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Research Article

## Basophils Membrane Expression of Epithelial Cytokines Receptors in Eosinophilic and Non Eosinophilic Asthma

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### Abstract

Basophils are effector cells most often associated with airway inflammation in patients with asthma and can have an important role in the initiation of Th2 inflammation, as these cells may be activated directly by tissue cytokines (IL-25, IL-33 and TSLP) released by epithelial cells in response to damage signals from allergens, bacteria, virus or pollutants.

We investigated whether IL-25, IL-33 and TSLP receptors expression levels on basophil membrane are associated with asthma phenotype.

24 patients with asthma (12 eosinophilic, 12 non eosinophilic) have been enrolled in the study. Basophils membrane expression of IL25 R, TSLP R and IL33 R was analyzed before and after IgE, fMLP, LPS, LTA-SA (Lipoteicoic Acid from *S. Aureus*) stimulation and correlated with FEV1 of each patient.

Following LPS stimulation a significant increase in IL25 R was observed in eosinophilic ( $p=0.001$ ) but not in non eosinophilic asthma. IgE stimulation significantly increased IL25 R expression on basophils membrane, in both eosinophilic and non eosinophilic asthma ( $p= 0.001$  and  $p= 0.001$ , respectively), the percentual increase being greater in eosinophilic compared to non-eosinophilic asthma ( $p=0.007$ ).

IL33 R and TSLP R expression on unstimulated basophils were inversely correlated with FEV1 in eosinophilic asthma ( $r= -0.45$ ,  $p= 0.04$  and  $r= -0.70$ ,  $p= 0.01$ , respectively).

In conclusion, in eosinophilic asthma, airway obstruction is inversely correlated to the membrane expression

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of TSLP and IL33 receptors on basophils. Whether the expression of epithelial cytokines on basophils membrane may be used as biomarker of severe eosinophilic asthma remains to be investigated.

*Keywords:* Basophils; Epithelial Cytokine Receptors; Asthma; FEV1

## 1. Introduction

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 (Schwartz et al.,2015). Basophils are not only effector cells, which release vasoactive amines and lipid metabolites through IgE and chemokine stimulation, but they play a role in the initiation and amplification of Th2 inflammation, as these cells may be activated directly by cytokines, such as IL25, IL33 and Thymic Stromal Lymphopoietin(TSLP), released by the airway epithelial cells in response to damage signals from allergens, bacteria, virus or pollutants (Licona-Limón et al.,2013). Thus, basophils are involved in the so-called 'Th2-high' expression profile of the specific phenotype of asthma which is associated with high levels of circulating IgE, atopy, and eosinophilia(Bhakta and Woodruff, 2011).

Our hypothesis is that basophils membrane expression of epithelial cytokines receptors could be increased in the eosinophilic phenotype of asthma. To test this hypothesis we used flow cytometry to compare spontaneous and stimulated expression of IL25, TSLP and IL33 receptors on basophils from patients with eosinophilic and non eosinophilic asthma, compared to normal controls.

## 2. Materials and Methods

### 2.1. Patients

Twenty-four consecutive patients in charge to Outpatient Asthma Clinic since at least 1 year, who had received a clinical diagnosis of asthma and well documented improvement in FEV1 of more than 12% and 200 mL after 200 µg inhaled salbutamol or a provocative concentration of inhaled methacholine needed to reduce FEV1 by 20% (PC20) of 8 mg/mL or less in the 12 months before study entry were admitted to the study. Twelve patients (50%) were selected based on an asthma-related peripheral blood eosinophil count of  $0.3 \times 10^9$  per L or more at study entry or in the previous 6 months. Sample size was calculated assuming a power of 0.8.

Spirometry, exhaled nitric oxide measurement and blood sample for basophil activation test were obtained in all the patients, who gave their informed consent to participate to the study, which was approved by the Institutional Review Board.

Spirometry was carried out by using Biomedin Instrument (Padua, Italy) in accordance with the American Thoracic Society criteria (ATS/ERS Task Force, 2005). Exhaled nitric oxide (FENO) was measured by using a portable electrochemistry-based sensor (NIOX MINO; Aerocrine AB, Solna, Sweden) according to the recommendation of American Thoracic Society/European Respiratory Society (ATS/ERS Recommendations, 2005) and the results were presented as parts per billion (ppb). Briefly, the patients were asked to exhale, then inhale through the device, and exhale steadily for 10 seconds at a flow rate of 50 mL/s and at a pressure of 10 cm H<sub>2</sub>O. The flow rate and pressure

were automatically controlled, and any incorrect performance was automatically rejected by the device. The measurements were taken with the participants in a sitting position in the morning, before the lung function tests and at least 1 hour after their breakfast.

## 2.2. Basophil activation test (BAT)

BAT was performed according to previously reported technique (Hoffmann et al., 2015). Briefly, endotoxin-free heparinized whole-blood samples were obtained from asthmatic patients. Cells were challenged with 100  $\mu$ l of anti-IgE (10  $\mu$ g/mL; clone G7-18 BD Bioscience, USA), fMLP (0.5  $\mu$ g/ml, Sigma Aldrich, Italy), or TLR ligands (lipoteichoic acid from *Staphylococcus aureus* (LTA-SA) 1  $\mu$ g/ml, InvivoGen, USA, and lipopolysaccharide from *Escherichia Coli* (LPS) 1  $\mu$ g/ml, Sigma Aldrich, Italy) for 20 minutes at 37°C in a warm bath (optimal stimulation time were assessed in previous experiments). The optimal stimulation concentrations of the anti-IgE, fMLP and TLRs ligands were established in preliminary experiments. As a negative control, Tyrode buffer (Sigma Aldrich, Italy) with 20 $\mu$ M HEPES and 7.5% NaHCO<sub>3</sub>, pH 7.4 was used to assess the spontaneous expression of the different markers. The reactions were terminated by chilling the cells on ice.

## 2.3. Immunophenotyping and flow cytometric analyses

Basophils were stained with anti-human Fc $\epsilon$ RI $\alpha$ -Pacific Blue (BioLegend, San Diego, CA), anti-human CD63-FITC (clone H5C6, BD Biosciences), anti-human CD203c-APC (clone NP4D6 Biolegend, San Diego, CA), anti-human TSLP R-PE (Biolegend, San Diego, CA), anti-human IL33 R-PE (MBL, Leuven, Belgium), and anti-human IL25 R-APC (R&D Systems, Minneapolis, USA) for 20 minutes on ice. The flow cytometric analyses 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 R, IL33 R and IL25 R positivity was set on basophils with a fluorescence intensity that was above the level of the 99th percentile of a fluorescence-minus-one (FMO) sample.

## 2.4. Statistics

Statistical analysis was performed by using GraphPad Prism 4.0c, GraphPad Software, Inc, CA, USA. Data are given as means  $\pm$  S.E.M. and the increase of expression is reported in terms of percent of baseline [(post stimulation- pre stimulation)/pre stimulation]\*100. Comparisons of paired data were carried out by using the t-test for paired data. In all the other calculations, an unpaired t test or the Mann-Whitney test was used. Correlation was evaluated by calculating Pearson correlation coefficient. A P values < 0.5 was considered significant.

# 3. Results

Basophils membrane expression of IL25, TSLP and IL33 receptors was not significantly different in asthmatic and controls (data not shown).

Clinical and demographic characteristics of the patients are reported in Table 1. Patients with eosinophilic asthma were significantly younger than patients with non eosinophilic asthma (42.58 $\pm$ 6.38 vs 63.17 $\pm$ 2.84 years, p= 0.007 ). Most patients with eosinophilic asthma were atopic

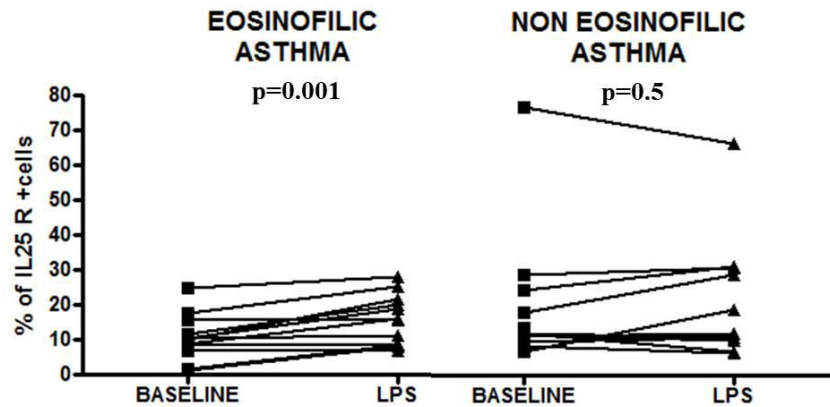
(9/12) and had FENO values >35 ppb (9/12) compared to patients with non eosinophilic asthma (3/12, p=0.014).

**Table 1** Clinical and demographic characteristics of the patients

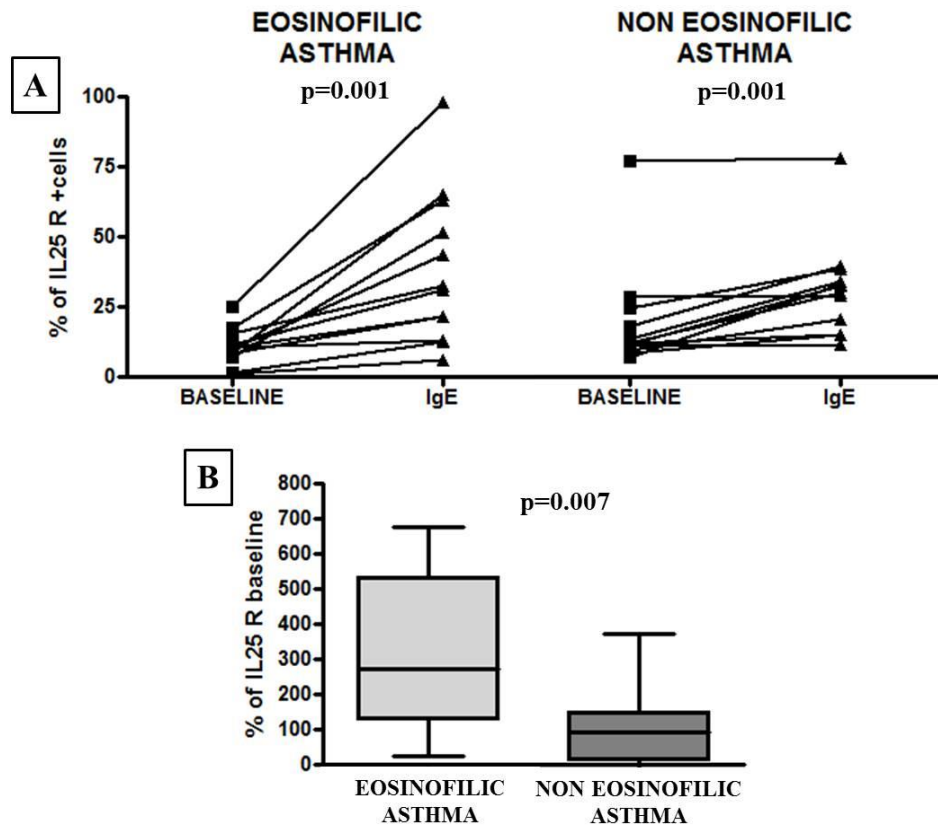
	<b>EOSINOPHILIC ASTHMA</b>	<b>NON EOSINOPHILIC</b>	<b>P VALUE</b>
<b>Number of patients</b>	<b>12</b>	<b>12</b>	
Age median, years (range)	42.58 ± 6.38	63.17 ± 2.84	<b>0.007</b>
Male, n (%)	7 (58.3)	5 (41.7)	0.414
Atopy, n(%)	9 (75.0)	3 (25.0)	<b>0.014</b>
Early onset asthma, n (%)	5 (41.7)	2 (16.7)	0.177
Asthma classification, n (%)			
Mild	4 (33.3)	2 (16.7)	0.345
Moderate	5 (41.7)	4 (33.3)	0.673
Severe	3 (25.0)	6 (50.0)	0.51
Frequent exacerbators, n (%)	6 (50.0)	7 (58.3)	0.205
Rhinitis, n (%)	4 (33.3)	4 (33.3)	1
CRSNP, n (%)	1 (8.3)	2 (16.7)	0.537
CRSWNP, n (%)	7 (58.3)	6 (50.0)	0.681
CTS therapy	3 (25.0)	6 (50.0)	0.205
FeNO>35 ppb	9 (75.0)	3 (25.0)	<b>0.014</b>
FeNO (mean ± SEM)	51.33 ± 8.89	31.61 ± 7.27	0.093
Eosinophils count (mean ± SEM)	733.2 ± 95.9	190.8 ± 27.9	<b>&lt;0.0001</b>

Before stimulation, no significant difference in receptors expression was observed between patients with eosinophilic and non eosinophilic asthma (IL33 R: 2.30 ± 0.54 vs 1.30 ± 0.38, p=0.08; TSLP R: 6.34 ± 0.96 vs 4.98 ± 1.20, p=0.07; IL25 R: 10.63 ± 1.89 vs 19.55 ± 5.55, p=0.09).

Following LPS stimulation a significant increase in IL25 R was observed in eosinophilic asthma (from 10.63 ± 1.89 to 15.74±2 % of IL25 R +cells, p=0.001) but not in non eosinophilic asthma (see Fig. 1).



**Fig. 1.** Percentage of IL25 R positive basophils before and after LPS stimulation in eosinophilic and non eosinophilic asthma.



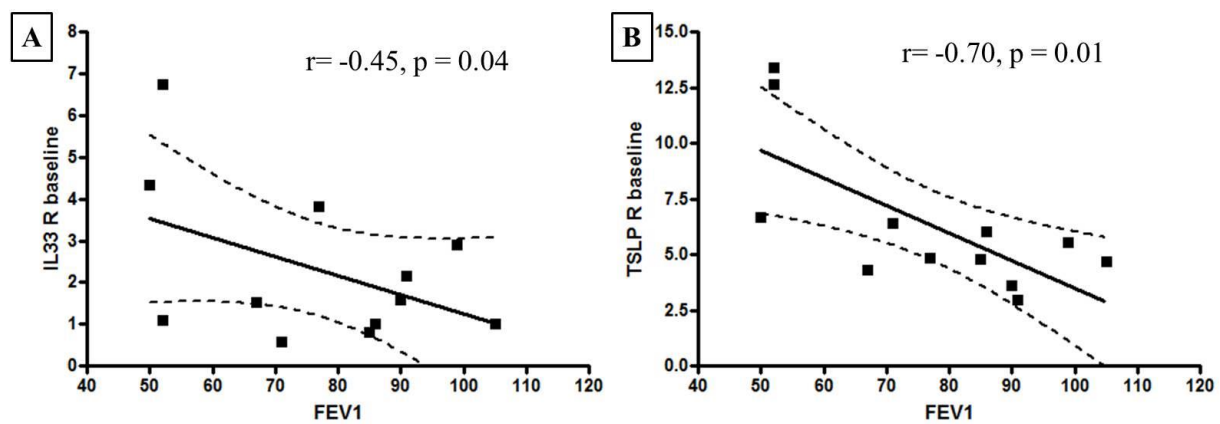
**Fig. 2.** Panel A) Percentage of IL25 R positive basophils before and after IgE stimulation in eosinophilic and non eosinophilic asthma patients. Panel B) IL25 R increase (in percent of baseline) in eosinophilic and non eosinophilic asthma patients.

IgE stimulation significantly increased IL25 R expression on basophils membrane, in both eosinophilic and non eosinophilic asthma (eosinophilic asthma: from  $10.63 \pm 1.89$  to  $38.29 \pm 7.23$ ,

$p = 0.001$ ; non eosinophilic asthma: from  $19.55 \pm 5.55$  to  $31.48 \pm 5.03$ ,  $p = 0.001$ ), the percentual increase being greater in eosinophilic compared to non-eosinophilic asthma ( $319.01 \pm 65.83$  vs  $103.43 \pm 30.31$  % of baseline,  $p = 0.007$ ; Fig. 2, panel A and B).

fMLP and LTA-SA stimulation did not change the expression of IL33, TSLP and IL25 receptors on basophils membrane.

IL33 R and TSLP R expression on unstimulated basophils were inversely correlated with FEV1 in eosinophilic asthma ( $r = -0.45$ ,  $p = 0.04$  and  $r = -0.70$ ,  $p = 0.01$ , respectively, see Fig. 3, panel A and B).



**Fig. 3.** Panel A) Correlation between baseline IL33 R and FEV1 in patients with eosinophilic asthma. Panel B) Correlation between baseline TSLP R and FEV1 in patients with eosinophilic asthma.

## 4. Discussion

Baseline expression of the three epithelial cytokines receptors on basophils membrane was not different between healthy controls and asthmatic patients, either with eosinophilic or non eosinophilic asthma.

Nevertheless, an inverse relationship between FEV1 and basophils membrane expression of TSLP and IL 33 receptors was observed in eosinophilic asthma. This finding suggests that the expression of TSLP and IL33 receptors on membrane of peripheral basophils mirrors the interaction between airway epithelial cells and basophils, which play a role in the pathogenesis of Th2 chronic airway inflammation underlying airways obstruction of eosinophilic asthma. Basophils are well known to be involved not only in the early asthmatic response, with release of histamine and cysteinyl leukotrienes, which contribute to bronchoconstriction, but also in chronic airway inflammation.

Both TSLP and IL33 interact with innate immunity cells, such as basophils, mast cells and innate lymphocytes type 2 (ILC2) promoting the production and release of Th2 cytokines by these cells (Salter et al., 2016).

The mechanisms of basophils membrane expression of epithelial cytokines receptors are not completely known. It has been shown that TSLP released by airway epithelial cells may upregulate epithelial cytokines receptors on basophils membrane (Wang et al., 2009). Moreover, allergen provocation has been shown also to increase the expression of epithelial cytokine IL25 and its receptor in the asthmatic bronchial mucosa (Corrigan et al., 2011). Different stimuli such as bacterial, viral, fungal infections and allergen challenges can trigger the release of IL33, which in turn may upregulate the expression of its receptor, which has been shown to be increased in bronchial epithelial cells of asthmatic patients (Préfontaine et al., 2010).

As epithelial derived cytokines, IL25, IL33 and TSLP, are considered strategic in driving Th2 response through the release of classical Th2 cytokines such as IL4, IL5 and IL-13 by Th2 CD4+ cells (Soumenlis et al., 2002) as well by innate type 2 lymphocytes (Saenz et al., 2010) we expected to find higher expression of epithelial cytokines receptors on basophils membrane of eosinophilic compared to non eosinophilic asthma. Unexpectedly we did not find any difference in baseline basophil receptors expression between patients with eosinophilic and non eosinophilic asthma, at least in baseline conditions. Actually, following LPS stimulation IL25 R was more expressed on basophils membrane of eosinophilic than non eosinophilic asthma and, following IgE stimulation, IL25 R expression increased more, in percent of baseline, on basophils membrane of eosinophilic compared to non-eosinophilic asthma. The meaning of this observation is not clear, as the baseline membrane expression of IL25 R was lower in eosinophilic asthma than in non eosinophilic asthma.

In conclusion, in eosinophilic asthma, airway obstruction is inversely correlated to the membrane expression of TSLP and IL33 receptors on peripheral basophils. Whether the expression of epithelial cytokines on basophils membrane may be used as biomarker of severe eosinophilic asthma remains to be investigated.

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