA and HC subjects, respectively. (C, D) representative graphs of MFIs of NK1R in (C) CCR3+ and (D) CCR3− PBMC, respectively. (E) and (F) demonstrated MFIs of NK1R on CCR3+ and CCR3− granulocytes from patients with AR, AA, ARA and HC subjects, respectively. (C, D) representative graphs of MFIs of NK1R expressed on CCR3+ and CCR3− granulocytes.

proportion of NK1R expressing basophils in their blood (Fig. 7E). FcεRI-KO AA mice seemed to have less basophils in their blood than WT AA mice when they were challenged by NS and OVA (Fig. 8B). Similarly, FcεRI-KO AA mice showed less NK1R+ basophils in their blood than WT AA mice regardless of whether they were sensitized or challenged by NS and OVA (Fig. 8D). As for BALB/c mice, C57 AA mice also exhibited increased proportion of NK1R expressing basophils in their blood following OVA sensitized and challenged (Fig. 8D).

**DISCUSSION**

Upregulated expression of SP in CCR3+ and CD123+HLA-DR+ granulocytes of patients with AA, but not with AR or ARA, was an unexpected result, which may implicate that these cells may contribute to the pathogenesis of AA via SP. Involvement of SP in AA has been previously reported. For instance, increased amounts of SP are found in sputum and BAL fluid of...
asthmatics, and SP causes many of the typical changes observed in asthmatic airways. Our finding suggests CCR3+ and CD123+HLA-DR− granulocytes may serve as the sources of SP in blood. Since expression of SP in CCR3+ and CD123+HLA-DR− PBMC of AR, AA or ARA patients had little changes in comparison to HC subjects, and CCR3+ cells and CD123+HLA-DR− cells are basophils in PBMCs. We anticipate that those upregulated SP expression cells in CCR3+ and CD123+HLA-DR− granulocytes could be a subtype of basophils which is different from those in PBMC, or includes some other cell types besides basophils. Indeed, it has been reported that AA patients exhibit phenotypically distinct basophil populations in the peripheral blood, CCR3+ cells in blood granulocytes include eosinophils and basophils. While information on CD123+HLA-DR− cells in blood granulocytes is not available, CD123+ granulocytes consist of eosinophils, immature neutrophils and basophils. Taking the information together, it is rather difficult to ensure the specific cell type which expresses SP in CCR3+ and CD123+HLA-DR− granulocytes at this stage.

In contrast, upregulated expression of NK1R is observed in CCR3+ and CD123+HLA-DR− PBMC of ARA patients, and enhanced expression of NK1R on CCR3+ and CD123+HLA-DR− PBMC of AR and AA patients is found in the present study, suggesting that basophils are involved in the pathogenesis of AA, AR and ARA via SP-related mechanisms. While information on basophil NK1R expression relating to AA, AR and ARA is not available, the reports that upregulated NK1R expression in asthmatic lung compared to normal controls, and that basophils are highly enriched in post-mortem lung tissue of patients who have died from asthma may indirectly support the anticipation that upregulated expression of NK1R in basophils could be involved in the pathogenesis of AA, AR and ARA. Therefore, enhanced
Fig. 6. Expression of NK1R on KU812 cells. The cells were incubated with various concentrations of OVA and Der p 1, IL-33, IL-37 and SP, and ASWE (3.0 µg/mL), HDME (3.0 µg/mL), IL-3 (3.0 ng/mL), IL-31 (10.0 ng/mL), TSLP (10.0 ng/mL), RANTES (0.1 µg/mL), tryptase (1.0 µg/mL), histamine (1.0 µg/mL), Olo (0.0 µg/mL), PGD2 (0.1 µg/mL), BAY (0.35 µg/mL), LTC4 (300 nM), Mon (900 nM), human IgE (1.0 µg/mL), human IgA (1.0 µg/mL), human IgD (1.0 µg/mL), human IgM (1.0 µg/mL), human IgG (1.0 µg/mL), CI (1.0 µM) or PBS (pH 7.4) for 2 or 16 hours at 37°C. (A, B) demonstrated percentages of NK1R+ KU812 cells at 2 and 16 hours following incubation. (C, D) showed MFI levels of NK1R expressed on KU812 cells at 2 and 16 hours following incubation. (E) represented NK1R mRNA expression in KU812 cells at 2 hours following incubation. The data are presented as the mean ± standard error for 4 separate experiments. SP, substance P; NK1R, neurokinin-1 receptor; OVA, ovalbumin; Der p, Dermatophagoides pteronyssinus; ASWE, Artemisia sieversiana wild allergen extract; HDME, house dust mite extract; IL, interleukin; TSLP, thymic stromal lymphopoietin; RANTES, regulated upon activation, normal T-cell expressed and secreted; Olo, olopatadine hydrochloride; PGD2, prostaglandin D2; BAY, BAY-u-3405 Ramatroban; LTC4, leukotriene C4, Mon, montelukast sodium; Ig, immunoglobulin; CI, calcium ionophore; PBS, phosphate buffered saline; MFI, mean fluorescence intensity.

* * P < 0.05 compared with the medium alone group; ** P < 0.05 compared with the IgE alone group.
expression of NK1R in CCR3+ and CD123+HLA-DR− granulocytes of patients with AR, AA and ARA should be at least in part contributed by basophils. The strong correlations between percentages of SP+CCR3+ and NK1R+CCR3+ granulocytes of AR and AA patients, between percentages of SP+CD123+HLA-DR− and NK1R+CD123+HLA-DR− granulocytes of AA and ARA patients imply that the increased SP+ and NK1R+ cells in granulocyte population may originate from the same cell types.

Originally, the flow cytometer system was used to separate unfixed and unstained human leukocyte cells into morphologically distinct populations based only on the intensity of 488-nm wavelength laser light simultaneously scattered by each cell at 2 different angles. Three populations were observed as distinct peaks in a 2-parameter pulse-height distribution. The 3 groups consisted of lymphocytes, monocytes and neutrophils. Later, the 2-parameters 90 degrees light scatter (SSC) reflecting primarily cell granularity vs. forward angle light scatter...
(FSC) correlating with cell size were found to clearly identify PBMCs including lymphocytes and monocytes, and granulocytes in white blood cell suspensions. It was observed that proportions of lymphocytes and granulocytes obtained by the flow cytometer correlated well with those obtained by both microscopic and automatic differential. Moreover, the
granulocyte population obtained by sorting on a plot of FSC/SSC was a mixture of eosinophils and neutrophils. Eosinophils were concentrated above the neutrophil cluster, but they are frequently found distributed throughout the neutrophil cluster. For the treatment regimen of allergen extracts, a 60-minute incubation period was adopted on the basis of our previous work. Sonneck et al. previously reported that blood basophils from allergic patients were exposed to various concentrations of recombinant grass pollen (Phl p 1, Phl p 5), birch pollen (Bet v 1) or HDM (Der p 2) allergens for 15 minutes. The concentrations of HDM, ASWE and PPAE used were based upon published papers.

It is difficult to obtain large quantities of highly purified inactive basophils. It has been reported that KU812 cells are a suitable model for studying the activation and degranulation of human basophils. Elevated percentages of NK1R+ KU812 cells and MFI of NK1R expression on KU812 cells induced by OVA and Der p 1, but not ASWE or HDME, suggest that purified allergens may be more efficient in induction of NK1R expression on basophils than allergen extracts. Induction of upregulated NK1R expression in KU812 cells by IgE, IgG, IgD and IgM implicates that Igs-associated mechanisms may be capable of contributing to regulate NK1R expression in basophils. Nevertheless, further work is required to prove the issue. The ability to induce enhanced NK1R expression on KU812 cells suggests that proinflammatory cytokines IL-33 and IL-37, and SP are likely to be involved in the modification of NK1R expression in basophils.

The influence of allergens on the expression of SP and NK1R in basophils has not previously been investigated. In the present study, allergen extract PPE upregulated SP expression in CCR3+ and CD123+HLA-DR− granulocytes of AR and ARA patients. ASWE and HDME augmented the proportions of SP+ cells in CD123+HLA-DR− granulocytes of AR and ARA patients, implicating that allergen extracts may affect more SP expression in AR than in AA patients. Unexpectedly, PPE also upregulated SP expression in CCR3+ and CD123+HLA-DR− granulocytes as well as ASWE augmented SP+ cells in CD123+HLA-DR− granulocytes of the HC subjects. Since these allergen extracts failed to induce upregulated SP expression in CCR3+ and CD123+HLA-DR− PBMCs of HC subjects, AR, AA and ARA patients, the elevated SP expression may not be in basophils. Considering that allergen extract can alter behavior of peripheral blood eosinophils during allergen-induced late-phase airway inflammation in patients with AA, that bronchial HDME challenge elicits significant increases in sputum eosinophils and ECP in AA patients, and that eosinophils express CCR3, we believe that these SP+CCR3+ granulocytes could be eosinophils. Nevertheless, these are rather confusing results, but we believe that the experiment using purified allergens and isolated cell types may help understand this issue.

It is observed that allergen extracts ASWE and PPE enhanced NK1R expression in CD123+HLA-DR− granulocytes of AR patients, as well as in CCR3+ and CD123+HLA-DR− granulocytes of HC subjects. HDME augmented NK1R expression in CD123+HLA-DR− granulocytes of HC subjects. Since PPE and HDME enhanced NK1R expression on CCR3+ PBMCs, ASWE and PPE raised NK1R expression on CCR3+ and CD123+HLA-DR− PBMC of HC subjects and AR patients, the enhanced NK1R expression in CCR3+ and CD123+HLA-DR− granulocytes of HC subjects and AR patients is at least partially on basophils.

It has previously been reported that allergens ASWE and HDME, but not PPE enhanced NK1R expression on CD14+ blood leukocytes regardless of atopic dermatitis (AD) or HC
Since AD, AA and AR all belong to atopic disease, it is likely that these allergens affect NK1R expression in AA and AR. Indeed, B cells appeared to play a major role in the adaptive immune response to inhaled HDM allergen in HDM-driven asthma by expanding allergen-specific T cells, and HDM allergen Der p 1 catalytically inactivated alpha 1-antitrypsin and promoted airway inflammation and asthma. It has been noticed that the production of IL-25, IL-33 and TSLP from airway epithelial cells was induced by the protease activity of an HDMs in HDM-induced asthma mouse model. Because IL-33 is one of the most highly replicated susceptibility loci for asthma, HDM-induced asthma may occur via IL-33-associated mechanisms. In a mouse asthma model, mice sensitized with ASWE had significantly increased IL-4, IL-5, IL-13 and allergen-specific IgE levels in the plasma. Moreover, Halogen immunoassay analysis demonstrated IgE binding to Platanus pollen in all Platanus-sensitized allergy subjects, and Pla a 1 and Pla a 2 were responsible for 79% of the IgE-binding capacity against PPE. These findings may help understand differences between the pro-asthma mechanisms of the allergen extracts. It was reported that FcεRI-activated mast cells upregulated NK1R transcripts and protein synthesis, without modifying SP expression, suggesting that the allergens may affect some transcriptional factors and upregulate NK1R transcripts in mast cells as allergens are likely to activate mast cells via FcεRI.

Upregulated NK1R expression in AR and AA mouse blood basophils upon OVA-sensitization and challenge confirms that allergen is capable of inducing AR and AA and upregulation of NK1R expression. A report that basophils play a direct role in promoting optimal Th2 cytokine responses in a murine model of HDM-induced airway inflammation may support the above observation. Considering that OVA can enhance NK1R expression in the basophil line of KU812, and a study has shown that the development of bronchial hyperreactivity was completely inhibited after 4 hours in mice treated with the NK1R antagonist L-732138 and stimulated with OVA, we anticipate that allergens may mediate AR and AA through a SP-NK1R mediated mechanism. FcεRI KO AA mice seemed to have less basophils and NK1R+ basophils in their blood than WT AA mice, when they were sensitized and challenged by NS and OVA. The result may be caused by the fact that the deletion of FcεRI gene in mice leads to the inability of IgE binding to their basophils or mast cells, and subsequently diminishing formation of sensitized basophils or mast cells, resulting in a significant decrease in NK1R expression in basophils.

In conclusion, upregulated expression of SP and NK1R in CCR3+ and CD123+HLA-DR− cells of patients with AA and AR implicates that these cells may contribute to the pathogenesis of AA and AR via SP-NK1R-related mechanisms. Allergens can modify behavior of these cells, particularly basophils, suggesting further that SP-NK1R related mechanisms are involved in these airway allergic diseases. Induction of upregulated NK1R expression in basophils by OVA and IgE in vitro as well as deletion of the FcεRI gene in mice decrease NK1R expression in basophils in vivo implicate that IgE-related mechanisms may be involved in upregulation of NK1R expression. Understanding SP-NK1R-related mechanisms will add novel content for discovery of roles of basophils in allergy.

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