

Basophil Membrane Expression of Epithelial Cytokine Receptors in Patients with Severe Asthma

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Keywords

Basophils · Basophil activation test · Epithelial cytokine receptors · Asthma · Severe asthma

Abstract

Background: Severe asthma is a heterogeneous disease, which is characterized by airway damage and remodeling. All triggers of asthma, such as allergens, bacteria, viruses, and pollutants, interact with the airway epithelial cells, which drive the airway inflammatory response through the release of cytokines, particularly IL-25, IL-33, and thymic stromal lymphopoietin (TSLP). **Objectives and Methods:** To investigate whether the expression of the IL-25, IL-33, and TSLP receptors on the basophil membrane are associated with asthma severity. Twenty-six patients with asthma (11 severe and 15 moderate/mild) and 10 healthy subjects (controls) were enrolled in the study. The results of the basophil activation test and flow cytometry analysis were assessed to

investigate basophil membrane expression of IL-25, TSLP, and IL-33 receptors before and after IgE stimulation. **Results:** IL-25 and IL-33 receptor expression on the basophil membrane at baseline were significantly higher in patients with severe asthma than in those with mild/moderate asthma or healthy subjects, independent of atopy, eosinophilia, asthma control, and exacerbation frequency. Following IgE stimulation, a significantly higher increase in the IL-25 and IL-33 receptors was observed in mild/moderate versus severe asthma. **Conclusions:** The high expression of the IL-25 and IL-33 receptors on the basophil membrane of patients with severe asthma indicates an overstimulation of basophils by these cytokines in severe asthma. This finding can possibly be used as a biomarker of asthma severity.

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Introduction

Asthma is recognized as one of the most common chronic diseases in adults and is a considerable health problem with increasing global prevalence [1]. Although the majority of asthma patients can be effectively treated with currently available medications, a substantial subset exists that remain difficult to treat. Overall, it is estimated that patients with severe asthma represent 5–10% of the entire asthma population. This small proportion of patients is accountable for a substantially higher use of healthcare resources than other asthmatic patients [2]. According to the International European Respiratory Society (ERS)/American Thoracic Society (ATS) Task Force, severe asthma is defined as asthma that requires treatment with high-dose inhaled corticosteroids (CS) plus a second controller and/or systemic CS to prevent it from becoming “uncontrolled,” or asthma that remains “uncontrolled” despite this therapy [3]. This definition does not contain any biological characteristic; it is disappointing that there is no clear definition distinguishing severe asthma from asthma in general. Severe asthma is not a homogeneous disease, and several studies have attempted to define its phenotypic biomarkers, based on triggers (allergens, exercise, infections, or aspirin), clinical presentation, or inflammatory markers (eosinophils or neutrophils) [4]. Indeed, airway epithelial damage and the consequent aberrant repair known as airway remodeling [5] appear to be the final pathway leading to severe asthma.

All triggers of asthma interact with the airway epithelial cells, which are now considered to play a pivotal role in driving the airway inflammatory response (i.e., T2 low versus T2 high) through the secretion of cytokines, chemokines, growth factors, and antimicrobial peptides, and also the recruitment of leukocytes [6]. The release of these epithelium-derived substances may be mirrored by the upregulation of their specific receptors on the membranes of cells which interact with airway epithelial cells, such as mast cells, smooth-muscle cells, fibroblasts, and basophils.

It is certainly easier to study the expression of cytokine receptors on the membrane of basophils, i.e., circulating cells of peripheral blood. Basophils have an important role in the development of airway inflammation, commonly found in bronchial biopsies of asthmatic patients where their number has been reported to be significantly higher than in healthy subjects [7]. Experimental data show that IL-25, IL-33, and TSLP upregulate the expression of their receptors on the basophil membrane [8, 9].

Our hypothesis is that the expression of epithelial cytokine receptors on the basophil membrane may increase in patients with severe asthma which is characterized by the chronic damage and repair of bronchial epithelium [10, 11]. To test this hypothesis, we analyzed the spontaneous and stimulated expression of the IL-25, TSLP, and IL-33 receptors on basophils from patients with severe asthma compared to patients with mild-to-moderate asthma.

Materials and Methods

Patients

Twenty-six consecutive patients attending the Outpatient Asthma Clinic for at least 1 year, with a confirmed diagnosis of asthma based on their symptoms and a well-documented improvement in FEV₁ of >12% and 200 mL after 400 µg inhaled salbutamol. Spirometry, exhaled nitric oxide (FeNO) measurement and blood samples for the basophil activation test (BAT) were obtained from all patients who gave their informed consent to participate in the study which was approved by the Institutional Review Board.

Spirometry was carried out using a Biomedin instrument (Padua, Italy) according to ATS criteria [12]. FeNO was measured by using a portable electrochemistry-based sensor (NIOX MINO; Aerocrine AB, Solna, Sweden) according to the recommendation of the ATS/ERS [13], and the results were presented as parts per billion (ppb). Briefly, the patients were asked to exhale, inhale through the device, and then exhale steadily for 10 s at a flow rate of 50 mL/s and at a pressure of 10 cm H₂O. The flow rate and pressure were automatically controlled, and any incorrectly performed test was automatically rejected by the device. The measurements were taken with the participants in a sitting position, in the morning before lung function tests and at least 1 h after their breakfast.

Severe asthma was defined according to the ERS/ATS [3] as asthma requiring treatment with guideline-suggested medications for GINA (steps 4–5, i.e., high-dose inhaled CS, long-acting β_2 -adrenergic receptor agonists, or leukotriene modifier/theophylline) for the past year or systemic CS for 50% of the past year to prevent the asthma from becoming uncontrolled, or asthma which remained uncontrolled despite this therapy.

Mild-to-moderate asthma was defined as asthma requiring treatment with guideline-suggested medications for GINA steps 2 and 3 [14].

Uncontrolled asthma was defined as at least 1 of the following: poor symptom control, as evaluated by the asthma control test, i.e., <20, frequent severe exacerbations (≥ 2 courses of systemic CS in the past year), serious exacerbations which required hospitalization, and airflow limitation (FEV₁ <80% predicted after appropriate bronchodilator withhold). We used a cut-off point of a blood eosinophil count of $>350 \times 10^6/L$ to define eosinophilic asthma patients. We defined frequent exacerbators as those patients who had ≥ 2 documented asthma exacerbation in the past year. Skin prick tests (ALK Abelló, Hørsholm, Denmark) for a panel of 14 inhalant standard allergens were performed in all patients. Atopy was defined as a wheal diameter of ≥ 3 mm in the presence of expected results in control solutions (histamine dihydrochloride 20

Table 1. Clinical and demographic characteristics of the patients

	Mild/moderate Asthma	Severe asthma	<i>p</i> value
Number of patients	15	11	
Median age (range), years	50.9 (13–78)	57.45 (40–77)	0.335
Males	4 (26.7)	7 (63.6)	0.0594
Atopy	11 (73.3)	3 (27.3)	0.0199
Early-onset asthma	8 (53.3)	0 (0)	0.0036
Eosinophilic asthma	8 (53.3)	5 (45.5)	0.691
Frequent exacerbators	6 (40.0)	8 (72.7)	0.0981
Rhinitis	6 (40)	4 (36.4)	0.851
CRS with nasal polyps	1 (6.7)	2 (18.2)	0.364
CRS without nasal polyps	8 (53.3)	5 (45.5)	0.691
FeNO >35 ppb	7 (46.7)	6 (54.5)	0.690
FeNO	36.20±8.06	45.09±8.22	0.457
Eosinophil count	431.67±87.59	436.36±109.79	0.973
Total IgE	483.17±275.37	219.10±41.61	0.397
FEV ₁ % predicted	83.87±4.96	74.18±4.57	0.164
MEF 25–75	49.18±7.62	37.36±7.03	0.268

Values are expressed as *n* (%) or mean ± SEM, unless otherwise indicated. CRS, chronic rhinosinusitis.

mg/mL as a positive control and solvent as a negative control). Ten healthy subjects, matched to the cases according to age and sex, were enrolled in the study as healthy controls.

Basophil Activation Test

The BAT was performed according to a previously reported technique [15]. Briefly, endotoxin-free, heparinized whole-blood samples were obtained from asthmatic patients. Cells were challenged with 100 µL of anti-IgE (10 µg/mL; clone G7–18 BD Bioscience, USA) for 20 min at 37°C in a warm bath (the optimal stimulation time was assessed in previous experiments). The optimal stimulation concentration of the anti-IgE was established in preliminary experiments. As a negative control, Tyrode buffer (Sigma Aldrich, Italy) with 20 µM HEPES and 7.5% NaHCO₃ (pH 7.4) was used to assess the spontaneous expression of the different markers. The reactions were terminated by chilling the cells on ice.

Immunophenotyping and Flow Cytometric Analysis

Basophils were stained with anti-human FcεRIα-Pacific Blue (BioLegend, San Diego, CA, USA), anti-human CD63-FITC (clone H5C6, BD Biosciences), anti-human CD203c-APC (clone NP4D6, BioLegend), anti-human TSLP R-PE (BioLegend), anti-human IL-33 R-PE (MBL, Leuven, Belgium), and anti-human IL-25 R-APC (R&D Systems, Minneapolis, MN, USA) for 20 min on ice. The flow cytometric analysis was performed on a Navios Beckman Coulter cytometer. Side-scatter and anti-IgE+/CD203c+ staining were applied to gate out at least 500 basophils. TSLP, IL-33, and IL-25 receptor positivity was set on basophils with a fluorescence intensity >99th percentile of a fluorescence-minus-one sample.

Statistics

Statistical analysis was performed by using GraphPad Prism v4.0c. Data are given as means ± SEM, and the increase of expression is reported in terms of percentage of baseline value: [(post-

stimulation – prestimulation)/prestimulation) × 100. Comparisons of paired data were carried out by using the Student *t* test for paired data. In all the other calculations, an unpaired *t* test or the Mann-Whitney U test was used. Correlation was evaluated by calculating the Pearson correlation coefficient. A *p* value <0.05 was considered significant.

Results

Clinical and Demographic Data

Clinical and demographic characteristics of the patients are reported in Table 1.

Of 26 patients, 11 were classified as having severe asthma and 15 as having mild/moderate asthma. There were no difference in age, gender, FeNO values, the frequency of exacerbations, rhinosinusitis comorbidity, and eosinophils blood count between patients with severe asthma and those with mild-to-moderate asthma. All patients with severe asthma reported a late onset compared to 47.7% of those with mild/moderate asthma (*p* = 0.0036), while atopy was more frequent (73.3%) in patients with mild/moderate asthma than in those with severe asthma (*p* = 0.01).

Immunophenotyping by BAT: Basal and Stimulated Basophils

At baseline, the expression of the IL-25 and IL-33 receptors on the basophil membrane was significantly higher in patients with severe asthma than in patients with

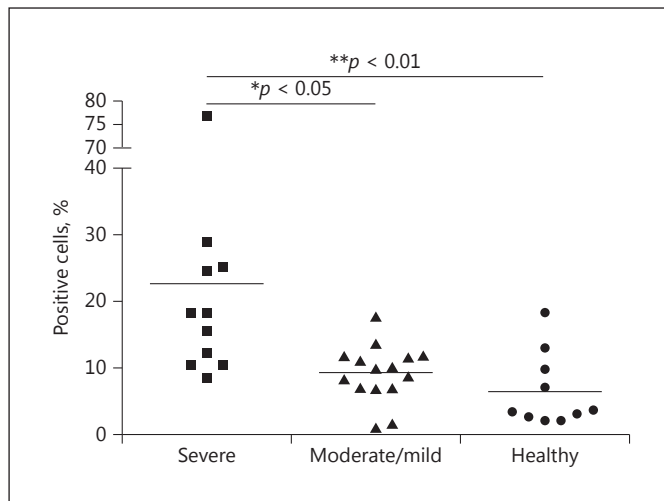


Fig. 1. Percentage of positive basophils expressing IL-25 receptor in severe asthmatic patients compared to patients with mild/moderate asthma and healthy controls.

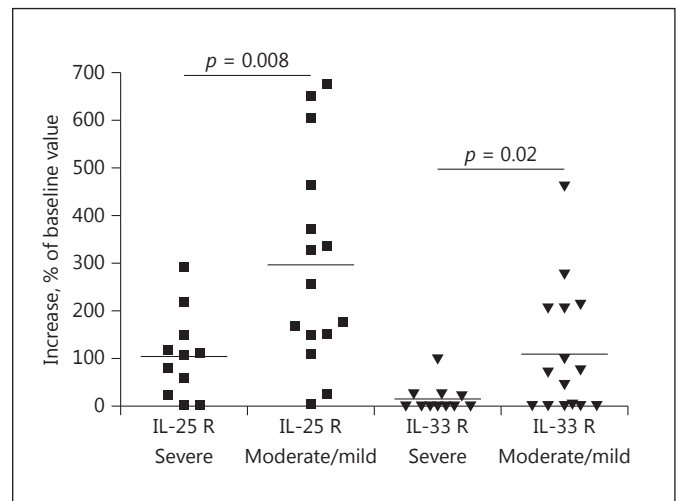


Fig. 3. IL-25 and IL-33 receptor increase (% of baseline value) after IgE stimulation in patients with severe and mild/moderate asthma.

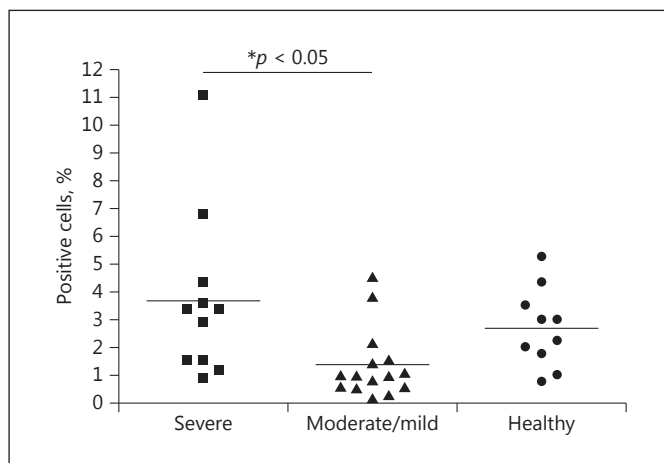


Fig. 2. Percentage of positive basophils expressing IL-33 receptor in severe asthmatic patients compared to patients with mild/moderate asthma and to healthy controls.

mild/moderate asthma and healthy controls (IL-25 receptor: 22.6 ± 5.7 vs. 9.2 ± 1.1 , $p < 0.05$; IL-33 receptor: 3.7 ± 0.8 vs. 1.3 ± 0.3 , $p < 0.05$) (Fig. 1, 2). No significant difference was observed in the baseline expression of TSLP receptor on the basophil membranes of asthmatic patients (severe and mild/moderate) or healthy controls.

Following IgE stimulation, IL-25 and IL-33 receptor expression, but not TSLP receptor expression, increased significantly in terms of percentage increase compared to at baseline, the increase being greater in mild/moderate

asthma than in severe asthma (297.5 ± 56.4 vs. $104.8 \pm 27\%$ of baseline for IL-25 receptor, $p = 0.008$; 110.2 ± 35 vs. $15.3 \pm 8.9\%$ of baseline for IL-33 receptor, $p = 0.02$) (Fig. 3). No significant difference was observed in baseline expression of the IL-25, IL-33, and TSLP receptors in controlled versus uncontrolled, atopic versus nonatopic patients, frequent versus nonfrequent exacerbators, and eosinophilic versus noneosinophilic asthma. Neither IL-25 nor IL-33 receptor expression correlated with spirometric parameters (FEV_1/VC , MEF_{50} , MEF_{25-75}), blood eosinophil count, FeNO, or total IgE.

Discussion

Our results show that baseline expression of the IL-25 and IL-33 receptors (but not the TSLP receptor) on the basophil membrane is significantly higher in patients with severe asthma than in patients with mild-to-moderate asthma. Interestingly, only the severity of asthma was associated with a higher expression of these receptors on the basophil membrane. In fact, the expression of these receptors was independent of asthma control, atopy, exacerbations, or eosinophilia.

Asthma severity is defined by the intensity of therapy needed to attain/maintain the control of disease [3], indicating a relative refractoriness to CS. Our results suggest that the circulating basophils of patients with severe asthma may be overstimulated by higher levels of airway epithelial cytokines, particularly IL-25 and IL-33, when

compared to patients with mild/moderate asthma and healthy controls, because the expression of the receptors of these cytokines is positively regulated by the levels of the cytokines themselves. It has been shown that allergen provocation is able to increase the expression of IL-25 and its receptor in the asthmatic bronchial mucosa [16] and also the expression of the TSLP receptor on the basophil membrane [9]. Salter et al. [8] investigated the effects of the alarmin cytokines IL-25 and IL-33 on basophils in a model of allergic asthma, and found that they increase basophil activation and migratory potential. In our patients, the overexpression of IL-25 and IL-33 on basophils was independent of atopy, which suggests causes other than allergy. Different stimuli, apart from allergen challenge, such as bacterial, viral, or fungal infections, can trigger the release of IL-25 and IL-33 which, in turn, may upregulate the expression of their receptors [17]. Both IL-25 and IL-33 cytokines have been related to airway changes which could be associated with the severity of asthma. Seys et al. [18] analyzed the mRNA expression of sputum cells from patients with asthma characterized by poor asthma control and refractoriness; they found that IL-25, together with IL-5 and IL-17A, is associated with asthma endotypes refractory to CS. Cellular targets of IL-25 are not only basophils and other cells of innate immunity, e.g., type 2 innate lymphoid cells, mast cells, macrophages, and dendritic cells, but also structural cells like epithelial cells, endothelial cells, smooth-muscle cells, and fibroblasts [19]. IL-25 also induces airway remodeling by acting directly on lung structural cells or indirectly on other immune cells [20].

Ultimately, the crosstalk between immune and structural cells creates a chronic inflammatory environment which might be the major cause of the airway changes commonly observed in patients with severe asthma. Several studies have shown that IL-33 is expressed more abun-

dantly in asthma patients than in healthy individuals [21–23]. Additionally, immunohistochemistry studies have reported elevated IL-33 expression in the bronchial epithelial cells of asthma patients versus healthy individuals [24]. The role of IL-33 in mast cell activation and airway smooth-muscle wound repair has also been reported [25], suggesting that IL-33 could be implemented for modulating mast cell-airway smooth muscle crosstalk in asthma.

In this study, following in vitro IgE stimulation, the increase of the expression of the IL-25 and IL-33 receptors was less pronounced in patients with severe asthma than in patients with mild/moderate asthma. This was likely due to the expression of these receptors already being near-maximal at baseline in the basophils of the patients with severe asthma. A limitation of our observation is the small sample size of our study population.

In conclusion, an overexpression of IL-25 and IL-33 receptors on basophil membranes may be a biomarker of asthma severity, independent of other clinical characteristics of asthma, such as atopy, eosinophilia, or control. In the incoming era of precision medicine, finding biomarkers is crucial for tailoring the therapy to an individual patient's characteristics. Due to their actions upstream of the T2 cytokine cascade and the production of IgE, IL-25 and IL-33 might be also a potential target for new therapeutic strategies for asthma.

Disclosure Statement

All the authors have no conflicts of interest to declare.

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