



# Immunoglobulin E and G autoantibodies against eosinophil proteins in children and adults with asthma and healthy subjects

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## ABSTRACT

**Background:** Autoimmune IgG response has been described in the pathogenesis of asthma in adults, but IgE autoimmunity has been little explored. Considering high levels of blood eosinophils and immunoglobulin E in asthmatic patients, the possibility of IgE autoantibody response to eosinophil proteins arises.

**Objective:** To explore the presence of IgE and IgG autoantibodies against Eosinophil peroxidase (EPX) and Eosinophil cationic protein (ECP).

**Methods:** Three steps were followed: 1) The frequency of IgE and IgG autoantibodies against EPX and ECP was investigated among asthmatic and healthy subjects. 2) The ability of IgE autoantibodies to induce an inflammatory response (basophil activation) was performed. 3) The capacity of autoantibodies to identify patients with severe asthma was evaluated.

**Results:** Asthmatic and healthy subjects had IgE and IgG autoantibodies against EPX and ECP. Anti-EPX IgE was significantly higher in asthmatic patients. Severe asthmatic patients had a higher frequency and higher levels of IgE and IgG autoantibodies compared to healthy subjects. There was not a correlation between autoantibodies and blood eosinophils. Children younger than 14 years of age had IgE and IgG autoantibodies against EPX and ECP. IgE autoantibodies to EPX and ECP induced basophil activation in asthmatic patients.

**Conclusion:** In this study, we identify for the first time IgE autoantibodies against EPX and ECP in adults and children patients with asthma; IgE and IgG autoantibodies against EPX and ECP could serve as a predictive biomarker of the clinical severity.

**Keywords:** Asthma, Autoreactivity, Autoantibodies, Eosinophils, Immunoglobulin E

## INTRODUCTION

Allergic asthma is a common respiratory disease with a high impact on quality of life.<sup>1</sup> The clinical

definition of asthma covers different phenotypes and endotypes that are strongly related to age. Based on the characteristics of the inflammatory process, Th2 cytokines are the most prevalent in

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patients with asthma (60%–80%),<sup>2,3</sup> especially in childhood. In this type of inflammation, specific IgE against environmental allergens and recruitment of eosinophils are important mediators of lung symptoms.<sup>4,5</sup> In recent years, new mechanisms have been proposed; IgG reactivity to several autoantigens has been reported in adult patients with asthma,<sup>6,7</sup> opening the hypothesis of an autoimmune endotype. IgE autoreactivity against autoantigens and its role in inflammation has been demonstrated in different diseases<sup>8–12</sup> but little is known about asthma and its presence in childhood population.

Mediators of the inflammatory asthma process include basophils, neutrophils, and especially eosinophils.<sup>13,14</sup> Eosinophils through their proteins such as major basic protein (MBP), eosinophil peroxidase (EPX) and eosinophil cationic protein (ECP) are capable of generating an inflammatory response.<sup>15,16</sup> Recently, sputum IgG response against EPX was detected in asthma and it was associated with severe asthma and corticosteroids resistance.<sup>6</sup>

Considering the central role of specific IgE and eosinophil cytoplasmic granules in the pathogenesis of asthma, we hypothesized that EPX and ECP may be possibly recognized by IgE autoantibody, and this response could be associated with chronicity and severity of the disease. The aim of this study was to investigate the presence of IgE and IgG autoantibodies against eosinophil proteins EPX and ECP and to explore the possible relationship with asthma inflammation and age.

## METHODS

### Study design and population

A cross-sectional study was performed using 2 groups: asthmatic and healthy subjects. The asthma group included patients with at least 2 years of asthma recruited from 3 medical institutions in Medellín, Colombia. Control subjects were students and workers invited from educational institutions. Severity of asthma was defined according to GINA 2020 recommendations by treatment (<https://ginasthma.org/>); Mild asthma corresponds to a disease that can be controlled with step 1 or 2 treatment, moderate asthma was step 3 or 4 and severe asthma was step 5

treatment. Patients with clinical indication for the use of a biological therapy for asthma were included if they had not started such therapy. Subjects in both groups consisted of people between 5 and 55 years without a clinical history of autoimmune diseases, atopic dermatitis, chronic obstructive pulmonary disease, or any other chronic pulmonary disease. Smokers were excluded in both groups.

Participants with a diagnosis of systemic diseases (eg, mastocytosis, dermatitis), infection within 4 weeks of participation, or pharmacologic treatment (eg, Cyclosporine), that could affect the interpretation of the laboratory results were not included.

### Antigen's production

ECP and EPX were obtained as recombinant proteins according to a previous protocol<sup>10,17</sup> using *Escherichia coli* BL21 (DE3) as an expression vector.

### Determination of the total IgE, specific IgE and IgG autoantibodies

Total IgE levels in the serum samples were determined using a fluoroenzyme immunoassay (ImmunoCap System, Thermofisher, Sweden). When the levels were above the reading range of the equipment ( $>100 \text{ KU}_A/\text{mL}$ ), the sample was diluted 1:5 or 1:10 depending on the case, and the total concentration was calculated by conversion.

We explored the presence of IgE autoantibodies against EPX, and ECP by ELISA technique after purification to avoid contamination of vector proteins.<sup>10,17</sup> The sera used for the quantification of IgE were previously depleted of IgG by immunoaffinity depletion; For each 500  $\mu\text{L}$  of serum and 1X PBS (1:5) were added 80  $\mu\text{L}$  of Protein G (Sigma-P3296, Protein G Sepharose®). After 60 min of incubation at room temperature (RT) on a rotating platform, the serum was recovered by centrifugation and stored at  $-20^\circ\text{C}$  until use. Residual IgG contents in protein G-adsorbed sera were measured by enzyme-linked immunosorbent assay (ELISA) assays and sera were accepted for anti-EPX/ECP IgE measure when IgG were found to be less than  $10 \mu\text{g}/\text{mL}$ . The specificity of the binding was evaluated by inhibition tests.

For ELISA, 100  $\mu$ L was loaded into wells by duplicated coated with the relevant antigen, and the plates were incubated overnight. Each plate had control and asthma samples. A calibration curve was done (0.001, 0.01, 0.1, 1 u/ml) for each antigen and PBS as negative controls was used. Considering previous studies,<sup>18,19</sup> antigens were reduced with SDS. The cut-off value for serum anti-EPX IgE and anti-ECP IgE were defined as the mean plus three standard deviations of absorbance values from 60 healthy controls (IgE-EPX 0.354, IgE-ECP 0.279, IgG-EPX 0.425, IgG-ECP 0.414). The results were expressed in optical density units (OD). The absorbance at 405 nm was determined using a spectrophotometer.

For the determination of anti-TPO IgG, the same protocol was followed, except for the IgG depletion and that the human sera adsorbed with *E. coli* lysate were diluted 1:50 and the secondary antibody was alkaline phosphatase-conjugated anti-IgG (Pharmigen).

### CD203c expression

Peripheral blood basophils were obtained from subjects from each group. We used the CD203c expression protocol previously reported.<sup>10,17</sup> Briefly, basophil activation test (BAT) was performed with the whole blood collected in EDTA tubes and red blood cells were lysed with a lysis buffer. The 100  $\mu$ L of the leukocyte suspension were added along with calcium ionophore, anti-human goat IgE antibodies and anti-IgG4. Basophil activation was stimulated with different concentrations of EPX/ECP antigens (0.01, 0.1 and 1  $\mu$ g/mL). After washing with PBS, the cells were stained with phycoerythrin (PE)-conjugated antihuman CD203c and fluorescein isothiocyanate (FITC)-conjugated antihuman CD123. CD203c expression was measured by flow cytometry.

The percentage of CD203c expression was defined as the percentage of basophils expressing more CD203c than the critical point, which was  $\geq 10.0\%$  of the basophils incubated with buffer only, similar to previous studies.<sup>10,17,20</sup>

### Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 21.0 (IBM, Inc, Chicago, IL) and GraphPad Prism 8 (La Jolla, CA).

The Shapiro-Wilk test was used to check for normality, and our descriptive and statistical analyses were chosen according to the results. The data are presented as the means and standard deviation for variables with a normal distribution and as the median and range when not. The Mann-Whitney *U* test were used to compare specific IgE levels. Fisher's  $\chi^2$  test was used to evaluate the differences among groups and proportions. Correlations were assessed with the Pearson or Spearman coefficient (*r*).

Given the results of previous studies,<sup>21-24</sup> we considered that a sample of at least 30 patients with asthma and 40 healthy subjects would be adequate to ensure a power of 80% and an alpha error of 0.05 for the primary outcome (the presence of IgE autoantibodies).

## RESULTS

### Patients' characteristics

Sixty control subjects and 55 asthmatic patients were recruited (Table 1). More sociodemographic characteristics were similar in both groups. The asthmatic group had higher levels of total IgE (mean 181 vs 139 IU/mL) and blood eosinophils (mean 126 vs 106 cells/ml) but these 2 variables were not significantly different. Atopy was present in 70.9% of the asthmatic patients and 20% of control subjects ( $p < 0.001$ ). Nasal diseases (rhinitis, rhinosinusitis, polyposis), positive anti-nuclear antibodies, and family history of autoimmunity comorbidities were more frequent in asthmatic group, but only rhinitis was significant ( $p < 0.001$ ).

### IgE against eosinophil autoantigens

Anti-EPX IgE and anti-ECP IgE levels were significantly higher in the asthma group versus control group (Fig. 1). Eight asthmatic patients have anti-EPX IgE and 5 have anti-ECP IgE above the detection threshold (Fig. 1); Three of these patients have both anti-ECP IgE and anti-EPX IgE. Despite the higher number of positive anti-ECP/EPX IgE in the asthma group, only anti-EPX IgE was statistically significant in the asthma group versus control group (anti-ECP IgE 0.04). Patients with IgE autoantibodies against EPX or ECP have a lower FEV1 than asthmatic patients without IgE

General characteristics	Asthma (n = 55)	Control (n = 60)	P value
<b>Age Median, (Range, min-max)</b>	20 <sup>6-44,44-50</sup>	23 <sup>6-44,44-50</sup>	0.3
<b>Age ≤ 14 years (%)</b>	17 (30.9%)	15 (25%)	0.3
<b>Sex: male, n (%)</b>	30 (54.5%)	26 (43.3%)	0.2
<b>Atopy (%)</b>	39 (70.9%)	12 (20%)	<0.001
<b>Rhinitis (%)</b>	43 (78.2%)	11 (18.3%)	<0.001
<b>Sinusitis (%)</b>	6 (10.9%)	2 (3.3%)	0.1
<b>CRS with polyps (%)</b>	2 (3.6%)	0	0.2
<b>ANAs (%)</b>	4 (7.2%)	1 (1.6%)	0.1
<b>Family history of autoimmunity</b>	8 (14.4%)	4 (6.6%)	0.2
<b>Total IgE (IU/ml), mean ± SD</b>	181 (174)	139 (84)	0.1
<b>Eosinophil serum (cells/ml) mean ± SD</b>	126 (71)	107 <sup>44</sup>	0.08
<b>Asthma severity according GINA steps</b>	42 (76.3%)	N/A	N/A
<b>Moderate</b>	13 (23.6%)	N/A	N/A
<b>Severe</b>			
<b>Patients with OCS in the last year</b>	11 (20%)	N/A	N/A
<b>Years with Asthma median, (Range)</b>	3 <sup>8</sup>	N/A	N/A
<b>FEV1 (%) median, (Range)</b>	89 <sup>30</sup>	N/A	N/A
<b>FEV1/FVC ratio median, (Range)</b>	0,80 (0.5)	N/A	N/A

**Table 1.** Sociodemographic characteristics. All subjects were over 6 years old. N/A: Not applicable. SD: Standard deviation. FVC: Forced vital capacity, FEV1: Forced expiratory volume. Atopy was defined as the presence of specific IgE to an allergenic source (mites, pets, etc.). The patients in the control group were not evaluated for lung function because they did not have respiratory symptoms

autoantibodies (91.1% vs 78.3%,  $p = 0.02$ ) and lower FEV1/FVC (0.82 vs 0.74,  $p = 0.03$ ).

The age minimum and maximum (min-max) of the subjects with IgE autoantibodies was 6-33 years; in the asthma group; most of them had severe asthma ( $n = 6$ ) (Table 2). Nine asthmatic and 3 subjects in the control groups had IgE autoantibodies and IgG autoantibodies.

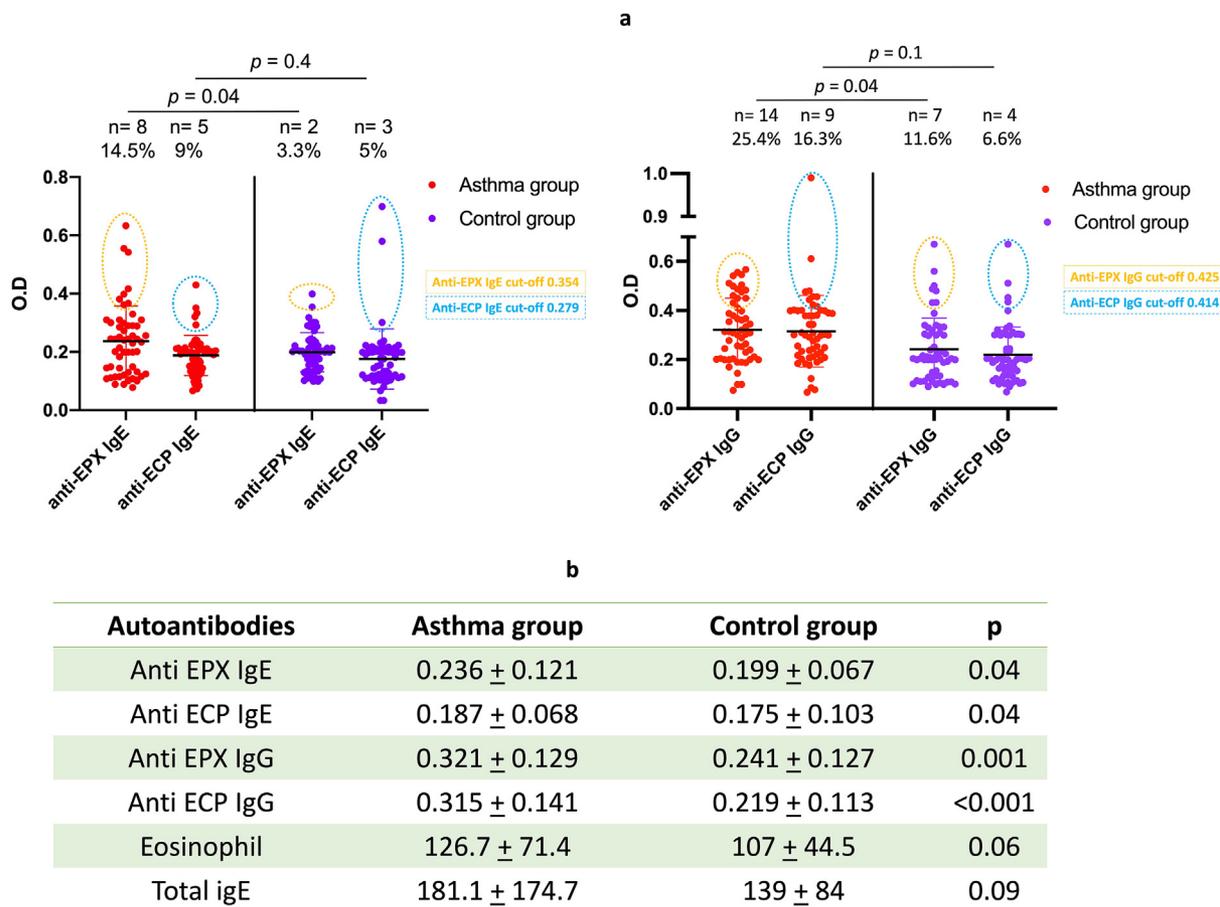
Asthma and control group showed a low but significant correlation among the total IgE levels and number of eosinophils in serum (Asthma group  $r = 0.480$ , CI 0.232 to 0.662,  $p = 0.002$ . Control group  $r = 0.360$ , CI = 0.109 to 0.568,  $p = 0.005$ ) (Fig. 3). There was not an association between autoantibody levels and blood eosinophils according to correlation or using different cut-off stratification (>150 cells/ml or >300 cells/ml eosinophils).

### IgG against eosinophil autoantigens

In the asthma and control group, anti-EPX IgG was the most prevalent autoantibody, the asthma group had a higher frequency of IgG autoantibodies than the control group (18 (32.7%) vs 10 (16.6%)  $p = 0.04$ ) (Table 3). The age minimum and maximum of the subjects with IgG antibodies was 7-50 years; 7 of them had severe asthma ( $n = 7$ ) (Table 3).

Asthmatic patients exhibited significantly higher levels of IgG autoantibodies than control subjects (Fig. 1). There was no correlation among autoantibodies levels (Fig. 2).

Patients with IgG autoantibodies against EPX or ECP have a lower FEV1 than asthmatic patients without IgG autoantibodies (91.1% vs 87.1%,  $p = 0.06$ ) and lower FEV1/FVW (0.82 vs 0.79,  $p = 0.1$ ) but it was not significant.



**Fig. 1 Asthma IgE and IgG against EPX and ECP.** In **a**, patients with IgE or IgG autoantibodies are presented in circles and were compared between groups. In **b**, concentration levels according to optical density units and eosinophil blood count are presented in mean and standard deviation and were compared between groups. Cut-off; IgE-EPX 0.354, IgE-ECP 0.279, IgG-EPX 0.425, IgG-ECP 0.414

### Basophil activation test (BAT)

We observed a similar concentration of basophils in total blood in the 2 groups, being slightly lower in the asthmatic group (28 cells/ul) compared to the control group (40 cells/ul), but without statistical differences.

For the BAT, basophils were obtained from different groups of subjects according to the presence (or not) of asthma, IgE, and IgG autoantibodies (Fig. 3). With the different dilutions of autoantigens used, CD203c expression from patients with asthma and IgE autoantibodies significantly increased in contrast to the other groups.

Basophils of control subjects with IgE autoantibodies have a CD203c expression over 10% when stimulated with the higher concentration of EPX or ECP (1 µg/mL), but it was significantly lower than the asthma group and without significant

difference with control subjects with negative IgE autoantibodies (Fig. 3). When analyzing all samples as a group, we observed a moderate correlation between basophil activation and the concentration of anti-EPX IgE ( $r\ 0.689\ p\ 0.03$ ) and anti-ECP ( $r\ 0.566\ p\ 0.04$ ).

### DISCUSSION

In this study, we described for the first time (i) IgE autoantibodies against eosinophil human proteins in children and adults; (ii) Basophil activation mediated by anti-EPX IgE and anti-ECP IgE; (iii) IgE and IgG autoantibodies against eosinophil proteins could be a biomarker of lung function in asthmatic patients.

In childhood, the eosinophilic endotype is frequent and different biomarkers in sputum or bronchopulmonary lavage have been proposed to detect this endotype of T2 response. However, less

Group	Age	Sex	Atopy	Rhinosinusitis	Severity	OSC	FEV1	FEV1/ FVC	Blood eosinophils (cell/mL)	Total IgE (IU/mL)	Anti- EPX IgE	Anti- ECP IgE	Anti- EPX IgG	Anti- ECP IgG
<b>Asthma</b>	16	Female	Yes	No	Severe	2	77	0.67	132	122	Yes	Yes	Yes	Yes
<b>Asthma</b>	25	Female	Yes	Yes	Moderate	0	80	0.81	221	300	Yes	Yes	Yes	Yes
<b>Asthma</b>	12	Male	Yes	Yes	Severe	1	95	0.89	140	50	Yes	No	Yes	No
<b>Asthma</b>	6	Female	Yes	No	Severe	2	55	0.6	92	223	Yes	No	No	No
<b>Asthma</b>	17	Male	No	No	Severe	2	70	0.61	128	111	Yes	Yes	Yes	No
<b>Asthma</b>	28	Female	Yes	Yes	Severe	1	75	0.7	314	1042	Yes	No	Yes	No
<b>Asthma</b>	17	Male	No	No	Severe	1	80	0.9	57	55	Yes	No	Yes	Yes
<b>Asthma</b>	24	Male	Yes	Yes	Moderate	0	98	0.85	315	888	Yes	No	Yes	Yes
<b>Asthma</b>	7	Female	Yes	No	Severe	2	77	0.7	205	333	No	Yes	No	Yes
<b>Asthma</b>	33	Male	Yes	No	Moderate	0	93	0.86	100	128	No	Yes	No	Yes
Control	10	Male	Yes	No	N/A	N/A	N/A	N/A	122	169	Yes	No	Yes	No
Control	30	Female	No	No	N/A	N/A	N/A	N/A	130	148	Yes	No	Yes	No
Control	20	Female	No	No	N/A	N/A	N/A	N/A	140	175	No	Yes	No	Yes
Control	11	Female	No	Yes	N/A	N/A	N/A	N/A	199	201	No	Yes	No	No
Control	23	Female	No	No	N/A	N/A	N/A	N/A	134	123	No	Yes	No	No

**Table 2.** Subjects with IgE autoantibodies. Asthma patients in control subjects with anti-EPX IgE or anti-ECP IgE. FVC: Forced vital capacity, FEV1: Forced expiratory volume. N/A: No apply. OSCs: Cycles of oral systemic corticosteroid

Group	Age	Sex	Atopy	Rhinosinusitis	Severity	OCS	FEV1	FEV1/ FVC	Blood eosinophils (cell/mL)	Total IgE	Anti- EPX IgE	Anti- ECP IgE	Anti- EPX IgG	Anti- ECP IgG
<b>Asthma</b>	16	Female	Yes	No	Severe	2	77,3	0,67	132	122	Yes	Yes	Yes	Yes
<b>Asthma</b>	25	Female	Yes	Yes	Moderate	0	80	0,81	221	300	Yes	Yes	Yes	Yes
<b>Asthma</b>	17	Male	No	No	Severe	2	70	0,61	128	111	Yes	Yes	Yes	No
<b>Asthma</b>	28	Female	Yes	Yes	Severe	1	75	0,7	314	1042	Yes	No	Yes	No
<b>Asthma</b>	17	Male	No	No	Severe	1	80	0,9	57	55	Yes	No	Yes	Yes
<b>Asthma</b>	24	Male	Yes	Yes	Moderate	0	98	0,85	315	888	Yes	No	Yes	Yes
<b>Asthma</b>	7	Female	Yes	No	Severe	2	77	0,7	205	333	No	Yes	No	Yes
<b>Asthma</b>	33	Male	Yes	No	Moderate	0	93	0,86	100	128	No	Yes	No	Yes
<b>Asthma</b>	12	Male	Yes	Yes	Severe	1	95	0,89	140	50	No	No	Yes	No
<b>Asthma</b>	18	Female	Yes	No	Moderate	0	102	0,78	156	156	No	No	Yes	No
<b>Asthma</b>	13	Male	Yes	No	Moderate	0	97	0,85	125	101	No	No	Yes	No
<b>Asthma</b>	14	Male	Yes	No	Moderate	0	89	0,85	126	101	No	No	Yes	No
<b>Asthma</b>	20	Female	Yes	No	Moderate	0	96	0,9	130	222	No	No	Yes	No
<b>Asthma</b>	35	Male	No	No	Moderate	0	93	0,71	135	132	No	No	Yes	No
<b>Asthma</b>	40	Male	Yes	No	Moderate	0	95	0,95	126	20	No	No	Yes	No
<b>Asthma</b>	50	Female	Yes	No	Severe	3	55	0,59	130	115	No	No	Yes	Yes
<b>Asthma</b>	45	Female	Yes	No	Moderate	0	101	0,8	120	113	No	No	No	Yes
<b>Asthma</b>	29	Female	No	No	Moderate	0	96	0,83	90	133	No	No	No	Yes
Control	10	Male	Yes	No	N/A	N/A	N/A	N/A	122	169	Yes	No	Yes	No
Control	30	Female	No	No	N/A	N/A	N/A	N/A	130	148	Yes	No	Yes	No

(continued)

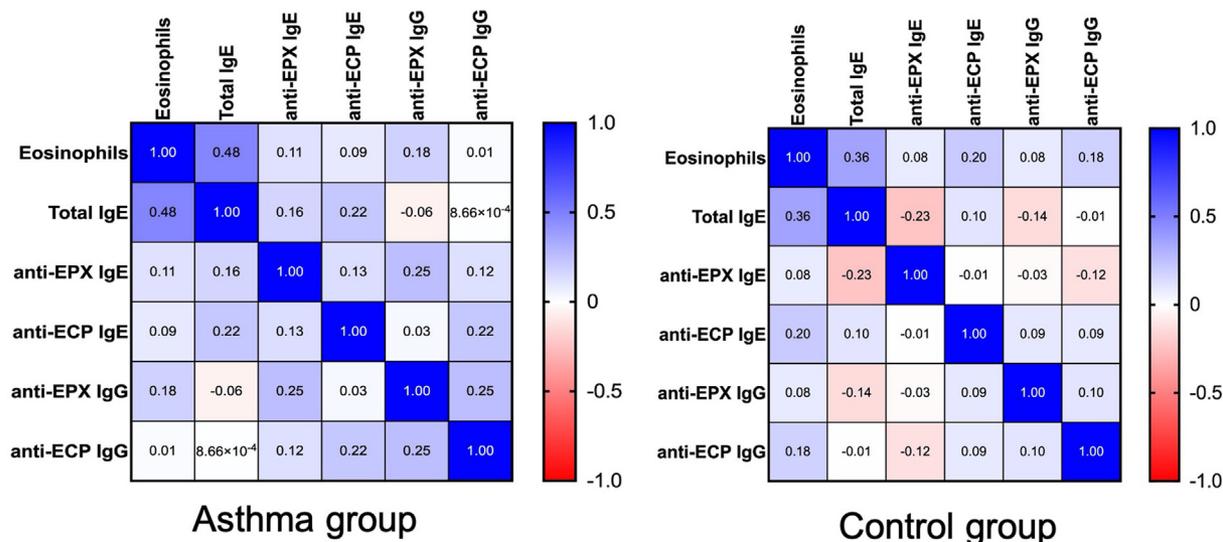
Group	Age	Sex	Atopy	Rhinosinusitis	Severity	OCS	FEV1	FEV1/ FVC	Blood eosinophils (cell/mL)	Total IgE	Anti- EPX IgE	Anti- ECP IgE	Anti- EPX IgG	Anti- ECP IgG
Control	20	Female	No	No	N/A	N/A	N/A	N/A	140	175	No	Yes	No	Yes
Control	24	Male	No	No	N/A	N/A	N/A	N/A	55	110	No	No	Yes	No
Control	33	Female	No	No	N/A	N/A	N/A	N/A	110	101	No	No	Yes	No
Control	41	Male	No	No	N/A	N/A	N/A	N/A	121	88	No	No	Yes	No
Control	46	Male	No	No	N/A	N/A	N/A	N/A	66	53	No	No	Yes	No
Control	30	Female	No	No	N/A	N/A	N/A	N/A	160	113	No	No	Yes	Yes
Control	33	Male	No	No	N/A	N/A	N/A	N/A	130	145	No	No	No	Yes
Control	34	Female	No	No	N/A	N/A	N/A	N/A	109	115	No	No	No	Yes

**Table 3. (Continued)** Subjects with IgG autoantibodies. Asthma patients in control subjects with anti-EPX IgG or anti-ECP IgG. FVC: Forced vital capacity, FEV1: Forced expiratory volume. N/A: No apply. OCS: Cycles of oral systemic corticosteroid

invasive methods have been used in children and blood eosinophils are the most common biomarker used to indicate the presence of predominantly eosinophilic asthma, but its specificity is low, as we could see in this study where there was no difference in the levels of blood eosinophils between the group with asthma and the control group.<sup>25,26</sup> The exploration of autoantibodies may be a starting point for new biomarker options of new biomarkers that allow improving the diagnosis, treatment selection and prognosis of patients. Of the 4 autoantibodies studied, anti-EPX IgE had the highest specificity for the group with asthma, which suggests that it could be useful for the diagnosis of the disease and could be signaling a new endotype with a T2 response but mediated by an autoimmune mechanism. The two IgE autoantibodies (anti-EPX/ECP IgE) were associated with lower lung function, which would indicate their usefulness as possible biomarkers of severity. In the case of IgG autoantibodies, although it was not statistically significant, similar to IgE autoantibodies, there was a tendency to be found more frequently in the group of patients with asthma and also to be present in patients with lower lung function, so that their usefulness as biomarkers cannot be ruled out.

Allergic and autoimmune response are part of a spectrum of immunological diseases and their involvement in the pathogenesis of asthma has been proposed based on presence of immunoglobulins against diverse environmental antigens and self-antigens.<sup>21</sup> However, the presence of antibodies or autoantibodies does not necessarily have a clinical impact or relevance in the pathogenesis of a disease. As have been observed with other allergens and auto-allergens, in both groups (asthma and healthy controls) the presence of IgE and IgG antibodies was observed. However, the median levels of these autoantibodies in the asthmatic group was higher than control group and basophil activation even in low concentrations was only present in asthmatic patients, suggesting that these autoantibodies are functional and inflammatory activity could participate in the pathogenesis of the disease.

Compared with other allergic and non-allergic diseases,<sup>11,27,28</sup> the role of IgE autoantibodies in asthma has not been studied in detail so far. In general, basophils in the control group presented



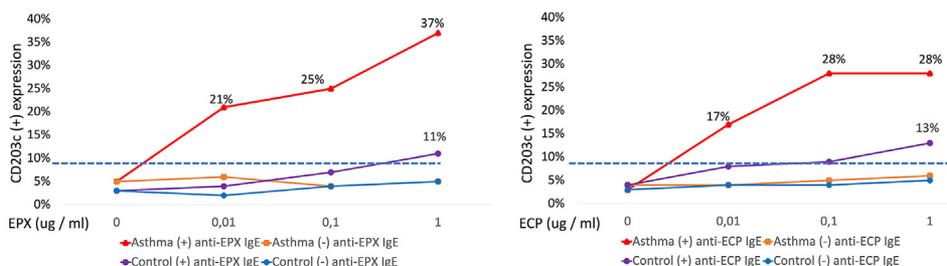
**Fig. 2 Correlations among autoantibodies and eosinophil count.** Concentration of IgE and IgG autoantibodies are presented according OD level. ECP: Eosinophil cationic protein. EPX: Eosinophil peroxidase

lower percentages of activation compared to asthmatic patients, however we observed activation using the highest concentration (1 µg/mL). Due to the enzymatic nature of EPX and ECP, a nonspecific degranulation could be the explanation for these results, but this hypothesis needs to be confirmed.

Recently, we described anti-EPEX and Anti-ECP IgE in patients with urticaria and atopic dermatitis,<sup>17</sup> so these autoantibodies are not pathognomonic for asthma. These 3 diseases have in common elevated levels of IgE and eosinophils in serum and/or local tissue (Skin or lung respectively). In atopic dermatitis the levels of anti-EPX/ECP were three times higher than in urticaria

or asthma. This suggests that the intensity of type 2 inflammation increases the probability of IgE autoimmunity.

Some previous studies suggest that IgG auto-immune response in asthma is present especially in non-atopic asthmatic patients with severe clinical presentations.<sup>29-34</sup> For most of IgG autoantibodies the clinical relevance is unknown and studies evaluating its role in the pathogenesis of asthma are necessary. However, some IgG autoantibodies have been associated with the severity of asthma and the use of high doses of steroids, therefore it could have an important clinical impact.<sup>21</sup> Anti-EPX IgG was previously reported in the sputum of 24/65 (37%)



BAT groups	0 ug/ml	0.01 ug/ml	0.1 ug/ml	1 ug/ml
Asthma (+) anti-EPX IgE (n 5)	5% ± 3	21% ± 7	25% ± 8	37% ± 11
Asthma (-) Anti-EPX IgE (n 5)	5% ± 2	6% ± 1	4% ± 3	5% ± 2
Control (+) Anti-EPX IgE (n 2)	3% - 4%	4% - 3%	7% + 6%	12% - 9%
Control (-) Anti-EPX IgE (n 5)	3% ± 1	2% ± 5	4% ± 3	5% ± 3

BAT groups	0 ug/ml	0.01 ug/ml	0.1 ug/ml	1 ug/ml
Asthma (+) anti-ECP IgE (n 5)	3% ± 4	17% ± 5	28% ± 9	28% ± 10
Asthma (-) Anti-ECP IgE (n 5)	4% ± 3	4% ± 4	5% ± 1	6% ± 3
Control (+) Anti-ECP IgE (n 4)	4% ± 1	8% ± 3	9% ± 3	13% ± 2
Control (-) Anti-ECP IgE (n 5)	3% ± 2	4% ± 3	4% ± 2	5% ± 1

**Fig. 3 Basophil activation test with EPX and ECP.** Basophil activation test using different concentrations of EPX and ECP. EPX: Eosinophil peroxidase. ECP: Eosinophil cationic protein. In the table CD203c activation is presented in median and range. "n" number of patients per group. For Control (+) anti-EPX IgE we put the value of CD203 expression for each patient

asthmatics,<sup>6</sup> we observed a similar frequency of anti-EPX IgG (25.4%) in asthmatic patients that was higher than control group but not statistically significant. The higher frequency of IgG autoantibodies, especially in the most severe cases, regardless of age, suggests that their formation is associated with the intensity of the inflammatory process and could potentially serve as biomarkers of severity.

IgE autoantibodies in asthma has been little studied<sup>7,35,36</sup> compared with other chronic diseases.<sup>11,12,27,28,37-39</sup> The origin of these autoantibodies is unclear; In some cases, it seems to be secondary to cross-reactivity with environmental proteins,<sup>40</sup> in others it seems to be the result of a high exposure of antigens usually hidden in a medium with a high number of inflammatory mediators.<sup>41-46</sup> This may occur with EPX and ECP antigens when they are released by eosinophils during inflammatory process in atopic dermatitis, urticaria, and asthma.<sup>17,47-51</sup>

Unlike other studies, we observed that blood eosinophil count and total IgE were high compared with other populations such as Europeans, both in the asthmatic group and in the control group. The study population is located in a tropical area where parasite exposure is endemic; therefore, this stimulus could influence the high levels found of both eosinophils and total IgE.<sup>4,52</sup> However, at the time of the study none of the subjects had active parasitosis infection. Despite EPX and ECP are both being produced by eosinophils during the inflammatory response, there was no correlation among the anti-ECP IgE and anti-EPX IgE levels, suggesting that although they are released by the same cell and probably their antigenic presentation occurs at the same time and context; the intensity and frequency of the IgE response to each antigen have different triggers.

The study has some limitations; The sample size was calculated to detect autoantibodies in patients with asthma; therefore, other analyses are only exploratory, for example to assess the association with asthma severity. However, the descriptive data obtained are useful as a starting point for further studies.

## CONCLUSIONS

We demonstrated IgE autoantibodies against eosinophil proteins EPX and ECP in adults and children. These antibodies can induce an inflammatory response and appear to be related to severe asthma. The detection of autoantibodies could have clinical implication in the diagnosis and therapeutic approach; therefore, more studies are necessary to evaluate its diagnostic performance and its possible usefulness as a predictor of severity.

### Abbreviations

ECP, Eosinophil cationic protein; EPX, Eosinophil peroxidase; FVC, Forced vital capacity, FEV1, Forced expiratory volume; OD, optical density units.

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### Authors' contribution

JS contributed the central idea. JL and JS evaluated and collected clinical data from patients. JL, MM, and AS organized the databases. JS, AS, EG and MM analyzed the data. JS, AS, and JL wrote the first draft. All authors were involved in writing, reviewing, and editing the final manuscript.

### Ethical approval

The Ethics committees of University of Antioquia approved the study (Code CII-07 2016). Written informed consent was obtained from all the participants or in the case of children, it was obtained from their parents.

### Authors' consent for publication

All authors have approved the submission of this manuscript. These results have not been previously published in another journal.

### Data availability statement

The datasets generated for this study are available on request to the corresponding author.

### Declaration of competing interest

Authors have not conflict of interest to declare.

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