ORIGINAL ARTICLE

IgE-Mediated Sensitization to Blo t 21 and Blo t 5 Is Associated With Asthma in the Tropics: A Case-Control Study

Zakzuk J[1], Donado K[1], Mondol E[1], Marrugo V[1], Regino R[1], López JF[1], Hernandez K[1], Mercado D[1], Dennis R[2], Puerta L[1], Caraballo L[1]

[1]Institute for Immunological Research, University of Cartagena, Cartagena, Colombia
[2]Fundación Cardioinfantil, Bogotá, Colombia

J Investig Allergol Clin Immunol 2024; Vol. 34(3): 157-166
doi: 10.18176/jiaci.0892

Abstract

Background: Sensitization to *Blomia tropicalis* is associated with asthma in various tropical and subtropical countries; however, information about the specific molecular components associated with this disease is scarce.
Objective: Using molecular diagnosis, we sought to identify *B tropicalis* allergens associated with asthma in Colombia.
Methods: Specific IgE (sIgE) to 8 *B tropicalis* recombinant allergens (Blo t 2, 5, 7, 8, 10, 12, 13, and 21) was determined using an in-house ELISA system in asthma patients (n=272) and controls (n=298) recruited in a national prevalence study performed in several Colombian cities (Barranquilla, Bogotá, Medellín, Cali, and San Andrés). The study sample included children and adults (mean [SD] age, 28 [17] years). Cross-reactivity between Blo t 5 and Blo t 21 was evaluated using ELISA-inhibition.
Results: Sensitization to Blo t 21 (aOR, 1.9; 95%CI, 1.2-2.9) and Blo t 5 (aOR, 1.6; 95%CI, 1.1-2.5), but not Blo t 2, was associated with asthma. sIgE levels to Blo t 21 and to Blo t 5 were significantly higher in the disease group. Cross-reactivity between Blo t 21 and Blo t 5 is on average moderate in frequency; however, analysis of individual cases indicates that it may be very frequent (>50%) in some cases.
Conclusions: Although Blo t 5 and Blo t 21 are considered common sensitizers, this is the first report of their association with asthma. Both components should be included in molecular panels for diagnosis of allergy in the tropics.

Key words: Asthma. Molecular diagnosis. Component-resolved diagnostics. Allergy. Blomia tropicalis. IgE. Recombinant allergens. Multiplex platform. House dust mites. Case-control study. Precision medicine.

Resumen

Antecedentes: La sensibilización a *Blomia tropicalis* está asociada con asma en diferentes países tropicales y subtropicales; sin embargo, la información sobre los componentes moleculares específicos asociados con esta enfermedad es escasa.
Objetivo: Mediante diagnóstico molecular se buscó identificar alérgenos de *B. tropicalis* asociados al asma en Colombia.
Métodos: Se determinó la IgE específica (sIgE) a ocho alérgenos recombinantes de *B. tropicalis* (Blo t 2/5/7/8/10/12/13 y 21) usando un sistema ELISA desarrollado internamente en pacientes asmáticos (n=272) y sujetos control (n=298) reclutados en un estudio de prevalencia nacional realizado en ciudades colombianas (Barranquilla, Bogotá, Medellín, Cali y San Andrés). La muestra del estudio incluyó a niños y adultos (edad media: 28±DE 17 años). La reactividad cruzada entre Blo t 5 y Blo t 21 se evaluó mediante ELISA inhibición.
Resultados: La sensibilización a Blo t 21 (aOR: 1,9; IC 95 %: 1,2 a 2,9) y Blo t 5 (aOR: 1,6; IC 95 %: 1,1 a 2,5) se asoció con asma, pero no a Blo t 2. Los niveles de sIgE para Blo t 21 y para Blo t 5 fueron significativamente más altos en el grupo de enfermedad. La reactividad cruzada entre Blo t 21 y Blo t 5 es en promedio moderada; sin embargo, el análisis individual indica que puede ser alto (>50 %) en algunos casos.
Conclusiones: Aunque Blo t 5 y Blo t 21 han sido descritos como sensibilizantes comunes, este es el primer estudio de su asociación con el asma. Ambos componentes deben incluirse en paneles moleculares para el diagnóstico de alergias en los trópicos.

Palabras clave: Asma. Diagnóstico molecular. Diagnóstico por componentes. Alergia. Blomia tropicalis. IgE. Alérgenos recombinantes. Plataforma multiplex. Ácaros del polvo doméstico. Estudio de casos y controles. Medicina de precisión.

Summary box

- **What do we know about this topic?**
  Sensitization to *Blomia tropicalis* is linked to asthma in tropical and subtropical regions. Previous research lacks detailed molecular analysis of the specific allergens involved.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  Using a case-control design, this study identifies Blo t 5 and Blo t 21 as key allergens in asthma, suggesting that their inclusion in diagnostic panels could enhance molecular diagnosis and management of allergy in the tropics.

Introduction

Blomia tropicalis is a major cause of sensitization in the tropics [1]. Sensitization to this house dust mite (HDM) is associated with acute asthma exacerbations in case-control studies [2-4], although few studies have identified which of its allergens are specifically associated with onset of disease. Precision medicine in allergy is based on the identification of clinically relevant allergens for applications such as the development of molecular reagents for allergen standardization and formulation, measurement of allergen exposure, and design of new strategies for allergen immunotherapy (eg, hypoallergenic vaccines) [5-7].

Early and strong IgE responses to *B tropicalis* are common in people living in tropical settings [8]. Previous investigations in the "Risk Factors for Asthma and Allergy in the Tropics" (FRAAT) cohort also indicate that children may be sensitized early to molecular components with no disease manifestations [8]. Therefore, it is important to define the *B tropicalis* allergens that are associated with allergic diseases.

Fourteen *B tropicalis* allergens have been officially recognized by the International Union of Immunological Societies [9]. Blo t 5 [10] and Blo t 21 are phylogenetically related allergens [11,12] and have been recognized as the most common sensitizers of this HDM in some populations [13]. Several epidemiological surveys have shown that other IgE-binding molecules from *B tropicalis* are frequent sensitizers [14-17]. Blo t 2, for example, shows low-to-moderate cross-reactivity with group 2 allergens from *Dermatophagoides* species [14] and has been proposed as clinically relevant in Brazil; in combination with Blo t 5 and Blo t 21, it is very sensitive in diagnosis of sensitization to *B tropicalis* using molecular panels [15]. Blo t 7 sensitized more than >50% of Singaporean allergic children [16], and Blo t 13, as well as other fatty acid–binding allergens, may activate biological pathways, thus promoting airway inflammation [17]. Blo t 12, a species-specific allergen of *B tropicalis* with chitin-binding activity [18], and Blo t 8, a mu-like glutathione transferase, are IgE-binding proteins with the ability to induce mast cell degranulation [19].

A group of investigators working on indoor allergens has suggested that the association between sensitization and onset of disease, as well as other factors, is an important criterion for definition of clinically relevant allergens in diagnosis and treatment [20], which is currently based mainly on the frequency of sensitization [21]. Using a panel of 8 *B tropicalis* allergens, we performed an exploratory analysis in a nested case-control study [22] to evaluate the association between IgE-mediated sensitization to specific components and onset of asthma in patients recruited in a tropical environment.

Methods

Study Design and Setting

From a nested case-control study within a population-based survey performed in 2009-2010, we randomly selected a subsample of 272 cases and 298 controls to compare the prevalence of sensitization to 8 allergens of *B tropicalis*. A description of the ancillary study has been published elsewhere [22]. The study participants were aged 1-59 years. People in acute care hospitals or institutions for the chronically ill or for people with disability at the time of the study were excluded, as were people with an altered mental state, dementia, or intellectual disability. Serum samples were collected from participants and stored at –80°C. In addition, we randomly selected a subsample of cases and controls from each city. This protocol was approved by the Ethics Committee of the University of Cartagena (Acta 10-05-2018).

Table 1. Descriptive Features of the Study Sample

Feature	Asthma cases (n=272)	Controls (n=298)	*P* Value
Mean (SD) age, y	28.0 (17.26)	28.8 (16.51)	.68
Males, No. (%)	89 (32.7)	124 (41.6)	.028
City, No. (%)			
Barranquilla	30 (11.0)	30 (10.1)	.98
Bogotá	149 (54.8)	168 (56.4)	
Cali	47 (17.3)	51 (17.1)	
Medellín	27 (9.9)	27 (9.1)	
San Andrés	19 (7.0)	22 (7.4)	
D pteronyssinus, No. (%)	119 (43.8)	67 (22.5)	<.0001
B tropicalis, No. (%)	100 (36.8)	57 (19.1)	<.0001

Asthma was defined according to a previously validated International Study of Asthma and Allergies in Childhood–based questionnaire [23,24]. A case was defined as a participant reporting current or past asthma symptoms. A control was defined as any participant who answered "no" to questions indicative of having symptoms of asthma, allergic rhinitis, or atopic eczema in the previous year and no clinical history of allergic diseases [22]. Descriptive features of the study participants are shown in Table 1. The study sample included children and adults (mean [SD] age, 28 [17] years). No differences in age were recorded between cases and controls, although male sex was significantly more frequent in controls (32.7% vs 41.6%)

Study Location

Colombia is a South American country. Given its location in the intertropical zone and the presence of the Andes Mountains, it has a large variety of weather types and ecosystems. This study was performed in 5 main cities: Bogotá, Barranquilla, Cali, Medellín, and San Andrés. Barranquilla and San Andrés, in the North region, are mostly at sea level and share similar hot (~28-29°C) and humid weather conditions. Medellín (~22°C), Cali (~24°C), and Bogotá (~13°C) are located on different branches of the Andes at 1480, 1018, and 2582 m above sea level, respectively. These cities tend to have a constant temperature throughout the year, with lower temperatures in the higher cities.

Recombinant Allergens

Blo t 5, Blo t 8, and Blo t 21 were isolated from a cDNA library built with mites collected in house dust obtained in Cartagena, Colombia and directly cloned from the PCR reaction into a pET100 vector. Blo t 12 and Blo t 13 were isolated from the same *B tropicalis* cDNA library [25], and their sequences were codon-optimized and cloned into pET45b+ [6,18]. Codon-optimized sequences of Blo t 2 (accession number: ABG76185.1), Blo t 7 (accession number: A1KXI4), and Blo t 10 (accession number ABU97466.1) were synthesized and cloned into the pET-45b+ vector using GenScript. Previous reports describe the production of Blo t 5 [26], Blo t 8 [27], Blo t 10 [28], Blo t 12 [18], and Blo t 13 [29]. Production of the remaining allergens is described here. Competent *E coli* BL21 (DE3) cells (for Blo t 7 and Blo t 21) and *E coli* Origami (DE3) cells (for Blo t 2) were transformed with the respective recombinant plasmids using electroporation (MicroPulser, Bio-Rad) and selected on Luria Broth-Agar plates containing 100 µg/mL ampicillin. Protein expression was induced in the logarithmic phase with 1 mM IPTG for 4-5 hours at 37°C in most cases, except for Blo t 7, whose production was induced with 0.5 mM IPTG at 25°C for 24 hours. Bacterial pellets of allergen cultures were recovered and resuspended in 20 mM NaH_2PO_4 0.3 M NaCl (NBB pH 8.0). Lysis and purification of each recombinant protein was standardized independently, although all molecules were generally lysed by sonication (Sonic Dismembrator FB-705, Thermo Fisher Scientific) and with lysozyme (100 ug/mL). Proteins were purified by affinity chromatography using a $Ni2^+$ NTA resin (Qiagen, Cat 30230) and 250 mM imidazole NBB pH 8.0 as elution buffer, before being further lyophilized and stored at –20°C in the "Silvia Jimenez" Recombinant Allergen bank.

Assessment of Sensitization

Levels of specific IgE antibody to the house dust mites *Dermatophagoides pteronyssinus* and *B tropicalis* were measured using the ImmunoCAP system, as previously described [21]. Serum specific IgE levels to the 8 recombinant allergens were detected in duplicate using a multiplex indirect ELISA-based platform. Each allergen (0.25 µg) was coated on carbonate/bicarbonate buffer (pH 9.2) by ON incubation. After 5 washes with 0.1% Tween 20 PBS, wells were blocked using 1% PBS bovine serum albumin with 0.02% sodium azide. After washing (4 times), serum (1:5) was added and incubated ON at room temperature (RT) in a humid chamber, before being washed 5 times and incubated for 2 hours with antihuman IgE Ɛ-chain specific alkaline phosphatase conjugate (Sigma), diluted 1:1000 in Tris buffer 50 mM 1% bovine serum albumin (BSA) $MgCl_2$ 1 mM with 0.02% sodium azide (pH 8), and developed with *p*-nitrophenyl phosphate substrate (1 mg/mL; Sigma) diluted in 10% diethanolamine 0.5 mM $MgCl_2$ (pH 9.8). The reaction was incubated in the dark and stopped after 60 minutes with 50 µL of 3N NaOH. Absorbance was measured at 405 nm in a spectrophotometer (Multiskan GO; Thermo Scientific). A negative control serum for all the allergens evaluated and *B tropicalis* extract (Lot #28, Institute for Immunological Research, University of Cartagena) was used in each experiment; PBS was used as a control for nonspecific binding of antihuman IgE alkaline phosphatase conjugate. As for the positive control, a curve with 2-fold serial dilutions (from 1:5 to 1:80) was constructed using serum with known IgE reactivity against *B tropicalis* extract. These dilutions were made for each experiment in duplicate using wells coated with 0.5 µg *B tropicalis* extract diluted in buffer carbonate/bicarbonate (pH 9.2). Cut-off values to define positive or negative IgE responses to the recombinant allergens were calculated as the mean optical density (OD) of 11 negative, non–mite-sensitized controls + 3 SD. The cut-off value to define sensitization was 0.09 OD units.

Cross-Inhibition Assays

Cross-reactivity between Blo t 5 and Blo t 21 was studied using inhibition ELISA according to a protocol described elsewhere [30]. Serum pools of double-sensitized patients were absorbed with 10-fold serial dilutions of allergens (from 1 ng/mL to 100 mg/mL). A description of the serum used in the pools is presented in Supplementary Table 1. We also performed end-point inhibition ELISAs using 5 individual sera from patients sensitized to both allergens, which were incubated with 0.1 mg/mL of Blo t 21 or Blo t 5. The results are shown as percentage of inhibition, calculated as follows: [OD without inhibitor – OD with inhibitor]/OD without inhibitor * 100. BSA was used as a nonrelated inhibitor.

Statistical Analysis

Univariate and multivariate binary logistic regression models were used to evaluate the association between onset of asthma as the outcome measure and sensitization

Table 2. Sensitization to *Blomia tropicalis* Components and Asthma Presentation: Univariate Logistic Regression Analysis.

Allergen	Biochemical identity	Controls		Asthma		OR	95%CI	*P* Value
		No.	(%)	No.	(%)			
Blo t 2	ML-domain protein	69	(23.9)	74	(27.3)	1.20	(0.82-1.75)	.35
Blo t 5	Unknown	48	(16.6)	66	(24.4)	1.62	(1.07-2.45)	.02
Blo t 7	Lipid-binding	38	(13.1)	39	(14.4)	1.11	(0.69-1.80)	0.67
Blo t 8	GST	36	(12.5)	34	(12.5)	1.01	(0.61-1.66)	.98
Blo t 10	Tropomyosin	28	(9.7)	28	(10.3)	1.07	(0.62-1.87)	.80
Blo t 12	Chitin-binding	26	(9.0)	25	(9.2)	1.03	(0.58-1.83)	.93
Blo t 13	Fatty acid–binding	44	(15.2)	47	(17.3)	1.17	(0.75-1.83)	.50
Blo t 21	Unknown	43	(14.9)	65	(24.0)	1.81	(1.18-2.77)	.007

Abbreviations: ML, MD-2–related lipid recognition; GST, glutathione S transferase.

Table 3. Association Between Sensitization to Blo t 21 and Blo t 5 and Asthma Presentation: Multivariate Logistic Regression Analysis.

Predictor	aOR	95%CI aOR	*P* Value
Blo t 21 model			
Socioeconomic status	1.01	0.98-1.04	.59
Male sex	0.65	0.45-0.92	.02
Age, y	1.00	0.99-1.01	.41
City of residence			
(Reference: San Andrés)	-	-	.99
Barranquilla	1.13	0.50-2.53	.77
Bogotá	1.06	0.55-2.06	.86
Cali	1.10	0.52-2.31	.81
Medellín	1.20	0.52-2.75	.67
Sensitization to Blo t 21, %	1.83	1.18-2.83	.007
Blo t 5 model			
Socioeconomic status	1.08	0.78-1.50	.63
Male sex	1.00	0.99-1.01	.46
Age, y	0.63	0.44-0.90	.01
City of residence			
(Reference: San Andrés)	-		1.00
Barranquilla	1.06	0.47-2.41	.89
Bogotá	0.99	0.50-1.95	.97
Cali	1.05	0.49-2.25	.90
Medellín	1.08	0.47-2.51	.86
Sensitization to Blo t 5, %	1.61	1.05-2.47	.03

to each molecular component of *B tropicalis*. The a priori adjusting variables were age, sex, socioeconomic stratum, and city of residence. Crude OR and adjusted OR (aOR) and their respective 95%CI are reported. An association was considered significant when the *P* value was <.05. Given the data distribution, nonparametric tests were chosen for analyses of specific IgE values. Allergen-specific IgE was compared between the groups using the Mann-Whitney test. Correlations for log-transformed IgE values were analyzed using the Pearson test. Statistical analyses were carried out using SPSS, Version 25.0 (IBM Corp.).

Regarding data visualization, UpSet plots were generated using the *ComplexUpset* package (version 1.3.3) in R version 4.1.1 (2021-08-10) to count the number of participants with a positive IgE response to 1 or multiple allergens (intersections). Bar and strip plots depicting the distribution of sensitization between groups were generated with seaborn version 0.11.2. in Python 3.9 (64-bit).

Results

The IgE Response to Blo t 21 and to Blo t 5 Is Associated With Asthma

Blo t 2 was the most common sensitizer in both patients and controls, with no significant differences in frequency between the groups (Figure 1, Table 2). In contrast, sensitization to Blo t 21 and Blo t 5 was significantly more frequent in the disease group (Table 2). After adjusting for potential confounders, and in independent models, sensitization to Blo t 21 (aOR, 1.83, 95%CI, 1.18-2.83, *P*=.007) and to Blo t 5 (aOR, 1.61, 95%CI, 1.05-2.47, *P*=.03) was significantly associated with asthma (Table 3). Although sex was associated with onset of disease in both models, it did not show a modifier effect on the associations detected (Table 3). The strength of the IgE response to *B tropicalis* allergens was also analyzed regarding disease status (Figure 2 Table 4). Specific IgE levels to Blo t 5 and Blo t 21 were also significantly higher in patients than in controls (*P*=.014 and .029, respectively). No differences between the groups were recorded for the remaining allergens (Table 4).

Sensitization to either of these 2 allergens (Blo t 5 or 21) was observed in 27.6% of asthma patients (n=75) and 18.8% of controls (n=56). In the adjusted model, the strength of association of sensitization for either of the 2 components was no higher (aOR, 1.61; 95%CI, 1.08-2.41; *P*=.02) than for Blo t 21 alone.

Table 4. Distribution of Specific IgE Levels to Components of *Blomia tropicalis* in Asthma Patients and the Control Group

Allergen	Control group		Asthma cases		*P* Value
	Geometric mean	IQR	Geometric mean	IQR	
Blo t 2	0.082	0.070-0.089	0.087	0.071-0.094	.078
Blo t 5	0.078	0.067-0.082	0.088	0.068-0.089	.015
Blo t 7	0.076	0.066-0.078	0.075	0.066-0.077	.855
Blo t 8	0.074	0.064-0.075	0.074	0.065-0.077	.569
Blo t 10	0.071	0.063-0.072	0.071	0.063-0.072	.508
Blo t 12	0.071	0.063-0.073	0.071	0.064-0.074	.135
Blo t 13	0.076	0.064-0.077	0.079	0.065-0.080	.148
Blo t 21	0.077	0.065-0.079	0.090	0.066-0.086	.022

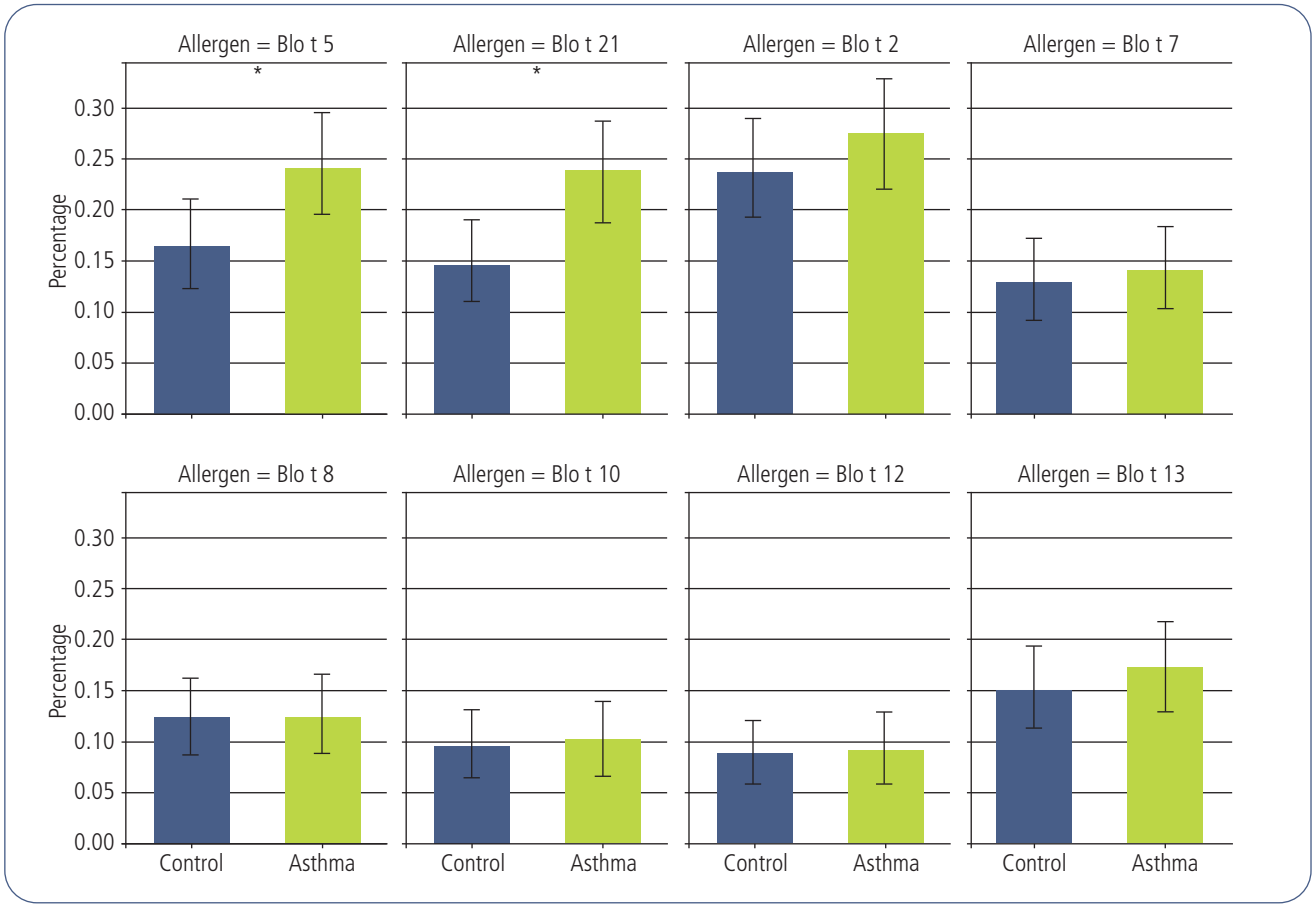

Figure 1. Sensitization to *Blomia tropicalis* allergens in asthma patients and controls. Bar graphs showing the frequency of sensitization in both groups. Comparisons were performed with the χ^2 test. *P<.05.

The Degree of Cross-Reactivity Between Blo t 21 and Blo t 5 Varies Between Individuals

Most patients sensitized to Blo t 21 were also sensitized to Blo t 5 (86.1%). In patients sensitized to Blo t 5, cosensitization to Blo t 21 was also common (80.3%). As shown in the correlation matrix (Figure 3A), the highest log values among all possible component pairs in specific IgE were for Blo t 5 and Blo t 21 (r=0.87, P<.001, Figure 3B). ELISA inhibition with a serum pool positive to both allergens revealed moderate inhibition of IgE binding to Blo t 5 by Blo t 21 (54%) at the highest inhibitor concentration. In contrast, Blo t 5 inhibited IgE binding to Blo t 21 at a lower rate (29%) (Figure 3C). However, by analyzing individual sera, we observed that Blo t 5 can also inhibit, to a large extent, Blo t 21, with values ranging from 2% to 81% (Figure 3D).

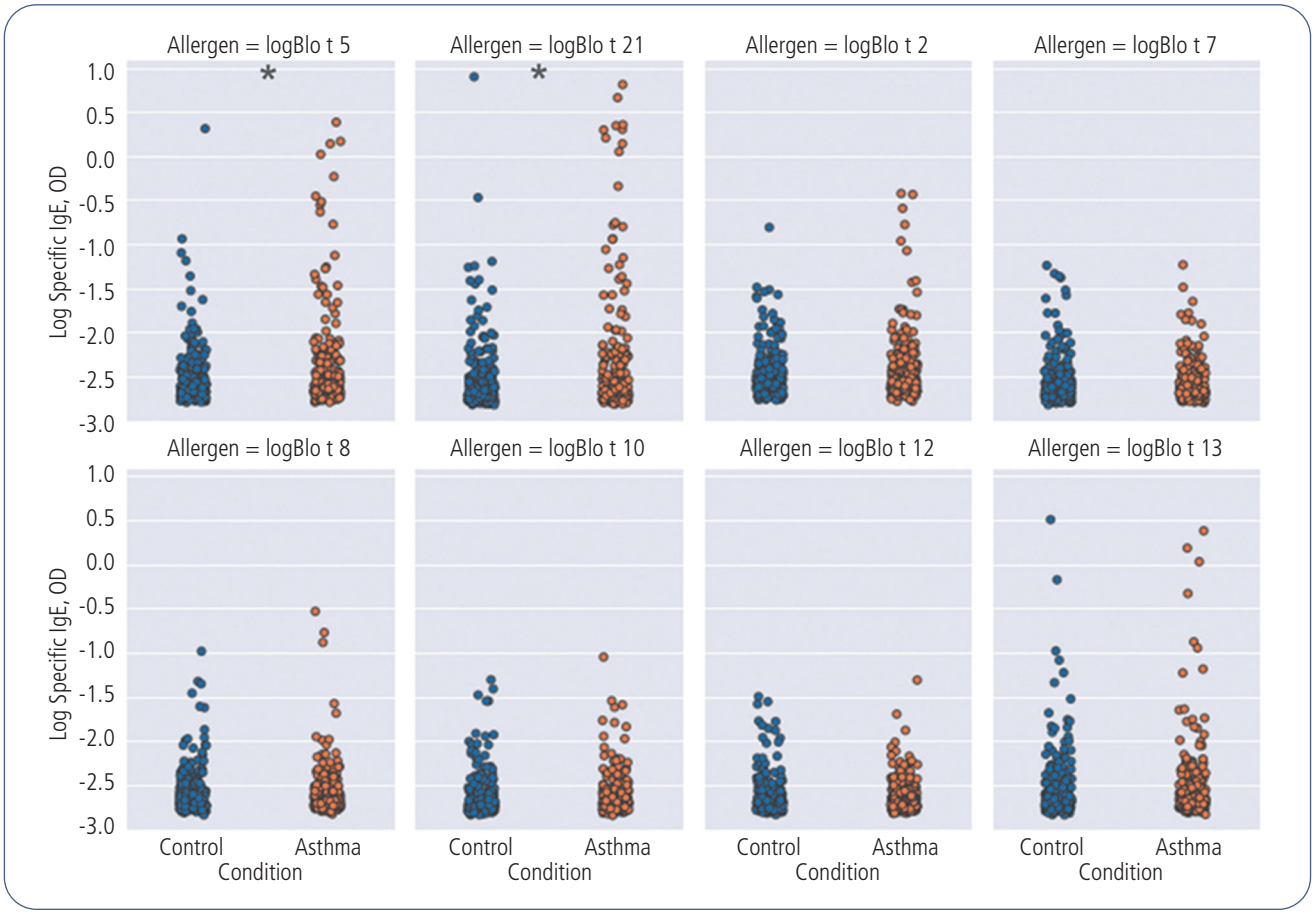

Figure 2. Allergen specific IgE values in asthma patients and controls. Strip plots showing individual values of specific IgE to each molecular component. Log-transformed values were used to improve distribution and visualization. *P<.05 (Mann-Whitney test).

The Response Rate to the Molecular Panel Was Higher Among Asthma Patients

In asthma patients, 108 out of 272 were positive for at least 1 allergen (39.7%); in the control group, 94 of 298 participants (31.5%) were sensitized (OR, 1.60; 95%CI, 1.13-2.26; P=.008). The frequency of sensitization detected with the *B tropicalis* panel was slightly higher than that found with the complete extract in the asthma group (36.8%) and ~12% higher in the control group (19.1%). Sensitization to any allergen was also associated with asthma even after adjustment for age, sex, city of residence, and socioeconomic status (aOR, 1.55; 95%CI, 1.09-2.22; P=.014).

We also explored other patterns of sensitization in cases and controls by analyzing intersection sets. As observed in Figure 4, monosensitization to Blo t 2 was the most frequent condition, both in cases (16/108 sensitized individuals, 14.8%) and in controls (21/94, 22.3%). In both groups, the second most common sensitization pattern was a positive IgE result for the 8 components. Sensitization to Blo t 2/Blo t 5/Blo t 21 was the third most frequent combination (followed by Blo t 5/Blo t 21) among the cases, but not among the controls.

There were significant differences in the rates of sensitization to molecular components between cities. The highest rates of sensitization to Blo t 2, Blo t 5, and Blo t 21 were recorded for Barranquilla and San Andrés (both at sea level) (Supplementary Figure 1). However, inclusion of city or residence in the multivariate logistic regression models did not show a modifier effect on onset of disease (Table 3).

Discussion

In this study, we report the associations between sensitization to Blo t 21 and to Blo t 5 and asthma in a tropical country. This is the most complete evaluation of a molecular repertoire of *B tropicalis* allergens using a case-control design. In most allergens, allergenic activity was demonstrated using at least a skin prick test or basophil activation test [12,19,20,31-34]. Among the 8 allergens evaluated, only 2 showed significant associations with asthma; in addition, the strength of the IgE response was also significantly greater among patients, suggesting that these allergens should be prioritized when designing platforms for diagnosis of allergy, especially for asthma, in tropical settings. Other studies have found that sensitization to Blo t 21 and Blo t 5 is frequent in asthma patients [8,13], although this

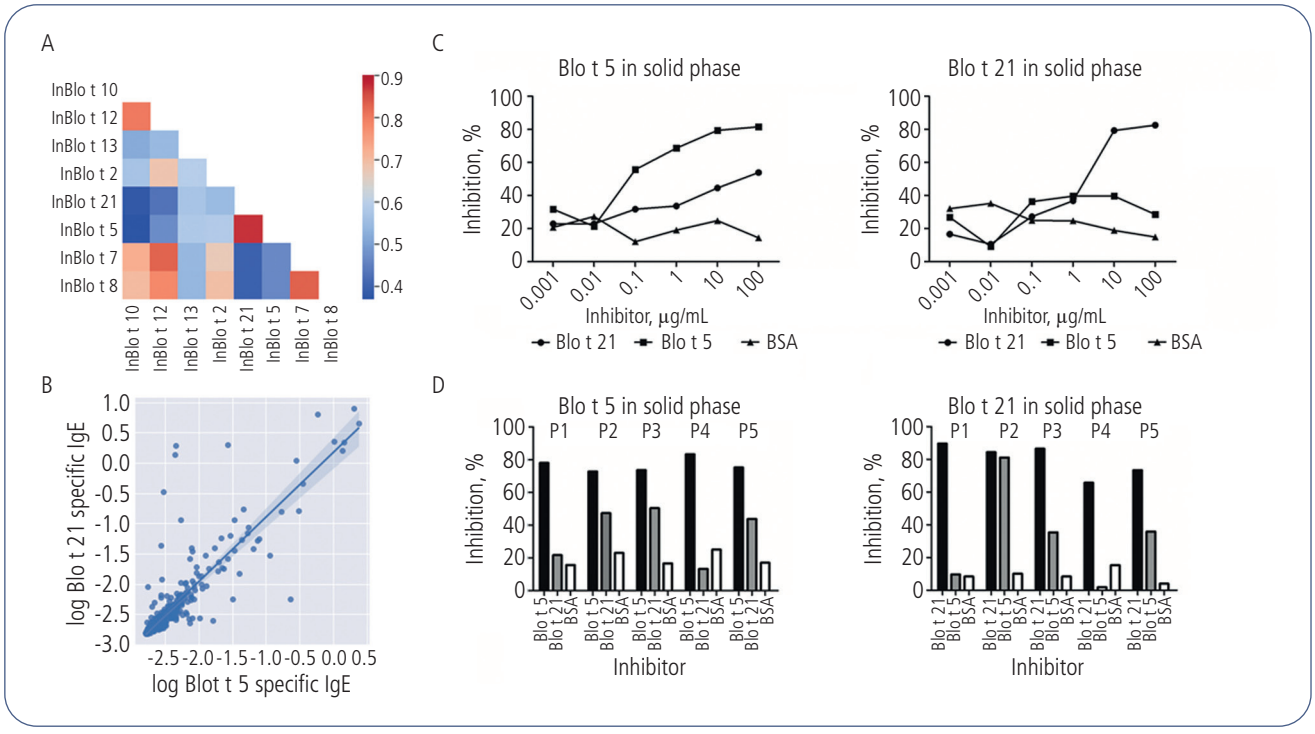

Figure 3. Assessment of cross-reactivity for Blo t 5 and Blo t 21. A, Correlogram of specific IgE values to the 8 allergens included in the *Blomia tropicalis* panel. Color bar indicates the r coefficient. B, Scatter plot depicting the correlation between log specific IgE to Blo t 21 and Blo t 5 in the sample analyzed (n=570). The regression line and its 95%CI are shown. C, ELISA inhibition curves for Blo t 21 and Blo t 5 using serum pools of double-sensitized asthma patients. D, Point inhibitions of IgE binding in 5 allergic patients. Each serum was inhibited with the homologous and heterologous condition as well as BSA as a nonrelated negative control. BSA indicates bovine serum albumin.

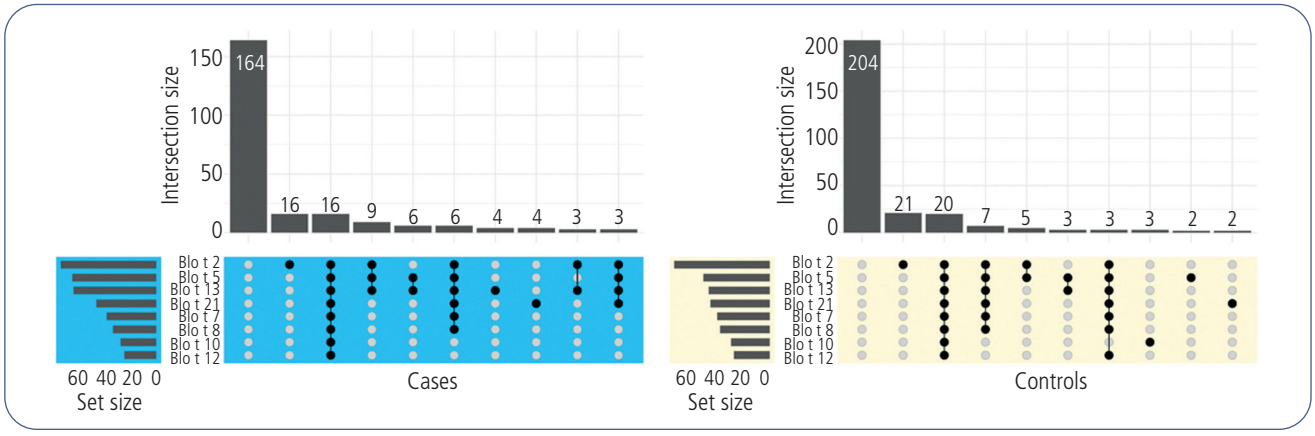

Figure 4. UpSet plot representing cosensitization patterns among the Blomia tropicalis allergens analyzed. Intersection sets are presented separately for asthma patients (A) and controls (B). Values above vertical bars indicate the number of participants sensitized to the allergens highlighted below with a black circle and connecting lines. The first column indicates the number of participants not responding to any allergen. Horizontal bars (left of each graph) indicate the number of participants responding to each allergen (set size) and are organized in descending order.

is the first time they have been analyzed together in a single sample population using a case-control approach.

Several efforts have been made to obtain a representative panel for diagnosis of HDM allergy in the tropics [15,16]. Our work contributes to this task with the identification of 2 allergens of possible importance in asthma (Blo t 21 and Blo t 5). Of course, we cannot rule out that other allergens in the platform used in this study have a clinical impact, although their effects may be influenced by non–IgE-mediated immune pathways [17,35].

We found that the frequencies of sensitization to Blo t 5 and Blo t 21 were 24.4% and 24%, respectively, that is, lower than those found in Singapore (Blo t 5 = 45% and Blo t 21 = 57%) and Brazil (rates close to 80% for both allergens) [13,16]. This could be due to differences in the technique used to detect specific IgE, although other reasons

include population age, inclusion criteria, differences in exposure, isoform variations, and even differences in the recombinant product used. Gao et al [12] found IgE reactivity to Blo t 21 by serology and skin prick test to be 92%-93% in a small sample of 42 individuals sensitized to *B tropicalis* with persistent allergic rhinitis whose clinical characterization included a nasal provocation test with *B tropicalis*. However, the frequency of IgE-mediated reactivity to Blo t 21 in a less clinically characterized sample of 494 allergic individuals in Singapore was 57.9%. It is also important to remark that given the extension of the Andes mountains, Colombia has different climates, and *B tropicalis* is more abundant in the warmer areas. In fact, in cities at high altitude, such as Bogotá, in contrast to *Dermatophagoides* species, *B tropicalis* has not been detected in house dust [36,37], thus potentially explaining why the rate of sensitization to Blo t 21 and Blo t 5 in Barranquilla, a city at sea level, was almost twice (40%) the average rate of all the other cities. These differences have been also observed in the rates of IgE response to the complete *B tropicalis* extract [22].

Other studies have evaluated cross-reactivity between Blo t 5 and Blo t 21, reporting marked differences in their results [11,38]. In our population, we found moderate cross-reactivity between Blo t 5 and Blo t 21, which may be high in some cases. Therefore, it is possible that part of the IgE-binding frequency exhibited by both allergens is due to this phenomenon. Structural studies indicate that Blo t 5 and Blo t 21 may share epitopes [38]. However, our analyses show that they are independently associated with asthma. Besides, since there is evidence suggesting the absence of cross-reactivity between these 2 molecules and *Ascaris* species extract [13], their use, together with other *B tropicalis*–specific allergens, is highly recommended for diagnosing *B tropicalis* allergy.

Since Blo t 2 was the most common sensitizer not only in asthma patients, but also in controls, it was not associated with onset of asthma. Compared to Der p 2 [26,39,40], there is less evidence about the intrinsic allergenic activity and biological functions of Blo t 2. However, other studies have reported that Blo t 2 sensitizes a considerable proportion of HDM-allergic patients. Reginald et al [14] found that 36% of Singaporean allergic patients were positive to Blo t 2, a rate that coincides with data from Gabon and Colombia in children with recurrent wheezing or asthma [41,42]. However, its clinical importance in asthma and other allergic diseases has not yet been evaluated.

This is the first report of sensitization to Blo t 7 in Latin America. Kidon et al [16] found Blo t 7 to be one of the most important sensitizers (57%) in Singaporean pediatric patients after Blo t 21 (56%) and Blo t 5 (45%) and concluded that with the combination of these 3 recombinant components, the sensitivity for diagnosis of sensitization to *B tropicalis* is about 70%. However, this was not replicated in our population, where sensitization rates were low (<15%) and similar between patients and controls. The presence of isoforms and differences in geographical distribution may play a role, as observed for other allergens [34].

Our study is subject to a series of limitations. The development of a multiplex assay to detect IgE may reduce optimal performance of the test for some antigens. Furthermore, since this a questionnaire-based study, there is a risk of selection bias for asthma and control groups. The frequency of sensitization may also be lower in this community-based study than in other reports, where patients are recruited in well-characterized samples from medical centers or specialized allergy departments. This study was designed to evaluate general rates of sensitization to each allergen; however, it is underpowered to report differences in sensitization rates between cities. Although our results may be generalizable to other allergic diseases, it must be recognized that these associations were analyzed only in the context of asthma. Similar analyses may be performed in the context of allergic rhinitis.

In conclusion, Blo t 5 and Blo t 21 were associated with asthma in a case-control study. Our results indicate that both components should be included in molecular panels for diagnosis of allergy in the tropics. Being a common sensitizer is not necessarily linked to clinical relevance, as is probably the case of Blo t 2 in the context of asthma.

Acknowledgments

We thank all the investigators of the Allergic Disease Prevalence study that gave rise to the initial research project through which samples were collected.

Funding

This research was funded by Minciencias (Grant Number 803-2018), Minciencias/SGR (Grant Number BPIN2020000100405), and the University of Cartagena.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Caraballo L, Zakzuk J, Lee BW, Acevedo N, Soh JY, Sanchez-Borges M, et al. Particularities of allergy in the Tropics. World Allergy Organ J. 2016;9:20.
2. Pefura-Yone EW, Mbele-Onana CL, Balkissou AD, Kenmegne-Noumsi EC, Boulleys-Nana JR, Kolontchang-Yomi BL, et al. Perennial aeroallergens sensitisation and risk of asthma in African children and adolescents: a case-control study. J Asthma. 2015;52(6):571-5.
3. Caraballo L, Puerta L, Fernandez-Caldas E, Lockey RF, Martinez B. Sensitization to mite allergens and acute asthma in a tropical environment. J Investig Allergol Clin Immunol. 1998;8(5):281-4.
4. Danansuriya MN, Rajapaksa LC, Weerasinghe A. Genetic, familial and environmental correlates of asthma among early adolescents in Sri Lanka: a case control study. World Allergy Organ J. 2015;8(1):19.
5. Martinez D, Cantillo JF, Herazo H, Wortmann J, Keller W, Caraballo L, et al. Characterization of a hybrid protein designed with segments of allergens from Blomia tropicalis and Dermatophagoides pteronyssinus. Immunol Lett. 2018;196:103-12.
6. Munera M, Martinez D, Labrada A, Caraballo L, Puerta L. Identification of B Cell Epitopes of Blo t 13 Allergen and

Cross-Reactivity with Human Adipocytes and Heart Fatty Acid Binding Proteins. Int J Mol Sci. 2019;20(24):6107.
7. Sanchez-Borges M, Fernandez-Caldas E, Thomas WR, Chapman MD, Lee BW, Caraballo L, et al. International consensus (ICON) on: clinical consequences of mite hypersensitivity, a global problem. World Allergy Organ J. 2017;10(1):14.
8. Zakzuk J, Mercado D, Bornacelly A, Sanchez J, Ahumada V, Acevedo N, et al. Hygienic conditions influence sensitization to Blomia tropicalis allergenic components: Results from the FRAAT birth cohort. Pediatr Allergy Immunol. 2019;30(2):172-8.
9. Santos da Silva E, Asam C, Lackner P, Hofer H, Wallner M, Silva Pinheiro C, et al. Allergens of Blomia tropicalis: An Overview of Recombinant Molecules. Int Arch Allergy Immunol. 2017;172(4):203-14.
10. Arruda LK, Fernandez-Caldas E, Naspitz CK, Montealegre F, Vailes LD, Chapman MD. Identification of Blomia tropicalis allergen Blo t 5 by cDNA cloning. Int Arch Allergy Immunol. 1995;107(1-3):456-7.
11. Kim CR, Jeong KY, Yi MH, Kim HP, Shin HJ, Yong TS. Cross-reactivity between group-5 and -21 mite allergens from Dermatophagoides farinae, Tyrophagus putrescentiae and Blomia tropicalis. Mol Med Rep. 2015;12(4):5467-74.
12. Gao YF, Wang de Y, Ong TC, Tay SL, Yap KH, Chew FT. Identification and characterization of a novel allergen from Blomia tropicalis: Blo t 21. J Allergy Clin Immunol. 2007;120(1):105-12.
13. Carvalho K dos A, de Melo-Neto OP, Magalhães FB, Ponte JC, Felipe FA, dos Santos MC, et al. Blomia tropicalis Blo t 5 and Blo t 21 recombinant allergens might confer higher specificity to serodiagnostic assays than whole mite extract. BMC Immunol. 2013;14:11.
14. Reginald K, Pang SL, Chew FT. Blo t 2: Group 2 allergen from the dust mite Blomia tropicalis. Sci Rep. 2019;9(1):12239.
15. Pinheiro CS, Silva ES, de Andrade Belitardo EMM, Pacheco LGC, Aguiar E, Alcantara-Neves NM, et al. En route to personalized medicine: uncovering distinct IgE reactivity pattern to house dust mite components in Brazilian and Austrian allergic patients. Clin Transl Allergy. 2021;11(1):e12004.
16. Kidon MI, Chiang WC, Liew WK, Ong TC, Tiong YS, Wong KN, et al. Mite component-specific IgE repertoire and phenotypes of allergic disease in childhood: the tropical perspective. Pediatr Allergy Immunol. 2011;22(2):202-10.
17. Smole U, Gour N, Phelan J, Hofer G, Kohler C, Kratzer B, et al. Serum amyloid A is a soluble pattern recognition receptor that drives type 2 immunity. Nat Immunol. 2020;21(7):756-65.
18. Zakzuk J, Benedetti I, Fernandez-Caldas E, Caraballo L. The influence of chitin on the immune response to the house dust mite allergen Blo T 12. Int Arch Allergy Immunol. 2014;163(2):119-29.
19. Acevedo N, Mohr J, Zakzuk J, Samonig M, Briza P, Erler A, et al. Proteomic and immunochemical characterization of glutathione transferase as a new allergen of the nematode Ascaris lumbricoides. PLoS One. 2013;8(11):e78353.
20. Caraballo L, Valenta R, Puerta L, Pomes A, Zakzuk J, Fernandez-Caldas E, et al. The allergenic activity and clinical impact of individual IgE-antibody binding molecules from indoor allergen sources. World Allergy Organ J. 2020;13(5):100118.
21. Caraballo L, Valenta R, Acevedo N, Zakzuk J. Are the Terms Major and Minor Allergens Useful for Precision Allergology? Front Immunol. 2021;12:651500.
22. Dennis RJ, Caraballo L, Garcia E, Rojas MX, Rondon MA, Perez A, et al. Prevalence of asthma and other allergic conditions in Colombia 2009-2010: a cross-sectional study. BMC Pulm Med. 2012;12:17.
23. Dennis R, Caraballo L, Garcia E, Caballero A, Aristizabal G, Cordoba H, et al. Asthma and other allergic conditions in Colombia: a study in 6 cities. Ann Allergy Asthma Immunol. 2004;93(6):568-74.
24. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J. 1995;8(3):483-91.
25. Caraballo L, Avjioglu A, Marrugo J, Puerta L, Marsh D. Cloning and expression of complementary DNA coding for an allergen with common antibody-binding specificities with three allergens of the house dust mite Blomia tropicalis. J Allergy Clin Immunol. 1996;98(3):573-9.
26. Buendia E, Zakzuk J, Mercado D, Alvarez A, Caraballo L. The IgE response to Ascaris molecular components is associated with clinical indicators of asthma severity. World Allergy Organ J. 2015;8(1):8.
27. Mueller GA, Pedersen LC, Glesner J, Edwards LL, Zakzuk J, London RE, et al. Analysis of glutathione S-transferase allergen cross-reactivity in a North American population: relevance for molecular diagnosis. J Allergy Clin Immunol. 2015;136(5):1369-77.
28. Ahumada V, Garcia E, Dennis R, Rojas MX, Rondon MA, Perez A, et al. IgE responses to Ascaris and mite tropomyosins are risk factors for asthma. Clin Exp Allergy. 2015;45(7):1189-200.
29. Múnera M, Martínez D, Labrada A, Caraballo L, Puerta L. Identification of B cell epitopes of Blo t 13 allergen and cross-reactivity with human adipocytes and heart fatty acid binding proteins. Int J Mol Sci. 2019;20(24):6107.
30. Acevedo N, Sanchez J, Erler A, Mercado D, Briza P, Kennedy M, et al. IgE cross-reactivity between Ascaris and domestic mite allergens: the role of tropomyosin and the nematode polyprotein ABA-1. Allergy. 2009;64(11):1635-43.
31. Martínez D, Cantillo JF, Herazo H, Wortmann J, Keller W, Caraballo L, et al. Characterization of a hybrid protein designed with segments of allergens from Blomia tropicalis and Dermatophagoides pteronyssinus. Immunol Lett. 2018;196:103-12.
32. Yi FC, Cheong N, Shek LP, Wang DY, Chua KY, Lee BW. Identification of shared and unique immunoglobulin E epitopes of the highly conserved tropomyosins in Blomia tropicalis and Dermatophagoides pteronyssinus. Clin Exp Allergy. 2002;32(8):1203-10.
33. Alvarez JRU. Obtenção, caracterização físico-química, imunológica e hipoalergização do alérgeno recombinante Blo t 2 visando o desenvolvimento de imunoterapia para alergia ao ácaro Blomia tropicalis.
34. Zakzuk J, Jimenez S, Cheong N, Puerta L, Lee BW, Chua KY, et al. Immunological characterization of a Blo t 12 isoallergen: identification of immunoglobulin E epitopes. Clin Exp Allergy. 2009;39(4):608-16.
35. Soongrung T, Mongkorntanyatip K, Peepim T, Buaklin A, Le Mignon M, Malainual N, et al. The Blomia tropicalis allergen Blo t 7 stimulates innate immune signalling pathways through TLR2. Clin Exp Allergy. 2018;48(4):464-74.

36. Charlet LD, Mulla MS, Sanchez-Medina M. Domestic Acari of Colombia: Population trends of house dust mites (Acari: Pyroglyphidae) in homes in Bogota, Colombia. Int J Acarol. 1978;4(1):23-31.
37. Poveda D, Chang A, Cuadros G, Acero V. Determinación de ácaros del polvo en 90 hogares de tres localidades de Bogotá, Colombia. Spei Domus. 2014;10(20):7-16.
38. Tan KW, Ong TC, Gao YF, Tiong YS, Wong KN, Chew FT, et al. NMR structure and IgE epitopes of Blo t 21, a major dust mite allergen from Blomia tropicalis. J Biol Chem. 2012;287(41):34776-85.
39. Vidal C, Lojo S, Juangorena M, Gonzalez-Quintela A. Association Between Asthma and Sensitization to Allergens of Dermatophagoides pteronyssinus. J Investig Allergol Clin Immunol. 2016;26(5):304-9.
40. Trompette A, Divanovic S, Visintin A, Blanchard C, Hegde RS, Madan R, et al. Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. Nature. 2009;457(7229):585-8.
41. Lopez J, Regino R, Guerrero L, Gracia M, Marrugo V, Zakzuk J, et al., editors. Sensitization to Blomia tropicalis and Ascaris spp. components in recurrent wheezing children living in a tropical city. Allergy. 2019;74:841-2.
42. Pauli G, Wurmser-Danion C, Roos A, Kokou C, Huang H-J, Lupinek C, et al. Molecular sensitization profile of asthmatic children in equatorial Africa. World Allergy Organ J. 2020;13(8):100137.

Manuscript received September 15, 2022; accepted for publication January 16, 2023.

Luis Caraballo

Universidad de Cartagena
Campus de la Salud. Cra. 50 #24-120
E-mail: lcaraballog@unicartagena.edu.co