Universidad de Cartagena
Instituto de Investigaciones Inmunológicas

Allergenic activity of Glutathione Transferase derived from *Blomia tropicalis* (Blo t 8)

Evaluación de la actividad alergénica de la Glutatión Transferasa derivada de *Blomia tropicalis* (Blo t 8)

TRABAJO PARA OPTAR AL GRADO DE MAGÍSTER EN INMUNOLOGÍA

PRESENTADA POR:

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Tutor:
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Cartagena, 2018

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To the memory of my father and my little warriors Maya and Belén

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LIST OF ABBREVIATIONS

AHR	Airway hyperresponsiveness
BALF	Bronchoalveolar lavage fluid
BRH	Bronchial Hiperresponsiveness
Bt	*Blomia tropicalis*
cDNA	*Complementary Desoxirribonucleic Acid*
ELISA	Enzyme-linked immunosorbent assay
GSH	Glutathione
HDM	House dust mite
HE	Hematoxylin Eosin
IFN-γ:	Interferon gamma
IgE:	Imunoglobulin E
IgG:	Imunoglobulin G
IgG1	Imunoglobulin G isotype 1
IgG2a	Imunoglobulin G isotype 2a
IgG4	Imunoglobulin G isotype 4
IL-10	Interleukin 10
IL-13	Interleukin 13
IL-4	Interleukin 4
OVA	Ovalbumin
PAS	Periodic acid–Schiff
PBS	0.15M phosphate-buffered saline, pH 7.4
PBS/BSA	PBS containing 1% of bovine serum albumin
PCA	Passive cutaneous anaphylaxis
pNPP	para-nitrophenyl phosphate
WHO/IUIS	World Health Organization/International Union of Immunological Societies

Abstract

BACKGROUND: The prevalence of allergic diseases has increased worldwide. *Blomia tropicalis* is a mite of allergenic importance in tropical and subtropical areas. Few allergens from this source have been fully characterized and there is a need to gain a deep insight on the immunological response to its components due to its clinical and academic implications. It has been previously reported that Blo t 8, the glutathione transferase of this mite, has allergenic properties. However, the influence of sensitization on asthma presentation remained unknown.

METHODS: The IgE-binding capacity of recombinant Blo t 8 (rBlo t 8) was evaluated by indirect ELISA in 574 subjects living in three different locations of the Department of Bolivar. The asthma case-control study involved 183 asthmatic patients and 200 non-asthmatic controls living in Santa Catalina or Cartagena. Additionally, we compared in 191 subjects the specific IgE response to Blo t 8 between two matched groups of African-descendent subjects (with parents and grandparents born in Palenque) who lived in this rural (93) town or in the urban (91) city of Cartagena. Additionally, we tested if Blo t 8 was able to induce airways inflammation. Three groups of BALB/c mice (n = 6 each) were sensitized i.p. and challenged i.n. with: 1. Alum-adsorbed Blo t 8, 2. phosphate buffer + alum (as negative control) or Alum-adsorbed ovalbumin (as positive control). Total and allergen-specific IgE, IgG1 and IgG2a antibodies were measured. The allergenic potential of Blo t 8 was also evaluated in a mouse model of passive cutaneous anaphylaxis and human basophil activation test.

RESULTS: Blo t 8 sensitization was higher in controls than in asthmatic patients (64,4% versus 23,9%, respectively). After adjustment by several potential confounders, Blo t 8 sensitization kept as a protective factor for asthma (aOR: 0.1 95%CI: 0.05-0.17). In the two related populations of Palenque origin, it was observed that Blo t 8 specific IgE response was more intense in the rural environment. In regard to the observed results in mice, Blo t 8 induced total and specific IgE production; however, contrary to OVA, it did not induce airway inflammation. In regard to the PCA results, the allergenic potential was intermediate, being the highest for OVA and negative for PBS.

CONCLUSION: The epidemiological relationship of rBlo t 8 with asthma presentation was in a protective direction. In contrast to classical allergens, sensitization to rBlo t 8 did not induce features of airway inflammation in experimentally sensitized animals.

I. INTRODUCTION

Asthma and other allergic diseases are increasing worldwide [1]. Two decades ago there was a belief that this raise was a particular situation of industrialized countries; nevertheless, basic and epidemiological research in different areas of the planet supports the idea that asthma epidemics affect many contexts of human populations [2–4]. The tropics, a wide area of the planet where about 65% of humans inhabit, have particular conditions that promote allergy development [5].

Humidity levels greater than 50% promote house dust mite (HDM) growth. In turn, HDM allergy has been associated with asthma development, severity and morbidity [6–8]. Its warm and humid climate favors mite growth, which includes potent allergenic sources such as *Blomia tropicalis* and *Dermatophagoides pteronyssinus*[5,6].

Socio-economic difficulties include deficiencies in hygienic conditions which in turn increase the risk of helminthic infections [9]. In addition, socio-demographical transitions [10–12], especially accelerated and unorganized urbanism, migrations [13] and health service problems difficult disease management[14]. Basic scientific research may contribute to ameliorate these problems and probably its impact on the tropics will be more relevant because the knowledge about its allergenic sources is still scarce. The characterization of local allergens would aid to develop better reagents for diagnosis, treatment and the identification of environmental risk factors for IgE sensitization will help to determine the most appropriate prophylaxis measures.

II. GENERAL CONCEPTS

Allergen

An allergen is an antigen (protein or glycoprotein) capable of inducing specific IgE antibody (*sensitization*) production in atopic or genetically predisposed individuals[15]. Allergen-IgE complexes may bind and cross-link high affinity receptors (FcERI) in the membrane of mast cells or basophils and trigger the release of mediators capable of producing local or systemic inflammation[16].

An antigen is officially defined as an allergen by the World Health Organization/International Union of Immunological Societies (WHO/IUIS) nomenclature committee database [17,18] once it has been established minimum conditions for its evaluation such as purity to homogeneity, physical-chemical characterization by molecular weight, isoelectric point and glycosylation pattern, nucleotide and/or amino acid sequence and when IgE reactivity in at least 5 subjects (allergic to the source where the potential allergen is derived) has been demonstrated [16,19]. Skin Prick Tests (SPT), histamine release test and Basophil Activation Test (BAT) results [16,18] are also considered in the evaluation, but they are not mandatory for its definition. We consider that evaluation of allergens should go beyond this minimum requirements that only demonstrate the potential to induce IgE production. Evaluation of allergenicity (or allergenic potential) should assess the ability of an allergen to induce symptoms or at least degranulation of effector cells.

Over the past few decades, the investigation about the important role of environmental allergens in the pathogenesis of allergic disease has been focused on the most important molecules in terms of frequency of sensitization (commonly named as *major* allergens), but for only few of them there are clues about a causal relationship with disease presentation [20]. Another aspect to keep in mind is that immune responses to certain allergens should not necessarily be associated positively with induction of disease traits. Recent studies by the group of Dr. Willis Karp indicate that the allergenic invertebrate tropomyosins may have protective roles on asthma depending on the genetic background of individuals [21]

House dust mite allergens

Allergens derived from HDM are the most important cause of IgE sensitization in the world[22], but especially in the tropics[5]. HDMs have been shown to be important sources of indoor allergens associated with asthma and other allergic conditions[23], being *Dermatophagoides pteronyssinus* (Dp), *D. farinae* (Df), *Euroglyphus maynei* (Em) and *Blomia tropicalis* (Bt) the most clinically relevant[24]. *B. tropicalis* belongs to the superfamily *Glyciphaoidea* and the family *Echimyopodidae* [25]. Bronswijk et al was first described in 1974 [26,27]. It is found in places where the temperature is 28 ° C and the percentage of relative humidity is high: 82%[7,8].

The WHO/IUIS (Allergen Nomenclature Sub-Committee. Allergen Nomenclature) has officially recognized 21 allergens from *Dermatophagoides pteronyssinus* and 14 allergens from *B. tropicalis* as well as multiple allergens from storage mite species.

Allergome database (www.allergome.org) has a wide number of potential allergens with any evidence of inducing IgE response. In terms of the ability of these allergens to individually induce an allergic T cell response there have been limited studies.

In Colombia, Puerta *et al* showed a prevalence of sensitization to *B. tropicalis* of 66% and 80% in patients with asthma or allergic rhinitis, respectively [28]. In an international collaborative study on HDM allergy, the prevalence of sensitization to *B. tropicalis* was 47% in Mexico City, 93.7% in Sao Paulo and 77.8% in Caracas [29].

At the extract level, there are few studies that simultaneously evaluate purified HDM allergens in the same population. In Colombia, the study of Jimenez, *et al* in 109 asthmatic patients from *Cartagena de Indias* found a prevalence of reactivity to *D. pteronyssinus* and *B. tropicalis* extracts was 86.6% and 84.4%, respectively [30]. BtM, a truncated form of Blo t 5, was found as the most frequent sensitizer among the three evaluated *B. tropicalis* components (Figure 1). However, this reactivity was not higher than 50% in contrast to what it was observed for Der p 1 and Der p 2 whose frequency of sensitization was more alike to international rates. As a conclusion of this study, more allergens of *B. tropicalis* should be evaluated.

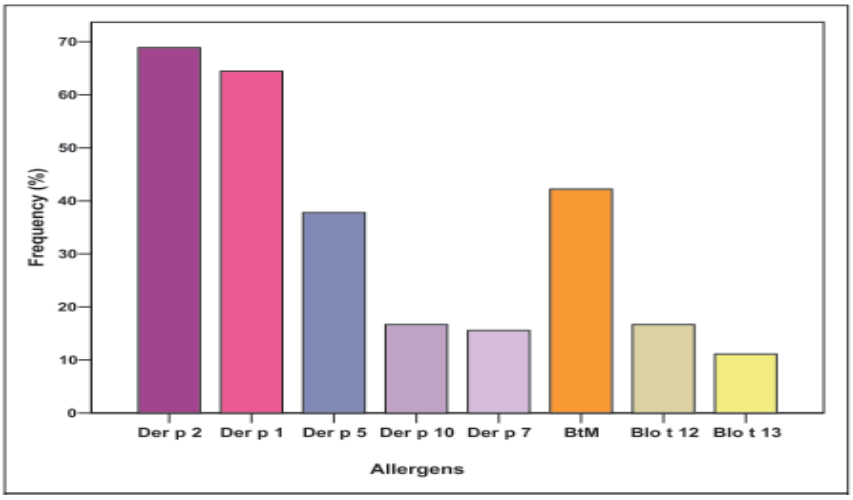

Figure 1. Data from epidemiologic study performed in Colombia with purified allergens by Jimenez et al. [30]

Blomia tropicalis *allergens*

Blomia tropicalis, a mite of allergenic importance in tropical and subtropical areas, belongs to the superfamily *Glyciphaoidea* and the family *Echimyopodidae* [31–33]. It is found in places where temperature is around 28°C and relative humidity is high (>80%)[6,8,34]. It is estimated that more than a billion people are regularly exposed to allergens of *B. tropicalis* in the tropics [22].

It has been detected in 16% to 96% samples of dust of domestic of tropical and subtropical environments, with variable rates of sensitization detected in many countries [34–36]. This HDM usually coexist with *D. pteronyssinus* in dwellings from tropical and subtropical regions explaining why dual sensitization to these species is so common [31,37–39].

This mite has been found in house dust from homes in Hong Kong, Brazil, Venezuela, Colombia, Taiwan, Malaysia, Spain, Egypt, and the United States [40]. High frequencies of positivity to *B. tropicalis* antigens in skin prick tests have been described in asthma and rhinitis patients, such as 68.1% in Cuba[41], 91.6% in Venezuela [42], 73.3% in Taiwan and 95.0%[43] in São Paulo, Brazil [44].

Currently, through the isolation and purification of single molecules, it has been found that *Blomia tropicalis* has more than 21 groups of allergens[31,45] (Table 1), with variable rates of IgE recognition. Despite molecular characterization in the last decades, just 14 *Blomia* allergens IUIS-acknowledged has been derived from predictions based on recent data published.

An unmet need in *B. tropicalis* allergens' research is a deep characterization of the allergenic potential of its components. Few allergens are well-characterized in terms of knowing if beyond IgE binding, the molecule induces an allergic reaction (i.e. basophil activation, skin prick test, histamine release).

Table 1 Blomia tropicalis allergens.

Allergen	MW (kDa)	IgE binding	Biochemical Activity
Blo t 1	25	61-92%	Cystein Protease
Blo t 2	14	7%	Lipid binding protein
Blo t 3	24-31	4.7-51%	Trypsine
Blo t 4	57	7.5-62%	α-Amilase
Blo t 5	14-17	10-100%	Unknown
Blo t 6	25	11%	Chimotrypsine
Blo t 8	26	80.2%	Glutathione-S-transferase
Blo t 9	30	ND	Serine protease
Blo t 10	33-37	10-29%	Tropomyosin
Blo t 11	98-110	10-86%	Paramyosin
Blo t 12	14	25-50%	Chitin binding protein
Blo t 13	14-15	13%-50%	Fatty acid binding protein
Blo t 14	117	ND	Vitellogenin
Blo t 15	62,5, 98, 105	ND	Chitinase
Blo t 18	60	ND	Chitinase
Blo t 19	7	3%	Antimicrobial peptide
Blo t 20	ND	ND	Arginin kinase
Blo t 21	13.2	57.9%	Unknown

*ND: Not described.

The family of Glutathione transferases (GSTs)

GSTs are cytosolic dimeric proteins involved in cell detoxification catalyzing the conjugation of glutathione (GSH) with a wide range of endogenous alkylating agents and xenobiotics, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress[46].

Studies have demonstrated that cytosolic GST are an integral part of a dynamic and interactive defense mechanism that protects against cytotoxic electrophilic chemicals and allows adaptation to exposure to oxidative stress. They have antioxidant, anti-inflammatory activities and recently some of them has allergenic properties.

Their substrates include halogen, nitrobenzenes, arenoxides, quinones and α, β-unsaturated carbonyls[47]. GST enzymes are proteins involved in cellular detoxification by catalysis of reduced glutathione conjugation (GSH) in non-compounds that contain a carbon, nitrogen or sulfur-electrophilic atom with a wide range of endogenous alkylating agents and xenobiotics, which include carcinogens, therapeutic drugs, environmental toxins and stress products oxidative[47,48].

They are classified into subfamilies: cytosolic, microsomal, mitochondrial, and bacterial. Cytosolic and mitochondrial GSTs comprise soluble enzymes that are distantly related [49–51]. The third family comprises microsomal GST and are now referred to as *membrane-associated proteins* in the metabolism of eicosanoids and glutathione (MAPEG) [52], the bacterial GST are represented by the bacterial phosphomycin resistance proteins FosA and FosB [46,47].

Cytosolic enzymes GST and MAPEG catalyze the isomerization of various unsaturated compounds [50] and are intimately involved in the synthesis of prostaglandins and leukotrienes [47]. At the same time they have subclasses named as α, κ, μ, π, Σ, Θ and ζ (*alpha, kappa, mu, pi, sigma, theta and zeta*) differ in their sequence immunological properties and physiological role[52].

Insect's GST play an important role in resistance to insecticides / drugs and stress [53]. Since invertebrates lack CYP450 activity, GST enzymes represent the most important detoxification mechanism of hydrophobic and electrophilic compounds, they are essential for the survival of species and in parasites the GST are recognized as an excretion-secretion antigen[54,55]. These GST which exhibit many peculiarities in contrast to mammalian GST [56].

In rodents and humans, cytosolic GST isoenzymes within a class typically share >40% identity, and those between classes share <25% identity [49]. Besides catalyzing conjugation, reduction, and isomerization reactions, cytosolic GST also bind, covalently and non-covalently, hydrophobic non-substrate ligands. GST provide targets for antiasthmatic, antitumor therapies and metabolize chemotherapeutic agents against cancer, insecticides, herbicides, carcinogens and by products of oxidative stress [47].

Since Blo t 8 is a Mu-class like protein, it is important to review the activity of this class of enzymes. GST Mu in humans have been reported to inhibit Ask1 and JNK during non-stressed conditions through physical interactions with the kinases. It has been shown that GSTM1 dissociates from Ask1 by heat shock [57]. Human Mu isoenzymes which occurred most frequently in patients with adenocarcinomas and the gene expressing class Mu isoenzymes may be a host determinant of genetic susceptibility to lung cancer among smokers GST overexpression in mammalian tumor cells has been linked to resistance to anticancer agents and chemical carcinogens [58–60].

It seems than humans induce strong antibody response to GST molecules derived from helminths. Several vaccines against different species of helminths has been developed, including the trematodes *Schistosoma haematobium* [61] and *Fasciola hepatica* [62] and the nematode *Necator americanus* GST-1 (Na-GST-1) [63]. It is a current concern that immunization with GST is accompanied of clinically meaningful IgE responses since the production of this isotype against different parasite GSTs have been demonstrated. Moreover, crossreactivity with airborne allergens has been demonstrated or suggested [64–67]

Allergenic GSTs

There are just 9 GST officially considered as allergens. They are: Alt a 13 (*Alternaria alternate, plant, rot and fungus*), Asc l 13 (*Ascaris lumbricoides*), Asc s 13 (*Ascaris suum*), Bet v 8 (*Betula verrucosa*), Bla g 5 (*Blattella germanica*), Blo t 8 (*Blomia tropicalis*), Der f 8 (*Dermatophagoides farinae*), Der p 8 (*Dermatophagoides pteronyssinus*) and Per a 5 (*Periplaneta Americana*) (Table 2).

The most important known sources of allergenic GSTs are cockroaches [68,69], house dust mites [65,70–72] and molds [73]. Based on the strength of the specific IgE response and the frequency of sensitization, the GST *German cockroach* (Bla g 5) [71] and GST *Periplaneta americana* (Per a 5) [74] are considered as *major* allergens, followed by Der p 8 [70]. One species usually contain different GST isoforms with moderate sequence conservation among them. This results in a wide repertoire of different epitopes with important variations among isoforms.

Proteomic analyses have been very useful to decipher the allergenic complexity of GST families. For example, the native GST of *P. americana* (nPer a 5) comprises three isoforms of delta and 2 sigma classes [65]. On the other hand, Acevedo *et al* founded at least six GST isoforms in *A. lumbricoides,* four isoforms were the most abundant and several aminoacid substitutions were found [67].

Given the structural homology between parasitic and non-parasitic GST, the hypothesis that helminth co-exposure could affect the frequency of sensitization and the strength of the serological response has been proposed [75]. There is experimental evidence about molecular mimicry between the GST of the *Wuchereria bancrofti* nematode and cockroach that could drive cross-sensitization. Human subjects infected by this filarial parasite showed higher levels of specific IgE to Bla g 5 than non-infected subjects [64].

Table 2 GST allergens, based on Allergome nomenclature.

Species	Allergen	MW(SDS-PAGE)	GenBank Protein	Allergenicity:
***Alternaria alternata* (Alternaria plant rot fungus)**				
	Alt a 13	26 kDa	AAR98813	No data available
***Ascaris lumbricoides* (Common roundworm)**				
	Asc l 13	23 kDa	P46436	26 (90%) of 29 patients exposed to A. lumbricoides with positive CAP to A. lumbricoides showed IgE binding to purified natural Asc l 13 in ELISA. Of 12 patients tested by SPT with nAsc l 13, 5 (42%) reacted. The identity of the natural purified Asc l 13 was established by sequencing 73% of the protein by MS/MS. [67]
***Ascaris suum* (Pig roundworm)**				
	Asc s 13	23 kD	CAA53218	20 of 29 Ascaris ImmunoCAP positive subjects showed IgE binding to GST by ELISA; 12 of 91 non-asthmatic controls were positive by ELISA to GST in vitro IgE binding; 42 of 215 Asthmatic subjects were positive to GST by ELISA; 3 of 10 asthmatics tested with recombinant GST by Skin Prick test were positive with at least 3mm diameter wheal [67]
***Betula verrucosa (Betula pendula)* (European white birch)**				
	Bet v 8	27 kDa	AHF71027.1	IgE binding by ELISA, positive for 29 of 217 SPT birch pollen allergic subjects (airway), using recombinant allergen. Three of three were positive by hRBL activation using recombinant allergen.[76]
***Blattella germanica* (German cockroach)**				
	Bla g 5	23 kDa	AAB72147	IgE Ab binding to natural and recombinant Bla g 5 was found in 27/40 (67.5%) and 29/40 (72.5%) of sera from CR-allergic patients, as measured by RIA. 5 out of 7 CR-allergic patients tested showed a positive skin reaction to rBla g 5. [77]
***Blomia tropicalis* (Storage mite)**				
	Blo t 8	27 kDa	ACV04860	Five of 20 subjects of a cohort of healthy and asthmatic volunteers reacted positively in a skin prick test with the purifed natural Blo t 8.[78]
***Dermatophagoides farinae* (American house dust mite)**				
	Der f 8	32 kD	AGC56215	Asthmatic patients selected (41) by SPT to extract of D. farinae, then tested by ImmunoCAP, a pool of sera of DP + subjects had clear IgE binding on 2D gel electrophoresis. However, 86% sequence identity to well proven D. pteronyssinus, Der p 8 glytathione S-transferase. Appears to be a partial sequence, no signal peptide[79]
***Dermatophagoides pteronyssinus* (European house dust mite)**				
	Der p 8	27 kDa	AAB32224	40% of mite-allergic sera recognized rDer p 8 on immunoblot.[80]
***Periplaneta americana* (American cockroach)**				
	Per a 5	23 kDa	AEV23867.1	Positive IgE tests with 15 of 15 cockroach allergic patients showing positive binding to natural and recombinant protein, with negative responses from 5 of 5. IgE binding tested by dot blot and ELISA. Subjects positive by SPT. NOTE: this submission was from Thailand in 2017, there was a previous paper by Wei et al., 2014 that showed IgE binding and basophil activity to a 98% identical sequence that Wei called Per a 5. That is now added as Per a 5.0102.[74]

The GST of *Blomia tropicalis*, Blo t 8

The interest in *Blomia tropicalis* GST begins when Acevedo *et al* [67] identified a component of *A. lumbricoides* that was cross-reactive with *B. tropicalis*, indicated by the presence of a 23 kDa band in the extract of this HDM that lacked recognition by IgE when serum was pre-incubated with *Ascaris* extract[66]. These results encouraged the search and isolation from a cDNA library of the Glutathione-S-Transferase of this HDM. Zakzuk *et al* identified a clone with 99% of identity to a GST sequence [78] published by Chew FT in Singapore [81].

After successful expression in *E. coli,* it was confirmed its IgE binding in allergic patients and also the high correlation of IgE levels with the natural counterpart and the GST from *A. lumbricoides* [78].Later, Mueller *et al*, by crystallography and X-ray diffraction[82], solved the three-dimensional structure of the allergen, observing it was well folded and similar to other mu GST isoforms present in nature. Blo t 8 is a dimeric protein with a molecular weight of 27 kDa composed of 236 residues containing an N-terminal domain TRX-fold and a C-terminal alpha helical domain, with an active site located in the cleft between the two domains[78].

Preliminary characterization in serology studies indicated that the IgE reactivity to recombinant Blo t 8 was common, but of low intensity and with a highly correlation with IgE titers against the native *B. tropicalis* GST [78].

Helminth infections and its role in sensitization

It is well known that intestinal parasitic diseases in low- and middle-income are still prevalent in vulnerable populations: pregnant women, low-income people and children's, for example, the roundworm *Ascaris lumbricoides* infects around 2 billion people and may influence the pathogenesis, evolution and diagnosis of allergic diseases [83,84].

Helminths and allergens have a strong th2 immune response, but helminths could induce immunoregulation [12,85,86] or allergic reactions[87,88] and allergens can induce uncontrolled chronic inflammation[89]. During infection by helminths, regulatory cells (macrophages Treg, Breg, M2) are induced through the damage induced by migration and the immunomodulatory molecules derived from helminths. Immunoregulation leads to preferential change to IgG4 antibodies[90,91], while allergens promote pro-inflammatory isotypes, such as IgG1 [91].

This knowledge of the molecular pathways triggered by mites and helminth parasites makes it possible to establish a parallel, for example: Acevedo, *et al* described that *B. tropicalis* and the extract of *Ascaris spp* had cross-reactivity IgE binding components (tropomyosine and GST) [67] without mentioning that epidemiological studies have shown these relationships [92];.This is a relevant topic in the Institute of Immunological Research and has been constructed from its lines of research suggesting an influence on the development and clinical presentation of allergic diseases.

Now, we know that intestinal parasites have an impact on the development of the allergic response[12,93–95]. Buendía et al, observed that sensitization to ascariasis was associated with more visits to emergency rooms and is an indicator to assess the severity of asthma[96]. This study shows that chronic and intense infection can produce immunosuppression and a pro-allergic state can be induced if the infection levels are mild. Zakzuk et al, (2018 submitted) showed that *A. lumbricoides* is a frequent agent of sensitization in asthmatic individuals in Santa Catalina (Colombia) when they were evaluated by serology (Immunocap®) and in the skin prick test.

Epidemiological studies have shown variations in the rates of sensitization and prevalence of these allergens in allergic diseases, so they have been refined each time to assess their factors, is the case of the evaluation in rural and urban contexts where differences exist [14,97–99]. Calvert et al, studied the relationship of infection and sensitization by *A. lumbricoides*, they showed positive association between infection and airway hyperreactivity in urban and rural children and was associated with greater probabilities of exercise-induced bronchoconstriction[100].

Therefore, comparing different populations in Colombia provides an opportunity to investigate factors that are relevant to the development of asthma. Although there is currently no unifying explanation for these observations, several factors that may be important for influencing the development of asthma in more urban than rural communities have been identified. The analysis of allergens components allows to know the physiological activity in tissues and if allergens have specific molecules that induce the clinical symptoms or cross-reactivity can contribute to this condition.

Cartagena, urban city

Urban it is a territory that has road infrastructure and primary networks of energy, aqueduct and sewerage with a population in the head equal or greater than 100,000 people [101]. For this study, Cartagena´s population was considered as urban. Is the capital of Bolivar, department in Colombia is a port of importance in Colombia, the Caribbean and the world. The total population is 971,700 inhabitants, it is a typical coastal area surrounded by the Caribbean Sea, the bay of Cartagena, lakes and lagoons, with a tropical climate with a temperature of 30 ° and a high relative humidity of more than 90%. Its most prevalent mites are Dp and Bt [102].

Santa Catalina as an Intermediate city

Intermediate city are those municipalities that have a regional importance and with access to different services. They have between 25.000 and 100.000 habitants in the headwaters or in spite of having smaller headwaters, they present high population density (more than 10 hab / km2)[101]. For this study, Santa Catalina´s population was considered as Intermediate. Is located 35 km from Cartagena. It has an estimated population of 12,500 inhabitants, distributed between its municipal seat (4,500) and the rest, in the 5 districts that comprise it. In 2008 Lopez-Agudelo reported a prevalence of intestinal parasitoses of 92%, including helminths and protozoa, in the corregimiento of Loma Arena. Infection by *A. lumbricoides* was frequent (56%), followed by Trichuris trichuria (53%) [103].

Palenque community

Is an Afro-descendant, Colombian community. Its climate is warm, semi-humid, has an ambient temperature that ranges between 29 and 30 ° C, with an average relative humidity of 80 to 85%. They are a community of approximately 3,500 people who have maintained cultural and ethnic identity with high inbreeding and little influence from other racial groups [104]. For this study they will be evaluated according to their area of residence, in this way those who live in Cartagena, Colombia, will be classified as *urban* and those who reside in rural will be classified as *rural*. Because rural have smaller headwaters (< 25,000 inhabitants) and have intermediate population densities (≈10 inhabitants / Km^2 and 100 inhabitants / Km^2) [101].

Animal model of hypersensitivity of the airways

Animal models of allergic respiratory disease help to understand the underlying pathophysiology, detect potential therapeutic targets and allow preclinical evaluation of new therapies. Disease model techniques in animals offer great tools for learning about asthma in ways that would not be possible in humans. Allergic airway disease has developed in several species, but murine models are particularly attractive due to their low cost, availability and well-characterized immune systems [105]. Experimental models, are a key tool to elucidate the molecular mechanisms through disease evolves, the development of diagnostic markers and the evaluation of efficacy and safety, unlike alternatives to evaluate and select drug candidates that affect these

processes and support its progression in clinical trials of treatment and prophylaxis [106].

Animal model of passive cutaneous anaphylaxis

One of most used preclinical studies on drug efficacy is the model of passive cutaneous anaphylaxis (APC) is a dermal response (local) and immediate to an allergen-IgE interaction and is characterized by increased permeability of the vessels within the skin, antiserum is challenged intradermally and changes are observed such as: local edema and erythema, at 24 h again a challenge is made by intravenous injection with the Evans blue dye (a dye that binds and extravasate with the plasma albumin) containing the antigen evaluated to visualize and quantify the increase in permeability characteristic of APC [107].

Anaphylaxis occurs as a consequence of an immune mechanism mediated by IgE and induce release of histamine, prostaglandins, leukotrienes, platelet activation factor, thromboxane A2 from mast cells and basophils. The antigen-antibody reaction fixed to the skin causes the release of histamine, which increases vascular permeability, allows leakage of the dye bound to albumin and produces a blue spot and edema at the site of the intradermal injection. (APC) is used as a tool to study sensitivity to allergens and the therapeutic efficacy of molecules in improving these responses [108].

III. Hypothesis

The IgE response to *B. tropicalis* GST (Blo t 8) is associated with asthma and its sensitization is influenced by urbanization context.

IV. Thesis aims

General aim

- To characterize the IgE immune response to the glutathione transferase from *Blomia tropicalis*, Blo t 8, and its relationship with the presentation of asthma.

Specific aims

1) To evaluate the relationship between sensitization to Blo t 8 and asthma in a case-control study design.

2) To compare the sensitization to Blo t 8 in populations with different contexts of urbanization.

3) To study the ability of Blo t 8 to induce airways inflammation in a murine model of sensitization.

4) To evaluate the allergenic potential of Blo t 8 in a model of passive cutaneous anaphylaxis.

V. MATERIALS AND METHODS

This thesis involves the characterization of the specific IgE response to rBlo t 8 in humans and its epidemiological relationship with asthma within a case-control study. The influence of urban/rural environment on sensitization was analyzed in Afro descendent subjects. Also, experimental procedures were performed in mice with the aim to evaluate its allergenic potential.

Section 1. Epidemiological studies.

Case-control asthma study

General design

This is a cross-sectional study to analyze the relationship between rBlo t 8 sensitization and asthma presentation in 392 subjects (189 with asthma and 203 healthy controls). The study population proceeds from two different communities of Bolívar: Cartagena de Indias (10° 23' 59″ North, 75° 30' 52″ West) and the rural municipality of Santa Catalina (10°36′14″North, 75°17′16″ West), a small tropical farming/fishing town at the North of Colombia (153 km2, 12.500 inhabitants) located at 44 kilometers from Cartagena.

Cartagena

Asthma patients were recruited as part of the research project "*Influence of sensitization by allergens of Ascaris in the allergic response and severity of asthma*" (Colciencias Code: 1107-493-26193. Contract 602 2009).

Subjects attending to health care centers in Cartagena were screened for eligibility by physicians of the research staff between June 2010 and March 2011.

Eligibility criteria were: subjects in the age range of 8 to 70 years who answered affirmatively to the question: *¿Have you ever been diagnosed with asthma?* Inclusion in the study depended on the confirmation of asthma diagnosis made by the physician. Eligible subjects were further evaluated by a physician of the research staff and asthma diagnosis was confirmed in those with at least two respiratory symptoms (cough, wheezing, dyspnea, and nocturnal cough/wheezing/dyspnea) or a history of recurrent asthma attacks.

Exclusion criteria: Patients with chronic obstructive pulmonary disease or another chronic respiratory co-morbidity were excluded as well as those patients belonging to the highest socio-economical strata of the city (4 to 6). Patients received an explanation about the investigation and signed a written informed consent to participate. Controls were recruited within the research program *"Candidate Genes for Asthma, CGA"* (PI, Luis Caraballo) and were individuals with no family or personal history of asthma and allergies. Both cases and controls lived in an urban setting, belonging to the lower three (out of six) socio-economic strata in the city.

Santa Catalina

Cases and controls were recruited from September 2014 to March 2015 as part of the research project: "*Ascariasis, anti-helminthic treatment and its effects on the allergic type immune response in a rural population of Bolívar* (Contract 590-013, Principal Investigator: Luis Caraballo)". Participants were identified from an epidemiological survey performed in Santa Catalina to estimate the prevalence of asthma and allergic rhinitis in a population-representative sample of 739 subjects using an ISAAC-based questionnaire previously validated in our country. Affirmative cases were confirmed by a physician of the research staff using the same criteria defined above for the population study of Cartagena de Indias.

Urban/rural study

Palenque

Participants were identified from a cohort of afro-descendant individuals whose parents and grandparents were from the village of San Basilio de Palenque. Two groups of subjects were recruited: one lived in this rural town and the other in urban neighborhoods of Cartagena de Indias. The presence of allergies in these subjects was evaluated by self-perception and confirmed by an allergist. Serum samples were taken as part of a research funded by the University of Cartagena, blood samples were taken for further studies and are part of the research funded by the University of Cartagena, according to Resolution No. 4396, 2010 (PI, Javier Marrugo, see Attach 2) and was approved by the Ethics Committee in institutional research.

Quantification of specific IgE to allergenic extracts

Subjects included in the asthma case-control study had information about serum specific IgE levels against *B. tropicalis*, *D. pteronyssinus* and *A. lumbricoides*, determined by ImmunoCap system (Phadia100, Thermo, Sweden). A cut-off value of 0.35 kU/L was used to define sensitization.

Determination of anti-rBlo t 8 specific IgE by indirect ELISA in humans

sIgE against rBlo t 8 was detected by ELISA as described previously [67]. Microtiter plates were incubated with 100 µL of the antigen per well ON (0.5 ug/mL). They were washed 5X with PBS-Tween 20-0.1% and blocked with 100 µl of 1% bovine-serum albumin, 0.02% sodium azide in PBS for three hours at room temperature. After washing again 5X, 100 µL of human sera (diluted 1:5 in PBS- 3% BSA) were added to each well with further overnight incubation at room temperature. Plates were then washed 5X and 100 µL of alkaline phosphate conjugated anti-IgE (diluted 1:500 in buffer Tris pH 8.0 0.05 M, BSA 1%, MgCl2 1 mM, sodium azide 0.02%) were added and incubated for two hours at room temperature. After the final wash (5X), colorimetric reaction was done adding 100 µL of para-nytrophenil diphosphate (pNPP) at RT for 30 minutes and blocking solution with NaOH3N. Absorbance at 405 nm was determined using a spectrophotometer. Results were expressed in optical densities (OD). The cut-off point (0.13 OD) was defined as the mean of 12 negative control sera plus 3 SD. All samples were assayed in duplicate, and inter-assay and intra-assay variation coefficients were lower than 15% and 10%, respectively.

Basophil activation test

The blood samples used to perform the basophil activation test were taken as part of the project "*Molecular characterization of the family of glutathione transferases derived from Ascaris lumbricoides: an IgE binding antigen*" (Code 110765842788, Contract number 201-2015, IP Josefina Zakzuk). Peripheral blood (6ml) was collected in ACD tubes (Vacutainer, Becton Dickinson, Meylan Cedex, France) from 3 mite allergic patients with IgE reactivity rBlo t 8 and 1 allergic patient without reactivity to rBlot8 and two healthy controls without IgE reactivity rBlo t 8, peripheral blood was refrigerated at 4ºC, until its use at 3 hours.

Basophil activation test was done by flow cytometry using the Allergenicity Kit® (Beckman Coulter, Inc. CA, USA) following instructions from the manufacturer. Allergenicity Kit is based on analysis of CD203c expression marker in CD3- CRTH2 + cells. 100 uL of whole blood were incubated with rBlo t 8 at different concentrations (0.1, 1 and 10 ug / mL), the positive control was anti-IgE and the negative control: PBS, with the antibody cocktail and the activation solution to 37 ° C for 15 minutes. then, the reaction was stopped with STOP solution®, the lysis of red blood cells was performed with Lysing Solution®, the sample was centrifuged at 200 g, washed with PBS and finally the obtained cells were re-suspended in PBS-paraformaldehyde.

The sample was read on a 9-color Cyan ADP flow cytometer.100 µL of blood was incubated with Bt, rBlo t 8(0.1 µg, 1 µg or 10 µg) or PBS, for 15 minutes at 37ºC. At least 500 basophils were counted in each assay (at least 10000 – 20000 events).

Allergen-induced upregulation of CD203c was calculated using median fluorescence intensities (MFI) obtained with stimulated (MFI stim) and unstimulated (MFI control) cells and expressed as the Stimulation Index (SI), (SI = MFI stim /MFI control). SI of ≥ 2.0 was considered indicative of a positive response.

Statistics Section 1

Most analyses were done using SPSS version 24.0 (Chicago, IL, USA). Frequency rates and their 95% confidence intervals (CI) were obtained with Epidat 3.1 (Xunta de Galicia, PAO/WHO). Specific IgE values were not normally distributed and reported as the median value and its inter-quartile range. Mann–Whitney U (MW) test was used for comparison of continuous variables. Differences between proportions were analyzed by Pearson chi-squared test. Multivariate binary logistic regression was used to analyze the relationships of rBlo t 8 sensitization and asthma presentation (dependent variable). Age, gender, place of residence (Cartagena, Santa Catalina) and HDM sensitization were included as co-variates in the multivariate model. Crude (OR) and adjusted odds ratios (aOR), their 95% CI and p-values were calculated. GraphPad Prism v7.0 (GraphPad Software, San Diego, CA). The analysis of basophil activation tests was performed using the FACS diva Software. Statistical significance were P value: P<0,05= *; P<0,005=**, P<0.001=***P > 0.05= no significance.

Section 2: Animal model of rBlo t 8 sensitization

Recombinant allergens

Recombinant Blo t 8 (rBlo t 8) sequence was isolated from cDNA library and was obtained as described previously [109]. Determination of endotoxin content in the protein solution, measured by a quantitative colorimetric assay (Thermo Scientific, USA), indicated it was pyrogen free (< 0.01 EU/mL). OVA was purchased to Thermo Scientific as an endotoxin free product.

ELISA for immunoglobulin isotypes to recombinant allergens

Total IgE, IgG1 and IgG2 was determined using commercial kits (eBioscience™ Mouse Total IgG2a, IgG1, ELISA Ready-SET-Go) following manufacturer instructions. For allergen specific IgE, IgG1 and IgG2a level determination, microtiter plates were coated with the allergens at 5 µg/mL diluted in PBS by overnight incubation at 4°C. Wells were then blocked with PBS 1% BSA 0.05% Tween 20 for 2 hours at RT. Plasma samples were incubated ON at 4°C. After 5 washes, wells were incubated with biotin labeled anti-mouse IgG1, IgG2a or IgE (diluted 1:1.000 in blocking buffer) for 1 hour at RT. After 5 washes, alkaline-phosphatase streptavidine (diluted 1:2.000 in 50 mM Tris 1% BSA 1 mM MgCl2 pH 8.0) was added to the wells. pNPP, dissolved in 10% diethanolamine 0.05 mM MgCl2 at 1 mg/mL, was used as substrate solution. After 30 minutes of incubation, the reaction was stopped with NaOH 3N. Optical densities were obtained by reading the plates in a spectrophotometer at 405 nm. To increase the sensitivity of IgE ELISA, avoiding the competitive effect of IgG for antigen binding, this immunoglobulin was depleted by incubation with protein G sepharose [110].

Animals

Balb/c mice (aged 6 weeks) were purchased from the "*Instituto Nacional de Salud"*, (INS, Bogota, Colombia). Throughout the study, six animals per cage were housed in a room with laminar air flow, a temperature of 22 ± 2°C, and relative humidity of 55 ± 5%. Animal care and treatments were carried out in accordance with the guidelines established by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care of Cartagena´s University.

Mice immunization

Female BALB/c mice (6 mice per antigen) were immunized intraperitoneally (i.p.) with: PBS (Negative control) rBlo t 8,OVA (Positive control) or adsorbed to Alum hydroxide (Imject® Alum, Thermo scientific). Animals received three injections each one (20 µg PBS, 20ug rBlo t 8 or 25 ug OVA) of antigen adsorbed onto 2 mg Alum, 200 µL every week. Seven days after the last immunization, blood was collected and mice sacrificed and the spleens were removed under aseptic conditions.

Lung Histology

Lungs were removed and immersed in 10% neutral buffered formalin. The tissue was cut into 4 µm thick sections and stained with Hematoxylin Eosin (HE) or Periodic Acid Schiff (PAS). Plates were visualized by light microscopy to evaluate lung inflammation and mucus production. To determine the severity of inflammatory cell infiltration, peribronchial and perivascular inflammatory cell counts were performed, based on a

modification of the 5-point scoring system described by Myou et al [111]. The peribronchial and perivascular inflammation was graduated independently according to the number of inflammatory cells: 0 = normal, 1 = few cells, 2 = a ring of inflammatory cells 1-2 cells deep, 3 = a ring of inflammatory cells 3 -5 cells deep and 4 = a ring of inflammatory cells> 5 cells deep. Total lung inflammation was defined as the sum of the peribronchial inflammation scores perivascular.

The extension of mucus production was determined by the blind quantification of the abundance of PAS-positive goblet cells in each airway (identified by magenta coloration), using the 5-point classification system described by Tanaka et al [112] ., obtaining numerical scores: 0 = 0- 5% Positive PAS cells, 1 = 5-25%, 2 = 25-50%, 3 = 50-75% and 4 = 75% Both scores, for inflammation and mucus production , will be calculated in an average of six fields in their respective sections of lung tissues. The images were captured in an Eclipse 400 microscope connected to a DS-Fi1 camera (Nikon, Japan) at 40x and 100x as final magnification.

Bronchoalveolar lavage (BAL)

Bronchoalveolar lavage (BAL) was done with 3× 600ul of PBS. Cellular infiltrate analysis was performed by flow cytometry for identification of macrophages, alveolar macrophages, eosinophils and neutrophils. The BAL was centrifuged at 1500 rpm for 5 minutes at 4 ° C, the supernatant was aspirated and stored at -80 ° C.

The cell pellet was treated with 1 ml of Lysis Buffer 1X and left in incubation for 5 minutes on ice, then centrifuged at 1,500 rpm for 5 min and washed with PBS. Cells were resuspendend in 160 µL of Stain buffer (PBS 0.5% BSA) and the following cocktail of monoclonal antibodies were added: efluor450 - CD45, FITC - CD11c, PE - CD11b, PerCPefluor - CD170 (SiglecF), APC - Ly6G Stain reaction was incubated for 30 minutes at 4° C in the dark and washed with 500 µL of Stain Buffer. Cells were analyzed in a BD FACS ARIA® cytometer. As detailed in Table 3, BAL cells were identified as follows: alveolar macrophages (Siglec-F+CD11c+), eosinophils (Siglec-F+CD11c-) and neutrophils (CD11b+Ly6G+).

Table 3. Gatting Strategy for BAL

Marker	Eosinophils	Neutrophils	Alveolars Macrophages
CD45	dim	+	+
CD11b	+	+	-
CD 11C	-	-	+
Ly6G	-	+	-
CD3	-	-	-
SiglecF	+	-	+

Passive Cutaneous Anaphylaxis (PCA) model

BALB / c consanguineous, 6- week female mice (weight range: 18 to 22gr) not exposed to some allergen previously were used. To induce local anaphylaxis, 100uL and 10uL in ear were administered intradermally in the ventral region of the abdomen,

the serum of the sensitized mice: PBS, rBlo t 8 and OVA, anaphylactic reaction was done 24 hours later by intravenous administration of 200µL of a 0.5% solution of the Evans blue dye (Panreac, USA) with 25µg of the allergen. After the challenge, at 2 hours later, extravasation was observed and the photographs are shown, the animals were sacrificed by lethal dose of Eutanex®, and skin portions of 1cm x 1cm were extracted and incubated in sterile tubes with 700µl of formamide. Also, the ears will be cut, placed in sterile tubes with lid containing 700µL of formamide, incubated at 37 ° C for 72h. At the end of this time the Evans blue dye at 620 nm will be quantified in a spectrophotometer (Multiscan GO, Thermo scientific). Titration curve was performed for extravasated Evans blue concentration values and expressed in mg/site.

Statistics Section 2

Statistical analysis were done with GraphPad Prism v7.0 (GraphPad Software, San Diego, CA). In order to verify differences among more than three mice groups, the results were analyzed using the one-way ANOVA test and the Tukey's post test. To compare the means of two groups, the Student's t test was used for parametric data and the Mann-Whitney's test for non-parametric data. Statistical significance were P value: P<0,05= *; P<0,005=**, P<0.001=***P > 0.05= no significance.

VI. Ethical considerations

All the procedures included were designed and executed in accordance with national and global ethical guidelines that protect the environment, the welfare of experimental animals and human health. The experimental models that contemplate the use of

laboratory mice are governed by the regulations in force established in the law 84 of 1989 of the Republic of Colombia, Chapter VI, referring to the use of live animals in experiments and research and according to the resolution 594 of July 11 of 1996.

We have the necessary infrastructure and conditions for the care and use of laboratory animals, in accordance with the legislations and recommendations stipulated by the CCAC (Committee on Care and Use of Laboratory Animals, US), as well as the operating manual established by our biotech.

In accordance with the Declaration of Helsinki; the CIOMS Guidelines and Resolution 008430 of October 4, 1993. This research is of minimal risk and this study expressed the risks and security guarantees that are provided to the participants prior to the signing of the Informed Consent by the person or their legal representative (See Attach1-4). The exceptions provided in Resolution 008430/93 and was carried out with the prior authorization of the institutional ethics committee. The confidentiality and privacy of the personal data were made according to the rules established in chapter III of Decree 1377 of June 27, 2013 and the personal data treatment policy of the University of Cartagena.

VII. RESULTS

Asthma study

A flowchart depicting the number of subjects according to asthma status and place of residence is shown in Figure 2. Descriptive information of participants is shown in Table 4.

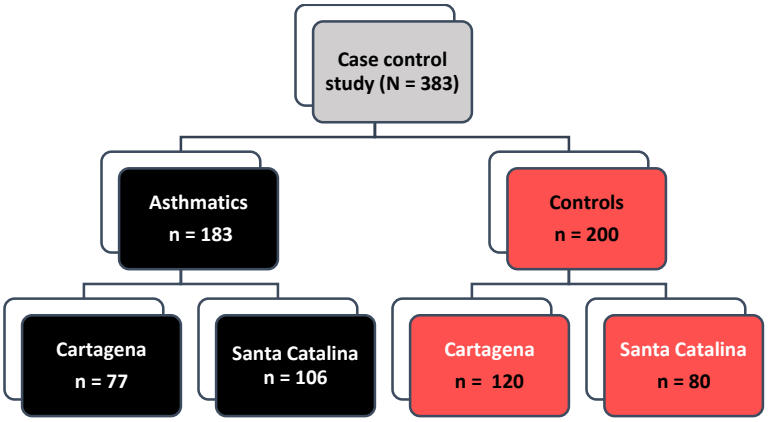

Figure 2 Design case control study

Table 4. Descriptive of the study population.

	Cartagena		St. Catalina	
	Cases (n = 77)	**Controls (n =120)**	**Cases (n = 106)**	**Controls (n = 80)**
Age (mean, SD)	30.6 (17.8)	32.6 (16.1)	24.1 (17.5)	30.7 (16.5)
Females (n, %)	47 (61%)	57 (47,5%)	70 (66%)	58 (72.5%)
***B. tropicalis* sensitization**	55 (71.4%)	11 (9,1%)	60 (56.6%)	25 (31.25%)
***D. pteronyssinus* sensitization**	43 (55.8%)	9 (7,5%)	66 (62.3%)	16 (20%)
***Ascaris* sensitization**	48 (62.3%)	21 (17.5%)	61 (57.5%)	28 (35%)
rBlo t 8 sensitization	**13 (16,8%)**	**70 (58.3%)**	**39 (36.8%)**	**59 (73.8%)**
***Ascaris* infection**	**ND**	N.D.	55 (51,8%)	47 (58,75)

Considering all subjects included in the serology study, the frequency of sensitization to rBlo t 8 was significantly higher in controls than in asthmatic subjects (64,4% versus 23,9%, respectively. $Chi^2 = 56,5$ $p < 0, 0001$).

In the multivariate logistic regression analysis, rBlo t 8 sensitization kept associated with asthma after adjustment by age, gender, place of residence and other causes of sensitization that has been found to be relevant for asthma presentation in our environment. (See Atachhments 5-9)

Table 5. Logistic regression of Cartagena and St. Catalina study.

	P value	**aOR****	**95% CI for OR**	
			Inferior Limit	Superior Limit
rBlot8 Sensitization*	**0,000**	0,090	0,047	0,174
Age	0,234	0,991	0,976	1,006
Female	0,188	1,501	0,819	2,751
Living in Cartagena (vs. St. Catalinas as reference)	0,043	0,546	0,304	0,982
Ascaris sensitization	0,017	2,355	1,165	4,759
B. tropicalis sensitization	0,001	3,732	1,730	8,052
D. pteronyssinus sensitization	0,024	2,458	1,128	5,358

*The power to study to detect the observed OR in relation with the sample size was 1.0. Epidat 3.1

**adjusted Odds Ratio

It was also observed that sensitization to rBlo t 8 was higher in subjects living in Santa Catalina (51,3%) than in those living in the urban center of Cartagena (42,3%), but this difference was not significant (p = 0,09). However after adjustment by age, gender and asthma, living in Cartagena was negatively associated with rBlo t 8 sensitization

(aOR: 0.48, 95%CI: 0,29 – 0,79, p = 0.004). Differences between asthmatics and controls it is also worth mentioning that evident in terms of the strength of the IgE response (Figure 3). Higher specific IgE levels were found in controls living in St. Catalina than in Cartagena, but this was not replicated in asthmatics.

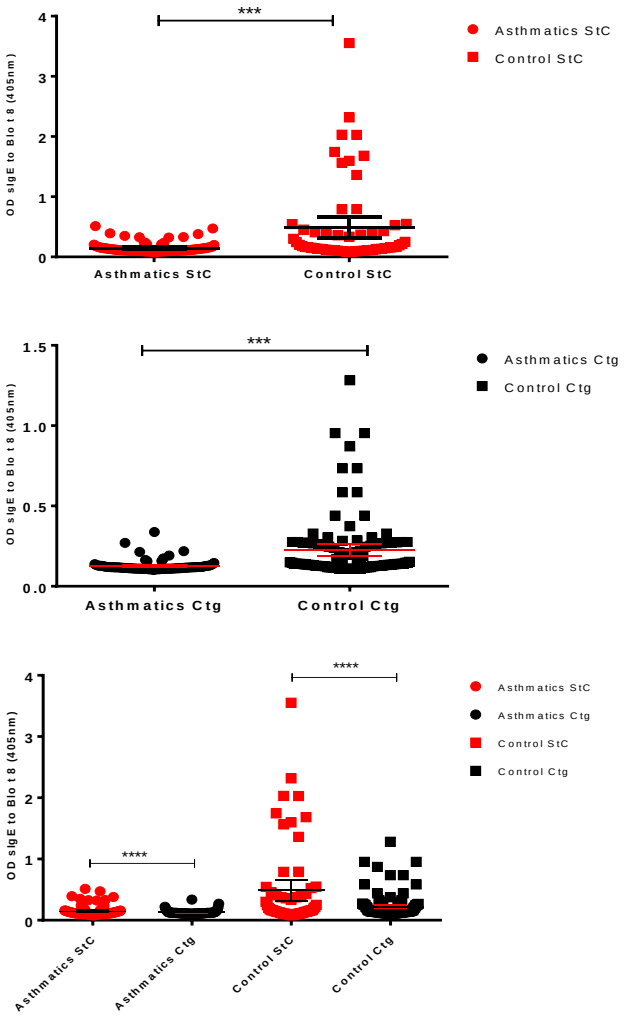

Figure 3. Case control study. *SIgE levels to rBlo t 8 in 2 populations A Cartagena, B Santa Catalina and C the merge of tow graphics. Each dot represents one individual, and the horizontal lines represent the IC 95% P value: P<0,05= *; P<0,005=**, P<0.001=***P > 0.05= n.s.*

Urban/Rural contexts and its influence on Blo t 8 sensitization

A flow diagram of selection of subjects for this analysis is provided in Figure. 4. The sIgE levels to rBlo t 8 was higher in those subjects living in the rural town of Palenque than participants living in the urban context ($p < 0.0001$). Higher specific IgE levels against Blo t 8 were found in those living in the rural environment (Figure 5). Results from the multivariate logistic regression analysis also indicate that living in the urban environment is negatively associated with Blo t 8 sensitization (Table 7).

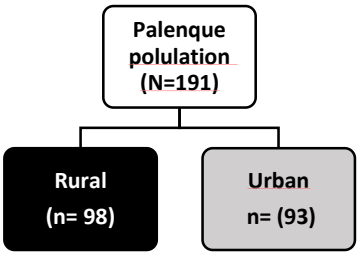

Figure 4. Design case control study in Palenque population in rural or urban context

Table 6. Descriptive of the Palenque population

	Population N(191)		P value
	Urban (n = 93)	**Rural (n =98)**	
Age (mean, SD)	11.9 (4,0)	11.7 (4,0)	0.824
Females (n, %)	45 (48.4%)	49 (50%)	0.295
***B. tropicalis* sensitization by SPT**	21 (22.6%)	6 (6.1%)	0.016
***D. pteronyssinus* sensitization by SPT**	11 (11.8%)	2 (2%)	0.007
***D. farinae* sensitization by SPT**	8(8.0%)	2 (2%)	0.042
P. Americana sensitization by SPT	1 (1.1%)	0	0.303
rBlo t 8 sensitization by ELISA	23 (21,7%)	45 (45.9%)	**0.002**
Atopy	21 (22.6%)	6 (7,5%)	**0.001**
Any allergy	36 (38,7)	20 (20,4%)	**0.006**

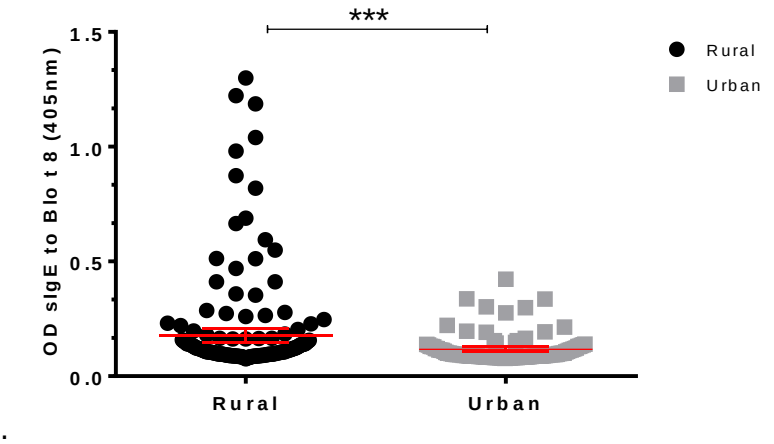

Figure 5. Case control study in Palenque population. Specific IgE levels to rBlo t 8 Each dot represents one individual, and the horizontal lines represent the IC 95% P value: P<0,05= *; P<0,005=**, P<0.001=P > 0.05= ns***

Table 7. Logistic regression for Palenque population

	P value	OR	I.C. 95% EXP(OR)	
			Inf	Sup
Gender	0,435	1,279	0,689	**2,371**
Age	0,231	1,049	0,970	**1,133**
Atopy	0,050	2,485	1,000	**6,177**
Living in Cartagena (REF. Palenque)	**0,001**	**0,320**	**0,165**	**0,621**

rBlo t 8 does not induce basophil activation

Table 8. Characteristics from selected BAT patients

Subject	Gender	Age	Allergies	sIgE to Blo t 8 (OD)
1	F	62	AR	1,565
2	F	58	AR	2,032
3	M	17	AR	3,552
4	F	34	AR	0,0922
Control 1	M	24	NONE	0,1084
Control 2	F	24	NONE	0,098

The allergenic activity of rBlo t 8 was analyzed using basophils from four HDM-allergic individuals (Table 9). The CD203c expression was measured after incubating the patients' cells with increasing concentrations of rBlo t 8 and *Bt* extract. In one of the five tested patients, rBlo t 8 induced a CD203c expression at a concentration of 10 µg/ml. Bt do not induced an expression of CD203c at concentrations 1 µg/ml.

Table 8. Basophil Activation Test Results in 4 patients and 2 controls.

A

Subject	PBS	Blo t 8 0,1ug/mL	Blo t 8 1ug/mL	Blo t 8 10ug/mL	Bt	AntiIgE
			% of Activation			
1	0,23	0,52	0,075	0	0,43	**52,4**
2	1,32	1,84	0,61	2,2	1,48	0,71
3	0,41	6,01	0,24	4,9	2,24	6,31
4	0,51	5,42	0,33	2,41	21,8	**73,2**
Control 1	0,7	0	0,4	0,5	0	**64,1**
Control 2	0,98	3,03	0,35	0	8,63	**74,8**

B

Subject	PBS	Blo t 8 0,1ug/mL	Blo t 8 1ug/mL	Blo t 8 10ug/mL	Bt	AntiIgE
			SI			
1	1,00	1,12	1,08	1,04	1,23	**5,05**
2	1,00	1,21	1,08	1,08	1,11	1,24
3	1,00	1,28	0,96	1,14	1,10	1,26
4	1,00	1,15	0,92	1,01	1,72	**7,76**
Control 1	1,00	0,91	0,98	0,96	1,02	**5,11**
Control 2	1,00	1,05	1,00	**2,93**	1,52	**11,60**

Panel A. Results are shown in percent of activation, which is defined as the relative frequency of basophils over-expressing CD203. Cut off are determined by results obtained in the negative control of each patient. Panel B. Results are shown in Stimulation Index (SI) values.

Experimental results

BHR animal model

Groups of BALB/c mice were sensitized subcutaneously with rBlo t 8, OVA or PBS co-adsorbed into alum at days 0, 7, 14 challenged intranasally with specific antigen on days 21, 22 and 23, and sacrificed 24 h later (Figure 6a). rBlo t 8 induced allergen-specific IgE, but do not induce airway hyperreactivity, bronchial inflammation or mucus secretion. (Figure 6b). Representative micrographs of tissue sections of PBS, rBlo t 8 or OVA-sensitized mice, stained with PAS and HE are shown respectively in figure 6b.

The total amount of inflammatory cells was significantly elevated in the OVA group, but not in mice sensitized with rBlo t 8. Goblet cell hyperplasia was also evident for OVA sensitized mice, but not for those receiving Blo t 8 or PBS. Results from the FACS analysis indicated that BAL from the OVA group was rich in eosinophils and neutrophils in contrast to Blo t 8 and PBS groups, in which only alveolar macrophages were detected. (Figure 7)

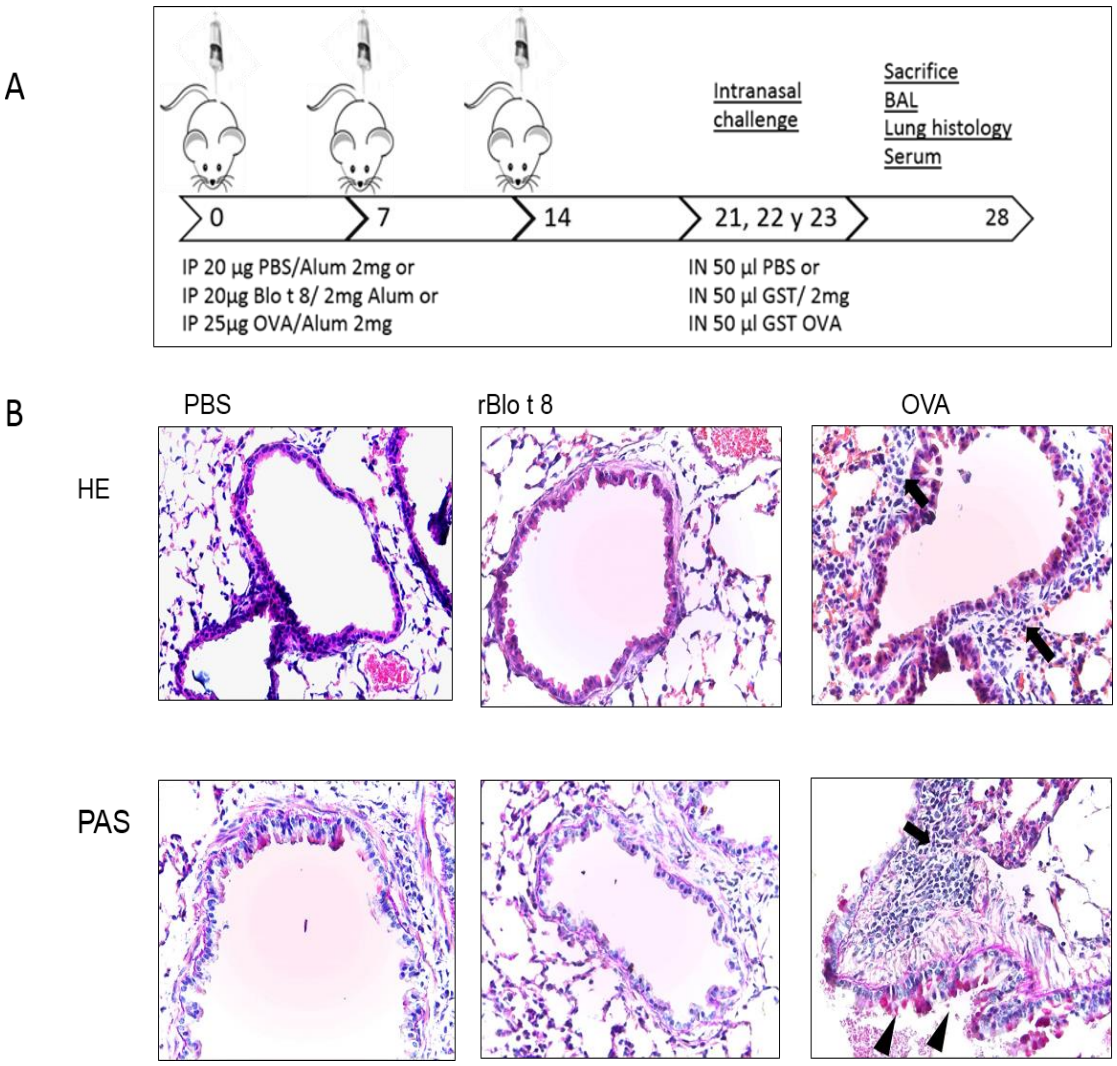

Figure 6. Animal model of HBR. *A. Scheme of the sensitization procedure. B. Histology of protocol groups exposed to different allergens challenged to the specific allergen stained with HE and PAS arrows indicate inflammatory infiltrate in OVA group and head arrows indicate goblet cells.*

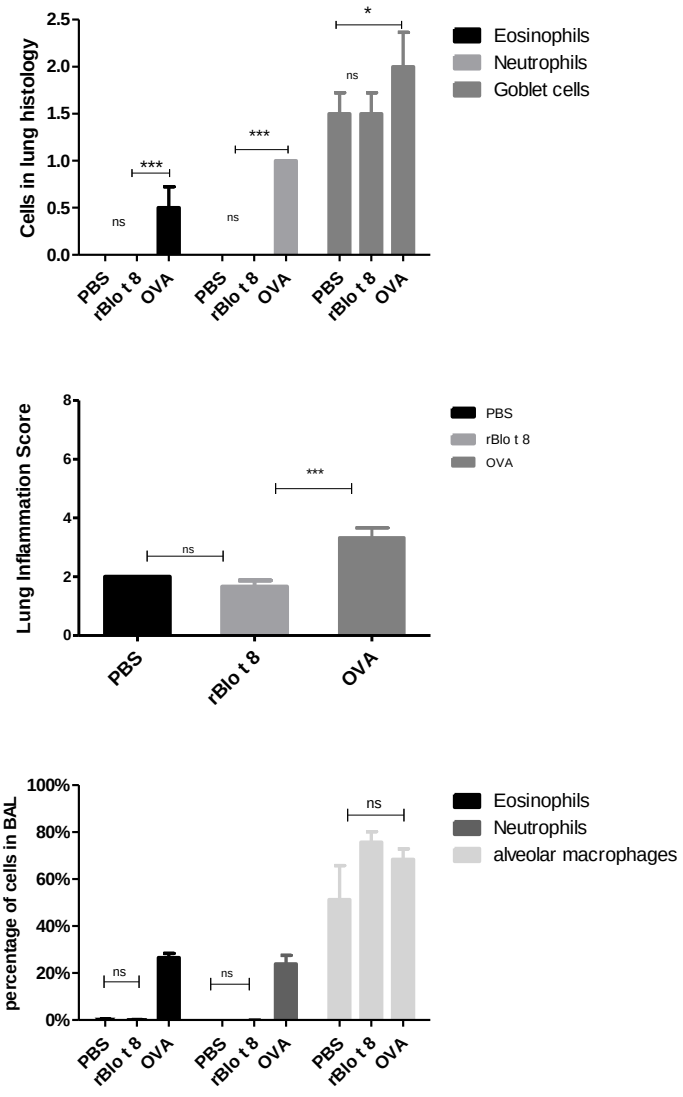

Figure 7. The score of lung inflammation and relative proportion of the inflammatory cell components in BAL. A. The score of lung inflammation B. Relative proportion of inflammatory cells components in BAL by flow cytometry. (N=18; n = 6) P value: P<0,05= *; P<0,005=**, P<0.001=***P > 0.05= ns.

There were significant differences in Total IgG1 levels among groups, being higher in the OVA group (Figure 8a). For Total IgG2a levels, there were no statistically significant differences between the three groups (p value= 0, 2555) (Figure 8b). Blo t 8 and OVA induced similar levels of total IgE, albeit higher that the PBS group (Figure 8c).

In regard to allergen specific antibodies, rBlo t 8 specific IgG1 (sIgG1) was higher than OVA- or PBS- sIgG1 and was statistically significant between the three groups, (Figure 9a) IgG1 specific levels to OVA was higher than rBlo t 8 or PBS group, (Figure 9b) and was statistically significant between three groups, IgG2a specific levels to rBlo t 8 it was not statistically significant between the three groups (Figure 9c). IgG2a specific levels to OVA it was not statistically significant between the three groups (Figure 9d). rBlo t 8 induced allergen-specific IgE (Figure 9e), however, rBlo t 8 induced higher specific IgE levels than OVA (Figure 9 e-f).

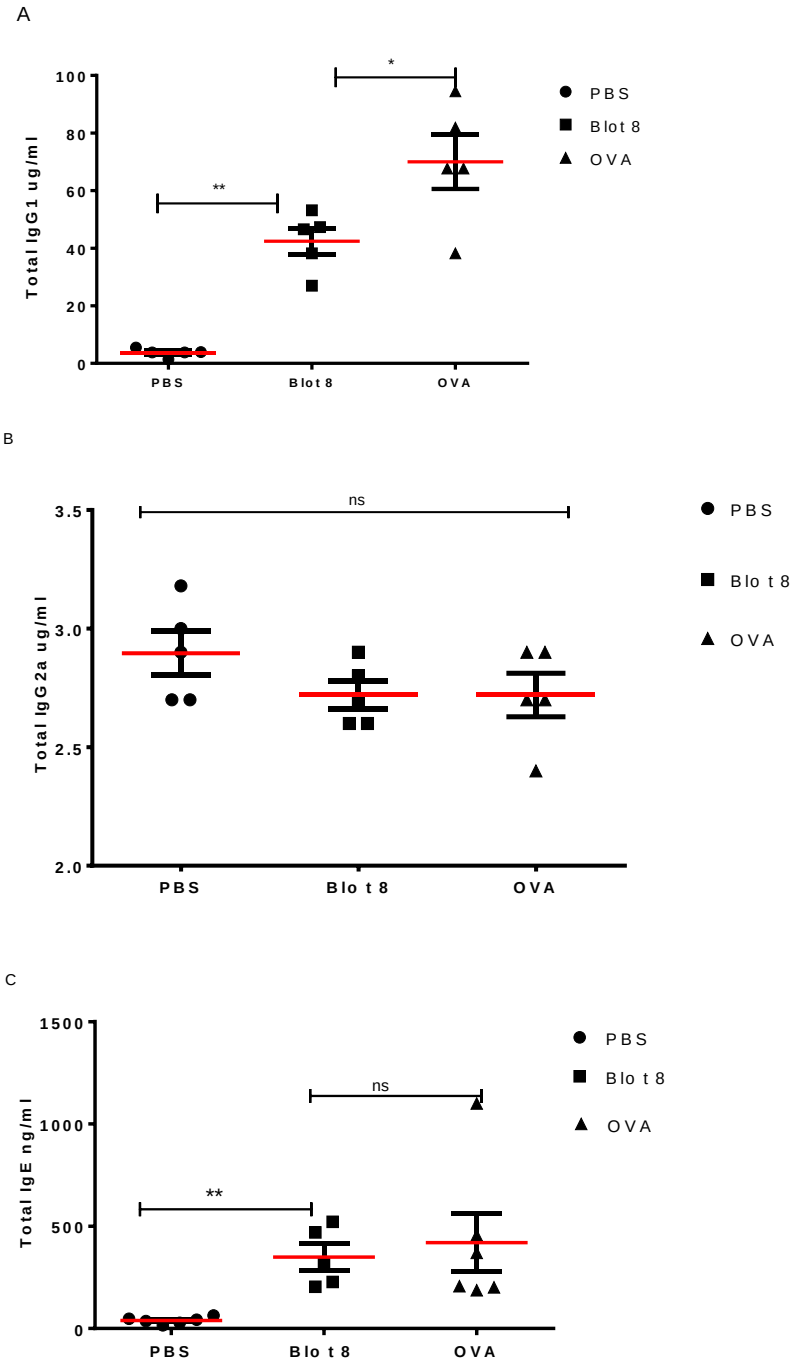

Figure 8. Total Abs in animal model. *Each dot represents one animal pooled from three independent experiments (N=18; n = 6). Red lines represent mean and Black Lines represent QI Confidence Interval 95%. The p values were calculated by ANOVA test. P value: P<0,05= *; P<0,005=**, P<0.001=***P > 0.05= ns.*

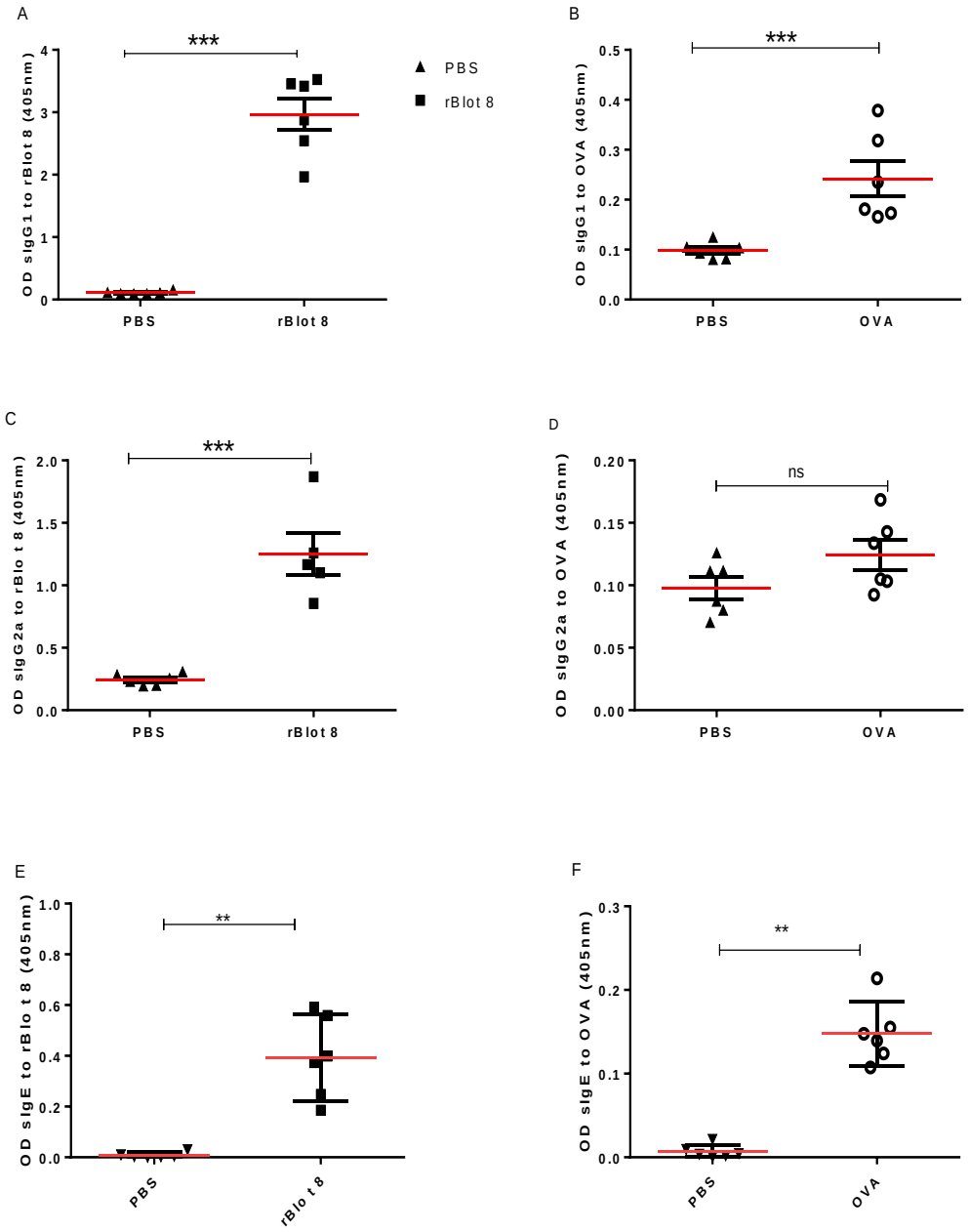

Figure 9. Specific antibodies to allergens in animal model. *Each dot represents one animal pooled from three independent experiments (N=18; n = 6). Red lines represent mean and Black Lines represent QI Confidence Interval 95%. The p values were calculated by ANOVA test, P value: P<0,05= *; P<0,005=**, P<0.001=***P > 0.05= ns*

rBlo t 8 induce passive cutaneous anaphylaxis *in vivo*.

Ear and skin pigmentation in PBS sensitized animals showed little to no dye extravasation. Ear and skin pigmentation in rBlo t 8 sensitized animals showed dye extravasation and OVA sensitized animals shows presence of blue dye extravasation. (Figure 10a).

Anti-serum was intradermally injected into the mouse ear and skin. After 24 h, an injection of 250 µg antigen in 0.5% Evans blue solution was administered into the mouse tail vein. Mice were euthanized 2 h after the antigen challenge, and the ear was then removed for measurement of the amount of dye extravased. (Figure 10a). Representative photographs of ears and skin after PCA and (figure 10b) quantitative data for skin or ear-tissue content of Evans blue (Figure 10c) as described in the "*Methods*" section. The values are expressed as the mean ± SEM from three independent experiments. P value: P < 0,05= *; P < 0,005=**, P<0.001=***P > 0.05= ns.

PBS do not induced passive cutaneous anaphylaxis, Ovalbumin induced passive cutaneous anaphylaxis and IgE-mediated passive cutaneous anaphylaxis model were used for the immediate-type allergic responses. rBlo t 8 group induced intermediate passive cutaneous anaphylaxis.

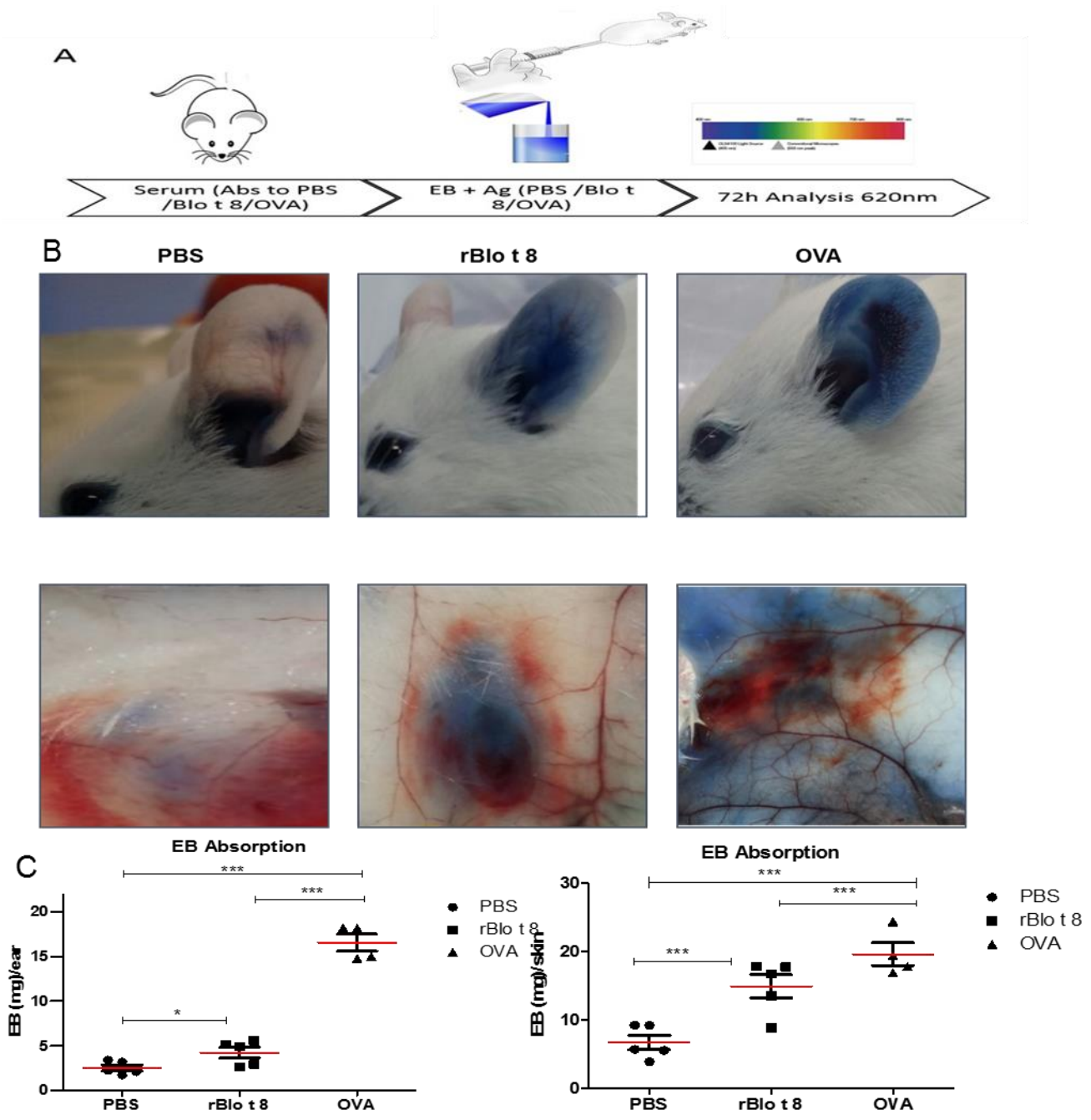

Figure 10. IgE-mediated PCA model*. A. Protocol Local Anaphylaxis Assay. B. Ear pigmentation in PBS, rBlo t 8 and OVA sensitized animals. Ear and skin pigmentation in PBS sensitized animals show little to no dye extravasation. Ear and skin pigmentation in rBlo t 8 sensitized animals shows dye extravasation and OVA sensitized animals shows presence of blue dye extravasation. There is allergic response because a bright blue coloring indicative of increased vascular permeability, a direct result of cell activation in the ear tissue. C. Values derived from spectrophotometric measurements taken at 620 nm and this values was interpolated into a curve, this measurements was done in mg/site. P value: P<0,05= *; P<0,005=**, P<0.001=***P > 0.05= ns.*

VIII. GLOBAL DISCUSSION OF RESULTS

This thesis makes contributions to the knowledge about the specific IgE response to the GST allergen derived from *B. tropicalis*, its epidemiological impact on asthma, the patterns of sensitization according to different urbanization contexts and its allergenic potential. We found a low prevalence of sensitization to rBlo t 8 in different cohort of patients living in the Department of Bolivar, including rural, intermediate and urban areas.

Less than 30% of asthmatic subjects who were living in Santa Catalina and Cartagena were sensitized to a locally isolated Blo t 8 isoform and unexpectedly, sensitization rates were higher in non-asthmatic subjects. Results from experimental sensitization in mice supported that this allergen lacks capacity to induce airway inflammation. In spite that Blo t 8 induces a high production of specific IgE (at least higher than OVA), its allergenic potential to induce PCA was lower than this allergen.

In line with our hypothesis that not all allergens necessarily induce pathological allergic response, the results of this work indicate that the specific IgE response to this molecule does not increase the risk for asthma presentation. Although our results support that Blo t 8 has allergenic activity, as observed previously by means of SPT in humans, it seems to be of low magnitude. As observed in the PCA, Blo t 8 induced - dye extravasation was about two times lower than the response elicited by the classical allergen OVA.

Possible explanations for the inverse effect on the presentation of asthma is rBlo t 8 induces the production of specific IgE, but this may be of low avidity. Also, the concomitant production of IgG2a – observed in the sensitization model – may be protective from airway inflammation and the induction of pathological IgE mediated responses. In line with this, Blo t 8 specific IgG titers must be measured in order to identify the role of this response on the observed results. Other aspects to be analyzed are if the homology of rBlo t 8 with the mammalian GSTM has an impact on tolerance mechanisms, especially those related with T cell regulation.

If the opposite direction on the response they seem to have protective effect (and its mechanisms) should be further investigated by more appropriate study designs that may get close to causality approximations. Longitudinal cohorts that follow the dynamics of Blo t 8 sensitization and if this prevents asthma presentation may be suitable for this purpose. Different authors, including our group, have previously found protective roles of IgE responses. Zakzuk *et al* found that children with higher total IgE levels in cord blood are protected from developing wheezing [113], but this is the first time that we know about a HDM allergen that induce sensitization, in a more frequent way, in non-asthmatic individuals than in patients with this disease.

There is so much to be investigated about allergens and disease pathogenesis and expected relationships are not generalizable[114]. Although it is not same case, the recent discovery that a classical allergen such as the HDM tropomyosin was a ligand

for a receptor coupled to anti-inflammatory responses in the lung that prevented asthma development in mice was also surprising. The genetic determination to respond in a different manner to invertebrate tropomyosins seemed to be the explanation for a same molecule to have contrary roles: being protective[21] and induce asthma [115].

Previous studies have shown that overexpression of GST[47,58,116] can protect cells *in vitro* against various agents, both therapeutic and carcinogenic but this results and mechanisms should be confirmed . Genetic variation of GST probably could influence their IgE binding and clinical reactivity. However, GST expression is not the only variable involved in the protection of asthma and allergic response [114,117].

We found that Blo t 8 sensitization rates markedly changed among environments. In the asthma case-control study, it was observed that IgE responses to rBlo t 8 were higher in the town of Santa Catalina than in Cartagena. This same pattern was observed when Palenqueros living in an urban context were compared with those living in the rural town of Palenque.

Our hypothesis for higher specific IgE responses to Blo t 8 in less urbanized populations is related with the fact that helminthiasis are more common in these environments[85,118,119]. ¿Could cross-reactivity (CR) of Blo t 8 with helminth GST explain this results? All species usually contain different GST isoforms with moderate sequence conservation among them. This results in a wide repertoire of different epitopes with important variations among isoforms. Proteomic analyses they must be

made to decipher the allergenic complexity of GST families. As far as we know there is no clarity of this phenomenon[85], some studies are in favor of CT and others that do not support it. Acevedo, et al described that, at the level of natural extracts, *B. tropicalis* and *Ascaris suum* has an IgE CR 23 kDa component identified as GST by mass spectrometry [66,120]. On the contrary, in the North American population obtained results were different. Mueller et al, examined the molecular structure of Bla g 5, Der p 8, Blo t 8, and Asc s 13 searching for possible sites of CR in sensitized patients of a temperate climate. Their results showed few contiguous surface residues shared by these allergens, which made cross-reactivity unlikely. Also, Bla g 5 sensitized North Americans did not recognize Blo t 8 or Asc s 13 [109]. A remarkable finding from this study was that Colombian´s serum patients did have positive specific IgE responses to the four GSTs that were evaluated (co-sensitization). Our opinion with these results is that CR among helminths GST and rBlo t 8 is still possible, but the CR isoforms have not been identified. Other GST with moderate sequence conservation are in fact cross-reactive. Santiago H et al demonstrated that filarial infected individuals has cross-reactive antibodies recognizing the GST from this parasite and Bla g 5 (a GST from cockroach [109] [64].

One limitation of the study is the analysis of only one isoform of Blo t 8. Preliminar proteomic analysis of the GST from *B. tropicalis* indicates that at least 6 isoforms are

expected. We had previously observed a high correlation between the specific IgE against the native and the studied recombinant isoform, but this tended to be of lower magnitude [120]. In this sense, our results about the immune response to this isoform may not be generalizable to the others.

IX. Perspectives

- To analyze the basophil activation response to rBlo t 8 in a greater number of sensitized subjects.
- To study the IgG response to rBlo t 8 and its relationship with the possible protective effect of sensitization in asthma.
- To replicate the animal model of airway sensitization in order to assess reproducibility.
- To measure cytokine levels (IL-6, IL-10, IL-17A, IFN-gamma y TNF-α, IL-5 and IL-13) in supernatants from spleen cell cultures and BAL, which are kept frozen.

X. CONCLUSIONS

- There is an inverse epidemiological relationship between sensitization to rBlo t 8 and asthma presentation.
- Mechanisms underlying this possible protective role of the specific IgE response to this GST should be further investigated.
- Results from the mouse model of asthma support that this allergen induces a strong specific IgE response, which does not translate in airway inflammation.
- If the intermediate potential to induce passive cutaneous anaphylaxis is involved in the lack of capacity to induce airway inflammation must be investigated.

XI. Conflict interest.

The authors declare that they have no conflict interest.

XII. REFERENCES

1 Mallol J, Crane J, von Mutius E, *et al.* The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three: A global synthesis. *Allergol Immunopathol (Madr)* 2013;**41**:73–85. doi:10.1016/j.aller.2012.03.001

2 Acevedo N, Sánchez J, Zakzuk J, *et al.* Particular characteristics of allergic symptoms in tropical environments: follow up to 24 months in the FRAAT birth cohort study. *BMC Pulm Med* 2012;**12**:13. doi:10.1186/1471-2466-12-13

3 Dennis RJ, Caraballo L, García E, *et al.* Prevalence of asthma and other allergic conditions in Colombia 2009 – 2010 : a cross-sectional study. *BMC Pulm Med* 2012;**12**:17–17. doi:10.1186/1471-2466-12-17

4 Eder W, Ege MJ, von Mutius E. The Asthma Epidemic. *N Engl J Med* 2006;**355**:2226–35. doi:10.1056/NEJMra054308

5 Caraballo L, Zakzuk J, Lee BW, *et al.* Particularities of allergy in the Tropics. *World Allergy Organ J* 2016;**9**:20. doi:10.1186/s40413-016-0110-7

6 Schnyder B, Schweri T, Thomann B, *et al.* [Allergy to house dust mites]. *Schweiz Med Wochenschr* 2000;**130**:443–7.http://www.ncbi.nlm.nih.gov/pubmed/10780059

7 Puerta L, Fernãndez-Caldas E, Mercado D, *et al.* Sequential determinations of Blomia tropicalis allergens in mattress and floor dust samples in a tropical city. *J Allergy Clin Immunol* 1996;**97**:689–91. doi:10.1016/S0091-6749(96)70315-9

8 Feng M, Sun W, Cheng X. Seasonal dynamics and distribution of house dust mites in China. *Biosci Trends* 2009;**3**:210–5.

9 Knopp S, Mohammed KA, Stothard JR, *et al.* Patterns and risk factors of helminthiasis and anemia in a rural and a peri-urban community in Zanzibar, in the context of helminth control programs. *PLoS Negl Trop Dis* 2010;**4**. doi:10.1371/journal.pntd.0000681

10 Cardoso LS, Costa DM, Almeida MCF, *et al.* Risk factors for asthma in a Helminth endemic area in Bahia, Brazil. *J Parasitol Res* 2012;**2012**:16–20. doi:10.1155/2012/796820

11 Hagel I, Di Prisco MC, Goldblatt J, *et al.* The Role of Parasites. 2004;**26**:75–83.

12 Caraballo L. Ascaris and Allergy. In: *Ascaris: The Neglected Parasite*. 2013. 21–50. doi:10.1016/B978-0-12-396978-1.00002-1

13 Rodriguez A, Vaca MG, Chico ME, *et al.* Rural to urban migration is associated with increased prevalence of childhood wheeze in a Latin-American city. *BMJ Open Respir Res* 2017;**4**. doi:10.1136/bmjresp-2017-000205

14 Cooper PJ, Rodrigues LC, Cruz AA, *et al.* Asthma in Latin America: A public health challenge and research opportunity. *Allergy Eur J Allergy Clin Immunol* 2009;**64**:5–17. doi:10.1111/j.1398-9995.2008.01902.x

15 Owen J. Kuby Immunology. *Kuby Immunol* 2012;**6**:574. doi:10.1016/S0076-6879(09)04815-0

16 Zakzuk J, Caraballo L, Coronado S. *Inmunoregulación inducida por helmintos Mecanismos, efectos y blancos celulares*. First. Berlin: : Editorial Académica Español 2016.

17 Radauer C, Nandy A, Ferreira F, *et al.* Update of the WHO/IUIS Allergen Nomenclature Database based on analysis of allergen sequences. *Allergy Eur J Allergy Clin Immunol*

2014;**69**:413–9. doi:10.1111/all.12348

18 Pomés A, Davies JM, Gadermaier G, *et al.* WHO/IUIS Allergen Nomenclature: Providing a common language. *Mol Immunol* Published Online First: 2018. doi:10.1016/j.molimm.2018.03.003

19 Cui Y. Structural biology of mite allergens. *Mol Biol Rep* 2013;**40**:681–6. doi:10.1007/s11033-012-2108-8

20 Buendía E, Zakzuk J, Mercado D, *et al.* The IgE response to Ascaris molecular components is associated with clinical indicators of asthma severity. *World Allergy Organ J* 2015;**8**:8. doi:10.1186/s40413-015-0058-z

21 Gour N, Lajoie S, Smole U, *et al.* Dysregulated invertebrate tropomyosin-dectin-1 interaction confers susceptibility to allergic diseases. *Sci Immunol* 2018;**3**:eaam9841. doi:10.1126/sciimmunol.aam9841

22 Wan Omar A, Patimah I, Rusliza B. Allergy to house dust mites and asthma. *Malaysian J Med Heal Sci* 2012;**8**:5–21.

23 Sánchez-Borges M, Fernandez-Caldas E, Thomas WR, *et al.* International consensus (ICON) on: Clinical consequences of mite hypersensitivity, a global problem. *World Allergy Organ J* 2017;**10**:1–26. doi:10.1186/s40413-017-0145-4

24 García E, Caraballo L. *Asma : enfoque integral y nuevas tendencias*. Editorial Médica Panamericana 2014.

25 Carnés J, Iraola V, Cho SH, *et al.* Mite allergen extracts and clinical practice. *Ann Allergy, Asthma Immunol* 2017;**118**:249–56. doi:10.1016/j.anai.2016.08.018

26 Bronswijk JEMH van., Cock AWAM de., Oshima S. genus Blomia Oudemans (Acari: Glycyphagidae). II. Comparison of its species. Acarologia. 1974.

27 Cardona G, Guisantes J, Postigo I, *et al.* Allergenic cross-reactivity between Blomia tropicalis and Blomia kulagini (Acari: Echymiopodidae) extracts from optimized mite cultures. *J Investig Allergol Clin Immunol* 2005;**15**:259–65.

28 Puerta L, Fernández-Caldas E, Gracia LRC, *et al.* Sensitization to Blomia tropicalis and Lepidoglyphus destructor in Dermatophagoides spp-allergic individuals. *J Allergy Clin Immunol* 1991;**88**:943–50. doi:10.1016/0091-6749(91)90252-J

29 Fernández-Caldas E, Lockey RF. Blomia tropicalis, a mite whose time has come. *Allergy Eur J Allergy Clin Immunol* 2004;**59**:1161–4. doi:10.1111/j.1398-9995.2004.00727.x

30 Silvia J, Leonardo P, Dary M, *et al.* IgE antibody responses to recombinant allergens of Blomia tropicalis and Dermatophagoides pteronyssinus in a tropical environment. Allergy Clin. Immunol. Int. 2007;**19**:233–8. doi:10.1027/0838-1925.19.6.233

31 Chua KY, Cheong N, Kuo I-C, *et al.* The Blomia tropicalis allergens. *Protein Pept Lett* 2007;**14**:325–33. doi:10.2174/092986607780363862

32 Arlian LG, Vyszenski-Moher DAL, Fernandez-Caldas E. Allergenicity of the mite, Blomia tropicalis. *J Allergy Clin Immunol* 1993;**91**:1042–50. doi:10.1016/0091-6749(93)90218-5

33 Fernández-Caldas E. Allergenicity of Blomia tropicalis. In: *Journal of Investigational Allergology and Clinical Immunology*. 1997. 402–4.

34 Thomas WR, Hales BJ, Smith W. Blomia tropicalis: More than just another source of mite allergens. *Clin Exp Allergy* 2003;**33**:416–8. doi:10.1046/j.1365-2222.2003.01634.x

35 Fernández-Caldas E, Lockey RF. Blomia tropicalis, a mite whose time has come. *Allergy Eur J Allergy Clin Immunol* 2004;**59**:1161–4. doi:10.1111/j.1398-9995.2004.00727.x

36 Santos Da Silva E, Asam C, Lackner P, *et al.* Allergens of Blomia tropicalis: An Overview of Recombinant Molecules. Int. Arch. Allergy Immunol. 2017;**172**:203–14. doi:10.1159/000464325

37 Stanaland BE, Fernandez-Caldas E, Jacinto CM, *et al.* Sensitization to Blomia tropicalis: Skin test and cross-reactivity studies. *J Allergy Clin Immunol* Published Online First: 1994. doi:10.1016/0091-6749(94)90200-3

38 Chew FT, Yi FC, Chua KY, *et al.* Allergenic differences between the domestic mites Blomia tropicalis and Dermatophagoides pteronyssinus. *Clin Exp Allergy* 1999;**29**:982–8. doi:10.1046/j.1365-2222.1999.00543.x

39 Caraballo L, Mercado D, Jiménez S, *et al.* Analysis of the cross-reactivity between BtM and Der p 5, two group 5 recombinant allergens from Blomia tropicalis and Dermatophagoides pteronyssinus. *Int Arch Allergy Immunol* 1998;**117**:38–45. doi:10.1159/000023988

40 Arruda LK, Chapman MD. A review of recent immunochemical studies of Blomia tropicalis and Euroglyphus maynei allergens. *Exp Appl Acarol* 1992;**16**:129–40.

41 Castro Almarales RL, Mateo Morejón M, Naranjo Robalino RM, *et al.* Correlation between skin tests to Dermatophagoides pteronyssinus, Dermatophagoides siboney and Blomia tropicalis in Cuban asthmatics. *Allergol Immunopathol (Madr)* 2006;**34**:23–6. doi:10.1157/13084223

42 Sánchez-Borges M, Capriles-Hulett A, Caballero-Fonseca F, *et al.* Mite and cockroach sensitization in allergic patients from Caracas, Venezuela. *Ann Allergy, Asthma Immunol* 2003;**90**:664–8. doi:10.1016/S1081-1206(10)61873-X

43 Tsai JJ, Wu HH, Shen HD, *et al.* Sensitization to Blomia tropicalis among Asthmatic Patients in Taiwan. *Int Arch Allergy Immunol* 1998;**115**:144–9.https://www.karger.com/DOI/10.1159/000023894

44 Rizzo MC, Fernandez-Caldas E, Sole D, *et al.* IgE antibodies to aeroallergens in allergic children in Sao Paulo, Brazil. *J Investig Allergol Clin Immunol* 1997;**7**:242–8.

45 Santos Da Silva E, Asam C, Lackner P, *et al.* Allergens of Blomia tropicalis: An Overview of Recombinant Molecules. *Int Arch Allergy Immunol* 2017;**172**:203–14. doi:10.1159/000464325

46 Mannervik B, Awasthi YC, Broard PG, *et al.* Nomenclature for human glutathione transferases. *Biochem J* 1992;**282**:305–6. doi:10.1111/j.1399-0039.2011.01760.x

47 Hayes JD, Flanagan JU, Jowsey IR. Glutathione Transferases. *Annu Rev Pharmacol Toxicol* 2005;**45**:51–88. doi:10.1146/annurev.pharmtox.45.120403.095857

48 Sherratt PJ, Hayes JD. *9 Glutathione S-transferases*. 2002. doi:10.1002/0470846305

49 Wu B, Dong D. Human cytosolic glutathione transferases: Structure, function, and drug discovery. *Trends Pharmacol Sci* 2012;**33**:656–68. doi:10.1016/j.tips.2012.09.007

50 Board PG, Menon D. Glutathione transferases, regulators of cellular metabolism and physiology. *Biochim Biophys Acta - Gen Subj* 2013;**1830**:3267–88. doi:10.1016/j.bbagen.2012.11.019

51 Sheehan D, Meade G, Foley VM, *et al.* Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. *Biochem J* 2001;**360**:1–16. doi:10.1042/bj3600001

52 Pearson WR. Phylogenies of glutathione transferase families. *Methods Enzymol*

2005;**401**:186–204. doi:10.1016/S0076-6879(05)01012-8

53 Ketterman AJ, Saisawang C, Wongsantichon J. Insect glutathione transferases. *Drug Metab Rev* 2011;**43**:253–65. doi:10.3109/03602532.2011.552911

54 Brophy PM, Patterson LH, Brown A, *et al.* Glutathione S-transferase (GST) expression in the human hookworm Necator americanus: potential roles for excretory-secretory forms of GST. *Acta Trop* 1995;**59**:259–63. doi:10.1016/0001-706X(95)00084-R

55 Aguayo V, Valdes B, Espino AM. Assessment of Fasciola hepatica glutathione S-transferase as an antigen for serodiagnosis of human chronic fascioliasis. *Acta Trop* Published Online First: 2018. doi:https://doi.org/10.1016/j.actatropica.2018.07.002

56 Torres-Rivera A, Landa A. Glutathione transferases from parasites: A biochemical view. *Acta Trop* 2008;**105**:99–112. doi:10.1016/j.actatropica.2007.08.005

57 Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology* 2000;**61**:154–66. doi:10.1159/000028396

58 O'Brien M, Kruh GD, Tew KD. The influence of coordinate overexpression of glutathione phase II detoxification gene products on drug resistance. *J Pharmacol Exp Ther* 2000;**294**:480–7.http://www.ncbi.nlm.nih.gov/pubmed/10900222

59 Townsend DM, Findlay VL, Tew KD. Glutathione S-transferases as regulators of kinase pathways and anticancer drug targets. *Methods Enzymol* 2005;**401**:287–307. doi:10.1016/S0076-6879(05)01019-0

60 Townsend DM, Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. *Oncogene* 2003;**22**:7369–75. doi:10.1038/sj.onc.1206940

61 Hong SJ, Lee JY, Lee DH, *et al.* Molecular cloning and characterization of a mu-class glutathione S-transferase from Clonorchis sinensis. *Mol Biochem Parasitol* 2001;**115**:69–75. doi:10.1016/S0166-6851(01)00270-5

62 Preyavichyapugdee N, Sahaphong S, Riengrojpitak S, *et al.* Fasciola gigantica and Schistosoma mansoni: Vaccine potential of recombinant glutathione S-transferase (rFgGST26) against infections in mice. *Exp Parasitol* 2008;**119**:229–37. doi:10.1016/j.exppara.2008.01.014

63 Diemert DJ, Freire J, Valente V, *et al.* Safety and immunogenicity of the Na-GST-1 hookworm vaccine in Brazilian and American adults. *PLoS Negl Trop Dis* 2017;**11**:1–22. doi:10.1371/journal.pntd.0005574

64 Santiago HC, Leevan E, Bennuru S, *et al.* Molecular mimicry between cockroach and helminth glutathione S-transferases promotes cross-reactivity and cross-sensitization. *J Allergy Clin Immunol* 2012;**130**:248–256.e9. doi:10.1016/j.jaci.2012.02.045

65 Sookrung N, Reamtong O, Poolphol R, *et al.* Glutathione S-transferase (GST) of American Cockroach, Periplaneta americana: Classes, Isoforms, and Allergenicity. *Sci Rep* 2018;**8**. doi:10.1038/s41598-017-18759-z

66 Acevedo N, Sánchez J, Erler A, *et al.* IgE cross-reactivity between Ascaris and domestic mite allergens: The role of tropomyosin and the nematode polyprotein ABA-1. *Allergy Eur J Allergy Clin Immunol* 2009;**64**:1635–43. doi:10.1111/j.1398-9995.2009.02084.x

67 Acevedo N, Mohr J, Zakzuk J, *et al.* Proteomic and immunochemical characterization of glutathione transferase as a new allergen of the nematode Ascaris lumbricoides. PLoS One. 2013;**8**. doi:10.1371/journal.pone.0078353

68 Arruda LK, Vailes LD, Ferriani VPL, *et al.* Cockroach allergens and asthma. *J Allergy Clin Immunol* 2001;**107**:419–28. doi:10.1067/mai.2001.112854

69 Pomés A, Mueller GA, Randall TA, *et al.* New Insights into Cockroach Allergens. *Curr Allergy Asthma Rep* 2017;**17**. doi:10.1007/s11882-017-0694-1

70 Huang CH, Liew LM, Mah KW, *et al.* Characterization of glutathione S-transferase from dust mite, Der p 8 and its immunoglobulin E cross-reactivity with cockroach glutathione S-transferase. *Clin Exp Allergy* 2006;**36**:369–76. doi:10.1111/j.1365-2222.2006.02447.x

71 Mueller GA, Pedersen LC, Glesner J, *et al.* Analysis of glutathione S-transferase allergen cross-reactivity in a North American population: Relevancefor molecular diagnosis. *J Allergy Clin Immunol* 2014;:1369–77. doi:10.1016/j.jaci.2015.03.015

72 Deifl S, Zwicker C, Vejvar E, *et al.* Glutathione-S-transferase: a minor allergen in birch pollen due to limited release from hydrated pollen. *PLoS One* 2014;**9**:e109075. doi:10.1371/journal.pone.0109075

73 Shankar J, Singh BP, Gaur SN, *et al.* Recombinant glutathione-S-transferase a major allergen from Alternaria alternata for clinical use in allergy patients. *Mol Immunol* 2006;**43**:1927–32. doi:10.1016/j.molimm.2005.12.006

74 Sookrung N, Reamtong O, Poolphol R, *et al.* Glutathione S-transferase (GST) of American Cockroach, Periplaneta americana: Classes, Isoforms, and Allergenicity. *Sci Rep* 2018;**8**:1–12. doi:10.1038/s41598-017-18759-z

75 Acevedo N, Caraballo L. IgE cross-reactivity between Ascaris lumbricoides and mite allergens: Possible influences on allergic sensitization and asthma. Parasite Immunol. 2011;**33**:309–21. doi:10.1111/j.1365-3024.2011.01288.x

76 Deifl S, Zwicker C, Vejvar E, *et al.* Glutathione-S-transferase: A minor allergen in birch pollen due to limited release from hydrated pollen. *PLoS One* 2014;**9**. doi:10.1371/journal.pone.0109075

77 Arruda LK, Vailes LD, Platts-Mills TA, *et al.* Induction of IgE antibody responses by glutathione S-transferase from the German cockroach (Blattella germanica). *J Biol Chem* 1997;**272**:20907–12.

78 Zakzuk J, Fernández-Caldas E, Caraballo L. Evaluation of IgE responses against Blo t 8, a Glutathione S Transferase (GST) from Blomia tropicalis (Bt) mite. *J Allergy Clin Immunol* 2010;**125**:AB6.

79 An S, Chen L, Long C, *et al.* Dermatophagoides farinae allergens diversity identification by proteomics. *Mol Cell Proteomics* 2013;**12**:1818–28. doi:10.1074/mcp.M112.027136

80 Huang CH, Liew LM, Mah KW, *et al.* Characterization of glutathione S-transferase from dust mite, Der p 8 and its immunoglobulin E cross-reactivity with cockroach glutathione S-transferase. *Clin Exp Allergy* 2006;**36**:369–76. doi:10.1111/j.1365-2222.2006.02447.x

81 Chew, F.T., Wang, W.-L., Kuay, K.T., Ong, S.T., Lua, B.L., Lim SH and. Blo t 8 allergen [Blomia tropicalis] - Protein - NCBI. Unpublished.

82 Pedersen LC, Mueller GA. Crystal structure of the gluthatione S-transferase Blo t 8. *doi.org* doi:10.2210/pdb4q5n/pdb

83 Brooker SJ, Pullan RL. Ascaris lumbricoides and Ascariasis: Estimating Numbers Infected and Burden of Disease. In: *Ascaris: The Neglected Parasite*. 2013. 343–62. doi:10.1016/B978-0-12-396978-1.00013-6

84 Holland C. *Ascaris: The Neglected Parasite*. 2013. doi:10.1016/C2011-0-06705-4

85 Nutman TB, Santiago HC. Human Helminths and Allergic Disease: The Hygiene Hypothesis and Beyond. *Am J Trop Med Hyg* 2016;**95**:746–53. doi:10.4269/ajtmh.16-0348

86 Coronado S, Barrios L, Zakzuk J, *et al.* A recombinant cystatin from Ascaris lumbricoides attenuates inflammation of DSS-induced colitis. *Parasite Immunol* Published Online First: 2017. doi:10.1111/pim.12425

87 Minciullo PL, Cascio A, David A, *et al.* Anaphylaxis caused by helminths: review of the literature. *Eur Rev Med Pharmacol Sci* 2012;**16**:1513–8.

88 Ahumada V, García E, Dennis R, *et al.* IgE responses to Ascaris and mite tropomyosins are risk factors for asthma. *Clin Exp Allergy* 2015;**45**:1189–200. doi:10.1111/cea.12513

89 Thomas WR, Hales BJ, Smith WA. Structural biology of allergens. *Curr Allergy Asthma Rep* 2005;**5**:388–93. doi:10.1007/s11882-005-0012-1

90 Hagan P, Blumenthal UJ, Dunn D, *et al.* Human IgE, IgG4 and resistance to reinfection with Schistosomahaematobium. *Nature* 1991;**349**:243–5. doi:10.1038/349243a0

91 Pereira EAL, Silva DAO, Cunha JP, *et al.* IgE, IgG1, and IgG4 antibody responses to Blomia tropicalis in atopic patients. *Allergy Eur J Allergy Clin Immunol* 2005;**60**:401–6. doi:10.1111/j.1398-9995.2005.00738.x

92 Leonardi-Bee J, Pritchard D, Britton J. Asthma and current intestinal parasite infection: Systematic review and meta-analysis. *Am J Respir Crit Care Med* 2006;**174**:514–23. doi:10.1164/rccm.200603-331OC

93 Caraballo L, Acevedo N. New Allergens of Relevance in Tropical Regions: The Impact of Ascaris lumbricoides Infections. *World Allergy Organ J* 2011;**4**:77–84. doi:10.1097/WOX.0b013e3182167e04

94 Acevedo N, Erler A, Briza P, *et al.* Allergenicity of Ascaris lumbricoides tropomyosin and IgE sensitization among asthmatic patients in a tropical environment. *Int Arch Allergy Immunol* 2011;**154**:195–206. doi:10.1159/000321106

95 Caraballo L, Acevedo N, Buendía E. Human Ascariasis Increases the Allergic Response and Allergic Symptoms. *Curr Trop Med Reports* 2015;**2**:224–32. doi:10.1007/s40475-015-0058-7

96 Buendía E, Zakzuk J, Mercado D, *et al.* The IgE response to Ascaris molecular components is associated with clinical indicators of asthma severity. *World Allergy Organ J* Published Online First: 2015. doi:10.1186/s40413-015-0058-z

97 Platts-Mills TAE, Cooper PJ. Differences in asthma between rural and urban communities in South Africa and other developing countries. *J Allergy Clin Immunol* 2010;**125**:106–7. doi:10.1016/j.jaci.2009.10.068

98 Perzanowski MS, Ng'Ang'A LW, Carter MC, *et al.* Atopy, asthma, and antibodies to Ascaris among rural and urban children in Kenya. *J Pediatr* 2002;**140**:582–8. doi:10.1067/mpd.2002.122937

99 Stevens W, Addo-Yobo E, Roper J, *et al.* Differences in both prevalence and titre of specific immunoglobulin E among children with asthma in affluent and poor communities within a large town in Ghana. *Clin Exp Allergy* 2011;**41**:1587–94. doi:10.1111/j.1365-2222.2011.03832.x

100 Calvert J, Burney P. Ascaris, atopy, and exercise-induced bronchoconstriction in rural and urban South African children. *J Allergy Clin Immunol* 2010;**125**:100-5.e1-5. doi:10.1016/j.jaci.2009.09.010

101 Dirección de Desarrollo Rural Sostenible – DDRS. Definición de categorías de ruralidad. 2014;:27.https://colaboracion.dnp.gov.co/CDT/Estudios Econmicos/2015ago6 Documento de Ruralidad - DDRS-MTC.pdf

102 Fernandez-Caldas E, Puerta L, Mercado D, *et al.* Mite fauna, Der pI Der fI and Blomia tropicalis allergen levels in a tropical environment. *Clin Exp Allergy* 1993;**23**:292–7.http://www.scopus.com/inward/record.url?eid=2-s2.0-0027319655&partnerID=40&md5=6b8e54ef92ae17a13314f5ad1722040c

103 Agudelo-Lopez S, Gómez-Rodríguez L, Coronado X, *et al.* Prevalencia de Parasitosis Intestinales y Factores Asociados en un Corregimiento de la Costa Atlántica Colombiana. *Rev Salud Pública* 2008;**10**:633–42. doi:10.1590/S0124-00642008000400013

104 Fang L, Martínez B, Marrugo J. Distribución de tres polimorfismos del gen TSLP en población afrodescendiente de San Basilio de Palenque, Colombia. *Biomédica* 2012;**33**:251–8. doi:10.7705/biomedica.v33i2.655

105 Aun M, Bonamichi-Santos R, Arantes-Costa FM, *et al.* Animal models of asthma: utility and limitations. *J Asthma Allergy* 2017;**Volume10**:293–301. doi:10.2147/JAA.S121092

106 Reddy AT, Lakshmi SP, Reddy RC. Murine Model of Allergen Induced Asthma. *J Vis Exp* 2012;:1–7. doi:10.3791/3771

107 Katayama S, Shionoya H, Ohtake S. A New Method for Extraction of Extravasated Dye in the Skin and the Influence of Fasting Stress on Passive Cutaneous Anaphylaxis in Guinea Pigs and Rats. *Microbiol Immunol* 1978;**22**:89–101. doi:10.1111/j.1574-6968.1984.tb00702.x

108 Evans H, Killoran KE, Mitre E. Measuring local anaphylaxis in mice. *J Vis Exp* 2014;:e52005. doi:10.3791/52005

109 Mueller GA, Pedersen LC, Glesner J, *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**:1369–77. doi:10.1016/j.jaci.2015.03.015

110 Lehrer SB, Reish R, Fernandes J, *et al.* Enhancement of murine IgE antibody detection by IgG removal. *J Immunol Methods* 2004;**284**:1–6.

111 Myou S, Leff AR, Myo S, *et al.* Blockade of Inflammation and Airway Hyperresponsiveness in Immune-sensitized Mice by Dominant-Negative Phosphoinositide 3-Kinase–TAT. *J Exp Med* 2003;**198**:1573–82. doi:10.1084/jem.20030298

112 Tanaka H, Masuda T, Tokuoka S, *et al.* The effect of allergen-induced airway inflammation on airway remodeling in a murine model of allergic asthma. *Inflamm Res* 2001;**50**:616–24.http://www.ncbi.nlm.nih.gov/pubmed/11822788

113 Zakzuk J, Acevedo N, Cifuentes L, *et al.* Early life IgE responses in children living in the tropics: A prospective analysis. *Pediatr Allergy Immunol* 2013;**24**:788–97. doi:10.1111/pai.12161

114 Lloyd CM, Snelgrove RJ. Type 2 immunity: Expanding our view. *Sci Immunol* 2018;**3**:1–12. doi:10.1126/sciimmunol.aat1604

115 Ahumada V, García E, Dennis R, *et al.* IgE responses to *Ascaris* and mite tropomyosins are risk factors for asthma. *Clin Exp Allergy* 2015;**45**:1189–200. doi:10.1111/cea.12513

116 Shen G-M, Shi L, Xu Z-F, *et al.* Inducible Expression of Mu-Class Glutathione S-Transferases Is Associated with Fenpropathrin Resistance in Tetranychus cinnabarinus. *Int J Mol Sci* 2014;**15**:22626–41. doi:10.3390/ijms151222626

117 Feldman AS, He Y, Moore ML, *et al.* Toward primary prevention of asthma: Reviewing the evidence for early-life respiratory viral infections as modifiable risk factors to prevent childhood asthma. *Am J Respir Crit Care Med* 2015;**191**:34–44. doi:10.1164/rccm.201405-0901PP

118 Azad MB, Konya T, Maughan H, *et al.* The gut microbiome and the hygiene hypothesis of allergic disease. Impact of pets and siblings on infant gut microbiota. *Ann Am Thorac Soc* Published Online First: 2014. doi:10.1513/AnnalsATS.201307-218MG

119 Bloomfield SF, Rook GA, Scott EA, *et al.* Time to abandon the hygiene hypothesis: new perspectives on allergic disease, the human microbiome, infectious disease prevention and the role of targeted hygiene. *Perspect Public Health* 2016;**136**:213–24. doi:10.1177/1757913916650225

120 Acevedo N, Caraballo L. IgE cross-reactivity between Ascaris lumbricoides and mite allergens: Possible influences on allergic sensitization and asthma. *Parasite Immunol* 2011;**33**:309–21. doi:10.1111/j.1365-3024.2011.01288.x

XIII. ATTACHMENTS

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ATTACHMENT 1: CONSENTIMIENTO INFORMADO 1. PALENQUE

DISTRIBUCIÓN DEL SNP - 847C >T DEL GEN TSLP EN POBLACIÓN AFRO-DESCENDIENTE DE LA REGIÓN CARIBE COLOMBIANA.

Consentimiento informado para la participación en el estudio:

Introducción:
Las enfermedades alérgicas son un problema de salud mundial, cuyas cifras de prevalencia han aumentado en los últimos años en muchos países. Estas enfermedades son complejas ya que en ellas intervienen factores genéticos y ambientales. Aun se conoce muy poco sobre las causas iníciales que desencadenan la respuesta alérgica, sin embargo, varios estudios muestran que algunas células y moléculas de la inmunidad innata son importantes en la desviación hacia una respuesta alérgica en individuos genéticamente susceptibles. Una de esas moléculas es la Linfopoyetina Estromal Tímica (TSLP) la cual es una citoquina producida principalmente por células epiteliales. Esta molécula ejerce su influencia sobre varios tipos de células de la respuesta alérgica entre las que se encuentran las células dendríticas, los linfocitos T y los granulocitos.
Estudios realizados en asmáticos identificaron variantes en la región promotora del gen TSLP que están asociados con asma atópica e hiper-reactividad bronquial. Otros análisis genéticos han reportado algunas variantes en el gen TSLP asociadas con bajos niveles de IgE específica a alérgenos e IgE total. De igual forma los polimorfismos rs3806933 y rs2289276 están significativamente asociados con atopia en niños y asma en adultos en población japonesa. De acuerdo a lo anterior, las variantes funcionales en el gen TSLP son consideradas como factores genéticos de riesgo para el desarrollo de enfermedades alérgicas. Por lo que este gen podría ser un candidato interesante para análisis. En Colombia, no existe información acerca de la distribución de los polimorfismos en el gen TSLP, lo cual es esencial para establecer cualquier tipo de asociación con el desarrollo de enfermedades alérgicas. Por otro lado, estudios realizados en nuestro grupo de investigación, muestran que los niveles elevados de IgE están significativamente relacionados con el componente ancestria africana en la población de Cartagena.

Procedimientos
Usted es elegible para participar en este estudio por ser hijo de ambos padres palenqueros con ancestria africana.

Queremos solicitarle comedidamente nos autorice a realizar las siguientes pruebas, que serán llevadas a cabo por personas debidamente entrenadas para este fin:

1. Historia Clínica. La cual será realizada por el Dr. Javier Marrugo, MD.

2. Pruebas cutáneas a 10 extractos de alérgenos comunes del ambiente y de

alimentos comunes en la dieta. Esta parte la realizará el Dr. Javier Marrugo, MD.

3. Toma de la muestra: se obtendrán 20 mL de sangre, de los cuales el suero o plasma se utilizara para la determinación de la respuesta inmune a diversos antígenos alimenticios y ambientales, en futuros proyectos que surjan para dar respuesta a interrogantes científicos. A partir del componente celular se extraerá el ADN (Acido desoxiribonucleico) genómico, del cual se genotipificaran alteraciones genéticas importantes que presenten asociación a diversas enfermedades crónicas. Además estas muestras de ADN permitirán desarrollar futuros proyectos de genómica y epigenética. Estas pruebas serán realizadas por profesionales Universitarios o estudiantes de maestría en inmunología.

4. Finalmente el médico Inmunólogo le dará al final del estudio a cada una de las personas evaluadas una nota por escrito sobre las recomendaciones y tratamiento necesario según el caso.

Riesgos y Beneficios

Si usted decide participar, deberá tener presente la siguiente información y aceptar los siguientes puntos:

a) La evaluación para la realización de la historia clínica no tiene ningún tipo de riesgo.

b) Manifiesto que he sido informado claramente sobre los riesgos que se pueden presentar, durante la realización de la prueba cutánea siendo estos: Reacciones locales, caracterizadas por prurito, ronchas hasta generalizadas como la anafilaxis que se puede presentar en un 0.008% de los individuos, y que el Médico responsable me ha manifestado que está preparado ya que cuenta con todos los elementos disponibles para manejar cualquier tipo de complicación.

c) Autorizo al investigador a tomar muestras de sangre en un volumen no superior a 25 ml. (Como posibles riesgo se encuentra la flebitis y lesiones del nervio), con el fin de que se adelanten algunos ensayos de investigación.

d) El principal beneficio que usted obtendría de esta evaluación es la posibilidad de detectarle o diagnosticar alteraciones de la respuesta inmune que pueda estar padeciendo.

Derechos

Si usted decide participar, tiene los siguientes derechos:

a) Los registros que lo puedan identificar serán mantenidos confidencialmente de acuerdo con las leyes colombianas y a las Buenas Prácticas Clínicas en investigación.
b) Su participación en esta investigación es completamente voluntaria y puede retirarse en cualquier momento.
c) Cualquier pregunta o duda por favor comuníquese con el Dr Javier Marrugo al Teléfono 3165222770.

Con su firma usted certifica que ha leído o alguien le ha leído el presente formato

de consentimiento informado; que le han sido resueltas todas sus preguntas satisfactoriamente y que acepta voluntariamente participar en el estudio.

_____
Lugar y fecha

Firma del participante: _____
Nombre del participante: _____
CC: _____ Huella:

Firma del testigo o responsable del participante: _____
Nombre (testigo/ responsable) del participante: _____
CC: _____ Huella:

Firma del médico o investigador principal: _____
Nombre del profesional: _____
CC:_____ Registro Medico Nº: _____

ATTACHMENT 2: CONSENTIMIENTO INFORMADO 2, SANTA CATALINA.

Título del proyecto: ASCARIASIS, TRATAMIENTO ANTI-HELMÍNTICO Y SUS EFECTOS SOBRE LA RESPUESTA INMUNE DE TIPO ALÉRGICA EN UNA POBLACIÓN RURAL DE BOLIVAR

Sub-proyecto: Estudio de intervención con albendazol en niños con sibilancia recurrente Investigadores: Luis Caraballo, Josefina Zakzuk y Nelson Alvis

El siguiente es un documento informativo acerca de la participación de su hijo (a) en un protocolo de estudio para estudiar los efectos de las parasitosis intestinales en el asma y las alergias. La participación en el estudio es voluntaria y no representa mayor riesgo para los individuos que en él participan. Los resultados del estudio no son divulgados con nombres propios y ellos no tienen influencia en el diagnóstico, tratamiento y pronóstico de la enfermedad.
Es importante que usted lea y entienda cuidadosamente el contenido de este documento. El documento se refiere a lo que usted necesita saber sobre el estudio. Si usted decide que su hijo o representado participen en este estudio, debe firmar este consentimiento informado. Su firma quiere decir que se la ha explicado y entendido en qué consiste el estudio y sus posibles riesgos.
Este estudio es una investigación de tipo intervención que se realizará por profesionales investigadores de la Universidad de Cartagena. Antes de ser realizado, el estudio cuenta con el aval y aprobación del comité de Ética de Universidad de Cartagena. Se incluirán niños que tengan síntomas respiratorios compatibles con asma, una enfermedad difícil de diagnosticar en la niñez, pero que puede manifestarse a través de sibilancia recurrente, lo que quiere decir, que el niño se aprieta frecuentemente. Teniendo en cuenta los criterios de la comunidad científica, solo se incluirán en la investigación aquellos niños que sibilen (se aprieten de los pulmones) más de 3 veces en el últ imo año de vida.

El objetivo del estudio es establecer como las infecciones por parásitos, especialmente el Ascaris lumbricoides, influye en el asma y en la respuesta inmune (las defensas naturales del cuerpo).

Los parásitos intestinales generan efectos en las personas que infestan, muchos de ellos perjudiciales para la salud. El impacto de las parasitosis, especialmente del helminto Ascaris, sobre el asma y las alergias es poco entendido. Por esa razón hemos decidido realizar este estudio en su comunidad, donde las parasitosis son frecuentes, seleccionando niños con síntomas de asma a los que se les administrarán varias dosis un medicamento antiparasitario denominado albendazol.

Este fármaco es el que recomienda la Organización Mundial de la Salud como parte de programas de desparasitación masiva en poblaciones donde las parasitosis son un problema frecuente. Se planean tomar varias muestras biológicas (del cuerpo) del participante del estudio. Con la muestra de sangre se harán exámenes para ayudar al médico a definir si su hijo es alérgico, esto se hará midiendo el anticuerpo IgE y un hemograma para indagar sobre problemas como anemia y conocer los valores de glóbulos blancos. En la muestra de heces se buscará si su hijo tiene parásitos.

Con este conocimiento avanzaremos en el conocimiento de factores de riesgo y protección para la presentación de asma y alergias, lo que podría resultar en un futuro en formas más eficaces de control de estas enfermedades.

De su hijo (a) o apoderado se obtendrá una muestra de sangre por punción venosa a nivel del antebrazo. La punción será realizada por una persona entrenada, con material desechable y adecuadas condiciones de limpieza. También se le harán algunas preguntas para completar un cuestionario. La muestra se sangre será marcada con un código especial del estudio. La punción venosa representa un riego muy bajo y como única consecuencia podría resultar un pequeño hematoma (morado) o infección en el sitio donde penetra la aguja.

En caso de presentarse alguna complicación con la punción venosa, el Instituto de Investigaciones Inmunológicas de la Universidad de Cartagena cubrirá la atención de esta complicación. Se le harán pruebas cutáneas de alergia, una herramienta diagnóstica confiable de alergia que no produce molestias salvo el prurito que aparece si la persona es alérgica. En este caso también se le formará una pequeña roncha que desaparecerá en dos horas. Esta prueba se utiliza en todos los países para el diagnóstico de alergias incluyendo niños y se ha utilizado en otros estudios como este sin reportarse efectos adversos. El medicamento que se le administrará a mi hijo tiene pocos efectos adversos sobre la salud humana, la mayoría de ellos menores. En algunas personas puede provocar dolor abdominal, náusea, vómito, cefalea, mareo, vértigo, dolor epigástrico y/o diarrea. Como cualquier otro medicamente puede provocar alergias, en personas sensibles; en todo caso la frecuencia de casos reportados es baja.

En caso de presentarse alguno de estos síntomas usted puede comunicarse con nuestros contactos, quienes se comprometen a brindarle la debida y oportuna atención a esta complicación.De manera voluntaria y sin coacción alguna, manifiesto mi disposición de que mi hijo o custodio participe en esta investigación y por lo tanto autorizo:

- Que se le tomen 3 muestras de sangre venosa, una cada 6 meses, con el fin de realizarle un hemograma para medir la hemoglobina, los glóbulos rojos y blancos, así como definir la presencia de anticuerpos específicos IgE contra alergenos ambientales y Ascaris lumbricoides y los niveles de IgE total.

- Que de la primera muestra de sangre recolectada se extraiga ADN genómico para el estudio de genes posiblemente relacionados con el asma, las alergias y la resistencia natural a las parasitosis.
- Que se le realicen pruebas cutáneas de alergias para diagnóstico de sensibilización alergeno-específica.
- Que antes y un año después de iniciar el anti-parasitario se analice la presencia de parásitos en 2 muestra de heces recolectadas en días consecutivos y traída por mí o el familiar responsable que lo acompañe al momento de la valoración clínica, o recogida en su hogar por el personal de apoyo que colabore con este proyecto. También que se guarde una parte de esta muestra para congelarse y posteriormente se aísle DNA de los microorganismos que habitan en el intestino.
- Que se le intente realizar la espirometría con el fin de confirmar el diagnóstico de asma y evaluar la severidad de la enfermedad.
- Que toda la información recolectada sobre las condiciones de vida, hábitos, características de la vivienda, antecedentes personales y familiares y los datos de la historia clínica de mi hijo, y por lo tanto de mi familia, sea incorporada a la base de datos del estudio, cuyo carácter es confidencial y que solo será empleada con fines de investigación biomédica o epidemiológica.
- Que se le administre una dosis única de albendazol 400 mg cada tres meses durante un año, lo cual en total suman cuatro dosis.
- Que sea seleccionado por los investigadores para tomarle dos muestras de sangre adicional para estudiar en sus células la forma de respuesta del sistema inmune a los parásitos. Una se tomará antes de iniciar el tratamiento con albendazol y otra una semana después de la última desparasitación.

Por favor marque una de las siguientes opciones:

- Sí autorizo la toma de muestras y la participación en el estudio.
- No autorizo la toma de muestras y la participación en el estudio.

La muestra que se está obteniendo de su hijo (a) puede ser utilizada en proyectos de investigación similares. Como estas muestras son tan valiosas, usted puede autorizar o no que se usen en estudios futuros. Por favor marque una de las siguientes opciones:

Sí deseo que la muestra sea almacenada en anonimato para otros estudios.

No deseo que la muestra sea almacenada para futuros estudios.

De qué manera se protegerá la privacidad de mi hijo(a)?

Los datos de este estudio serán publicados. El nombre o cualquier forma de identificación de su hijo o apoderado no serán utilizados sin su expresa autorización. La información consignada en la historia clínica de su hijo o apoderado podrá ser utilizada por el investigador para el estudio. Esta información puede incluir el

nombre, fecha de nacimiento, la dirección, el número de teléfono, la historia médica de su hijo(a). Se utilizará un código que identifica a su hijo que solo conocerán los investigadores.

Usted puede hablar con los investigadores en cualquier momento y hacer cualquier pregunta que tenga en relación con el estudio. Los investigadores no tendrán ningún tipo de compensación económica por este estudio. Los investigadores: A la Doctora Josefina Zakzuk, el Doctor Luis Caraballo y el Doctor Nelson Alvis los puede encontrar en los siguientes teléfonos: 6698491 y 3215412280 o por correo electrónico: j.zakzuk@fundemeb.org, lucaraballo@yahoo.com y nalvis@yahoo.com.

Me ha sido dada la oportunidad de hacer todas las preguntas y éstas han sido respondidas. Me han dado una copia de este consentimiento informado. Estoy de acuerdo con la participación de mi hijo o apoderado en el estudio y el almacenamiento de las muestras biológicas que se le tomen:

Nombre del Voluntario
No del Documento de Identidad Fecha

Firma del Padre o Tutor	Firma de la madre o tutor
Nombre:	Nombre:
C.C. No.	C.C. No.
Fecha	Fecha:
Testigo No. 1	Testigo No. 2
Nombre:	Nombre:
C.C. No.	C.C. No.
Dirección	Dirección

ATTACHMENT 3: FORMATO DE ASENTIMIENTO DEL MENOR

Título del proyecto: ASCARIASIS, TRATAMIENTO ANTI-HELMÍNTICO Y SUS EFECTOS SOBRE LA RESPUESTA INMUNE DE TIPO ALÉRGICA EN UNA POBLACIÓN RURAL DE BOLIVAR

Investigadores: Luis Caraballo, Nelson Alvis y Josefina Zakzuk

A mí se me ha pedido participar en esta investigación. Los Dres. Josefina Zakzuk y Luis Caraballo en persona u otro médico del estudio me han explicado en qué consiste esta investigación. Este estudio se está llevando a cabo para establecer si la infección por parásitos intestinales, entre estos *Ascaris lumbricoides*, se relaciona con el desarrollo y empeoramiento del asma y las alergias. Este estudio puede no ayudarte inmediatamente para el asma. Pero será de gran ayuda para las próximas generaciones, ya que nos ofrecerán nuevas alternativas de tratamiento. Me darán cuatros dosis, una cada tres meses, de un medicamento llamado albendazol que se usa para combatir los parásitos intestinales. Puedo sentir un sabor amargo cuando lo tome. Se me pedirá que acuda al Centro de Salud de mi municipio para la obtención de una muestra de sangre. La punción para la muestra de sangre representa un riesgo bajo y como única consecuencia podría resultar un hematoma o morado o infección en el sitio de punción. Se me realizarán también pruebas cutáneas de alergia, un examen no doloroso que se hace en la piel y que me puede ocasionar picazón en el lugar del cuerpo donde me las hagan. Esta sensación no durará más de 30 minutos. Se intentará hacerme el examen de espirometría, que estudia la capacidad de los pulmones para manejar el volumen de aire que respiramos. El éxito de este examen depende de mi colaboración, por eso en niños es algunas veces difícil obtener resultados confiables. Se me recogerán muestra de heces para saber si tengo parásitos y congelar una parte para otros estudios científicos.
No es obligatorio que participes en este estudio si no lo deseas, aunque tus padres digan que sí. Puedes hacer cualquier pregunta sobre este estudio en cualquier momento, así como negarte a seguir participando cuando lo desees. Los datos de este estudio serán publicados. El nombre o cualquier forma de identificación no serán utilizados sin su expresa autorización. La información consignada en tu historia clínica podrá ser utilizada por los investigadores para el estudio.

Nombre del Voluntario _____
Firma _____
Fecha_____Firma del Investigador que administra en asentimiento

ATTACHMENT 4: CONSENTIMIENTO INFORMADO 4, CARTAGENA Proyecto "Influencia de la sensibilización por alérgenos de Áscaris en la respuesta alérgica y severidad del asma",

En este documento expreso mi consentimiento para participar en el Proyecto **"Influencia de la sensibilización por alergenos de Áscaris en la respuesta alérgica y severidad del asma"**, el cual hace parte del Programa de Prevalencia y Factores de Riesgo de Asma y Alergias adelantado por el Instituto de Investigaciones Inmunológicas de la Universidad de Cartagena y dirigido por el Dr. Luis Caraballo.

Se me han explicado los objetivos de esta investigación, los procedimientos y actividades que se necesitan para realizarla, los beneficios y los posibles riesgos. Sé que sus resultados ayudarán al conocimiento sobre cómo influye la infección por el parásito *Ascaris lumbricoides* en el desarrollo y severidad de las enfermedades alérgicas y el asma en nuestro medio. También se me ha informado que los exámenes y procedimientos serán realizados por personal experto, no tendrán ningún costo para mí y sus resultados serán confidenciales.

Tengo conocimiento que esta investigación es de riesgo mínimo, pero algunos procedimientos pueden generar molestias. Que la toma de sangre se realizará como se hace rutinariamente en los laboratorios clínicos, por una persona debidamente entrenada. También se me ha explicado que la prueba cutánea es la herramienta diagnóstica más confiable de alergia y que no produce molestias salvo el prurito que aparece si la persona es alérgica. En este caso también se le formará una pequeña roncha que desaparecerá en dos horas. Esta prueba se utiliza en todos los países para el diagnóstico de alergias incluyendo niños y se ha utilizado en otros estudios como este sin reportarse efectos adversos.

Se me ha garantizado que cualquier pregunta o duda que tenga sobre los procedimientos, riesgos, beneficios y otros asuntos relacionados con la investigación puedo preguntarlos a los teléfonos 6698491 y 310 352 7373 del Instituto de Investigaciones Inmunológicas, los investigadores han colocado a mi disposición toda la información disponible sobre otras investigaciones como esta que se han realizado en otros países y cualquier material educativo que me interese revisar. Se me ha explicado que puedo retirar mi consentimiento cuando desee y dejar de participar en el estudio sin que por ello tenga ningún perjuicio o se afecten mis derechos legales, mi reputación o mi acceso a los servicios de salud. De acuerdo a lo establecido por la ley, en caso que se generen daños a mi persona como consecuencia de los procedimientos realizados durante esta investigación, el

tratamiento o la indemnización a la que legalmente tendría derecho será asumida por la institución responsable de la investigación.

De manera voluntaria y sin coacción alguna, manifiesto mi disposición a contribuir con las actividades necesarias para la realización de esta investigación y por lo tanto autorizo:

1. Que se me tome una muestra de sangre venosa con el fin de definir la presencia de anticuerpos específicos IgE contra alergenos ambientales y *Ascaris lumbricoides,* niveles de IgE total así como extraer de esta ADN genómico para el estudio de genes posiblemente relacionados con el asma.

2. Que se me realicen pruebas cutáneas de alergias para diagnóstico de sensibilización alergeno-específica.

3. Que se analice la presencia de parásitos en una muestra de heces recolectada y traída por mí al momento de la valoración clínica.

4. Que se me realice espirometría con el fin de confirmar el diagnóstico de asma y evaluar la severidad de mi enfermedad.

5. Que toda la información recolectada sobre mis condiciones de vida, hábitos, características de la vivienda, antecedentes personales y familiares y los datos de la historia clínica sea incorporada a la base de datos del estudio, cuyo carácter es confidencial y que solo será empleada con fines de investigación biomédica o epidemiológica.

Este proyecto fue revisado y aprobado por un comité de Ética Médica y todos sus protocolos se ajustan a las leyes internacionales y a las disposiciones vigentes en Colombia según la Resolución Nº 008430 de 1993, que establecen las normas científicas, técnicas y administrativas para la investigación en salud.

ACEPTO Y FIRMO EN SEÑAL DE APROBACION:

Nombre de la madre:_____

CC:_____

Firma de la madre:_____

Nombre del padre:_____

CC:_____

Firma del padre:_____

Nombre del Testigo # 1: _____

CC:_____

Firma del Testigo # 1: _____ Parentesco:

_____

Nombre del Testigo # 2: _____ CC:

_____

Firma del Testigo # 2: _____ Parentesco:

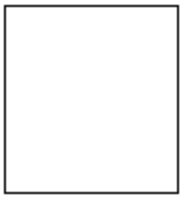
_____

Coloque la huella digital de la madre o el padre si no puede escribir

Persona designada que escribe su nombre:

Nombre:_____ CC: _____

Firma: _____ Parentesco: _____



Índice Derecho

Nombre del investigador o delegado: _____

CC: _____ Firma: _____

Firmado en Cartagena de Indias, el día____ del mes_____ del año 20___

ATTACHMENT 5: OR Case control Study: Cartagena

Sensitization to rBlo t 8

	Case	Control	Total
Expuestos		13	70	83
No expuestos		64	50	114
Total	77	120	197	

	Estimación	IC(95,0%)		
Proporción de casos expuestos	0,168831	-	-	
Proporción de controles expuestos	0,583333	-	-	
Odds ratio	0,145089	0,072192	0,291595	(Woolf)
	0,072782	0,289741	(Cornfield)	
Fracción prevenida en expuestos	0,854911	0,708405	0,927808	
Fracción prevenida poblacional	0,459784	0,317075	0,572671	

Prueba Ji-cuadrado de asociación	Estadístico	Valor p
Sin corrección	33,0528	0,0000
Corrección de Yates	31,3746	0,0000

Prueba exacta de Fisher	Valor p
Unilateral	0,0000
Bilateral	0,0000

ATTACHMENT 6: OR Case control Study: St Catalina

Sensitization to rBlo t 8

Tipo de estudio : Caso-control
Nivel de confianza: 95,0%

	Case	Control	Total
Expuestos		39	59	98
No expuestos		67	21	88
Total	106	80	186	

	Estimación	QI(95,0%)		
Proporción de casos expuestos	0,367925	-	-	
Proporción de controles expuestos	0,737500	-	-	
Odds ratio	**0,207184**	0,109739	0,391160	(Woolf)
		0,110129	0,389929	(Cornfield)
Fracción prevenida en expuestos	0,792816	0,608840	0,890261	
Fracción prevenida poblacional	0,507202	0,268518	0,668003	

Prueba Ji-cuadrado de asociación	Estadístico	Valor p
Sin corrección	24,9808	0,0000
Corrección de Yates	23,5202	0,0000

Prueba exacta de Fisher	Valor p
Unilateral	0,0000
Bilateral	0,0000

ATTACHMENT 7: OR Case control Study: Cartagena-St Catalina

Sensitization to rBlo t 8

	Case	Control	Total
Exposed	52	129	181
Not exposed	131	71	202
Total	183	200	383

	OR	IC(95,0%)		
Proporción de casos expuestos	**0,285326**	-	-	
Proporción de controles expuestos	0,644279	-	-	
Odds ratio	**0,220430**	**0,143246**	**0,339201**	**(Woolf)**
	0,143374	**0,338905**	**(Cornfield)**	
Fracción prevenida en expuestos	0,779570	0,660799	0,856754	
Fracción prevenida poblacional	0,439801	0,310797	0,544658	

Ji-square Asociation Statistics		p Value
Sin corrección	49,6571	0,0000
Corrección de Yates	48,2274	0,0000

Fisher	p Value
Unilateral	0,0000

ATTACHMENT 8: Logistic regression for Cartagena population

	Sig.	Exp(B)	I.C. 95% EXP(B)	
			Inferior	Superior
CutoffBlot8	**0,000**	0,048	0,013	0,180
Age	0,748	1,005	0,977	1,032
Gender (female)	**0,014**	3,794	1,305	11,030
Ascaris Sensitization	0,219	2,185	0,629	7,595
Bt Sensitization	**0,000**	13,581	3,763	49,022
Dp Sensitización	0,003	7,752	2,012	29,878
Constant	**0,004**	**0,118**		

ATTACHMENT 9: Logistic regression in St. Catalina population

	Sig.	Exp(B)	I.C. 95% EXP(B)	
			Inferior	Superior
Blot8Sens	**0,000**	0,146	0,070	0,302
Age	**0,004**	0,975	0,958	0,992
Gender (female)	0,445	0,743	0,347	1,592
Dp Sensitization	0,729	1,204	0,421	3,441
Bt Sensitization	0,143	2,138	0,774	5,903
Ascaris Sensitization	0,162	1,846	0,782	4,357
Constante	0,001	4,989		