

ORIGINAL ARTICLE Asthma and Rhinitis

IgE responses to *Ascaris* and mite tropomyosins are risk factors for asthma

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Clinical
&
Experimental
Allergy

Summary

Background The relationship between helminthiasis and allergy is a matter of considerable interest and research. In the tropics, house dust mite exposure, a known risk factor for asthma, is frequently concurrent with helminth infections. It remains to be defined whether infection with the common roundworm *Ascaris* or its bystander immunological effects influence the prevalence and pathogenesis of asthma independently of mite sensitization.

Objective To investigate the relationship between the IgE responses to *Ascaris* and its purified allergens and the risk of asthma in a tropical country.

Methods A nested case–control study was performed in 356 subjects who reported current and past asthma symptoms (asthmatics) and 435 controls that had never experienced such symptoms. They were tested for serum levels of total IgE and specific IgE to *Ascaris* extract, Asc s 1 (ABA-1), Asc l 3 (tropomyosin) and GST (glutathione transferase). In addition, specific IgE to *Dermatophagoides pteronyssinus*, *Blomia tropicalis* and their tropomyosins Der p 10 and Blo t 10 was measured. Sensitization was defined as a positive specific IgE result to any extract or recombinant allergen.

Results Sensitization to *Ascaris* and *D. pteronyssinus* was independently associated with asthma after adjustment for age, gender, socio-economic stratum, city and other IgE levels (adjusted ORs: 2.17; 95% CI 1.37–3.42 and 2.46; 95% CI 1.54–3.92), respectively. There was also a significant association with sensitization to the highly allergenic and cross-reactive tropomyosins Asc l 3, Blo t 10 and Der p 10 (aORs: 1.76; 95% CI 1.21–2.57, 1.64; 95% CI 1.14–2.35 and 1.51; 95% CI 1.02–2.24), respectively.

Conclusion and Clinical Relevance IgE responses to *Ascaris* are associated with asthma symptoms in a population living in the tropics. Sensitization to the cross-reactive *Ascaris* and mite tropomyosins partially underlies this finding. These results have potential relevance in asthma diagnosis and management.

Keywords allergen, ascariasis, *Ascaris*, asthma, IgE, mite, recombinant allergens, tropomyosin

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Introduction

Allergic diseases are important health problems around the world. In Colombia, a tropical developing country,

their prevalence, as in other countries, has risen in recent decades [1, 2]. Patients with asthma in the tropics are sensitized (i.e. have positive IgE antibodies) to house dust mite (HDM), such as *Blomia tropicalis* and

Dermatophagoides pteronyssinus [3, 4]. Also, ascariasis (caused by the helminth *Ascaris lumbricoides*) is present [5–7] being the most common helminth intestinal infection [8, 9], inducing a strong IgE response from an early age [10]. These features presumably result from the genetic background of the population combined with climatic and socio-economic conditions of tropical countries (where around 40% of people are currently living) that lead to permanent exposure to parasites and HDM [11]. Therefore, a comprehensive analysis of these characteristics is required ultimately to explain the inception, pathogenesis and rising incidence of allergy.

Anti-*Ascaris* IgE has been associated with asthmatic symptoms [12, 13] and also with the frequency and severity of asthma [14, 15], but it remains to be formally established whether this reactivity is independent of mite exposure and whether there is a true cause and effect relationship. Besides, it has been suggested that during helminth infection, allergic symptoms (mainly detected by skin testing) decrease [16–18], indicating that there is some degree of parasite-induced immunomodulation, although there are reports of a boosting effect of ascariasis on the IgE response to bystander antigens [19–21]. Nevertheless, the available data suggest that ascariasis and its concurrent IgE response influence asthma pathogenesis [10, 22], and further investigation to define the specific targets of these antibodies will hopefully clarify the mechanisms involved.

Because of the cross-reactivity between *Ascaris* and mite extracts [23], a molecular diagnosis approach is needed to accurately define the sensitization profile. Asc s 1 (also known as ABA-1) is a fatty acid binding protein [24, 25] of a protein type only found in nematodes and shows no cross-reactivity with allergens of mites extracts [23]. Tropomyosins from *Ascaris* (Asc l 3), *B. tropicalis* (Blo t 10) and *D. pteronyssinus* (Der p 10) are, however, cross-reacting allergens of clinical relevance [4, 26]. Asc l 13 (glutathione S-transferase) is a protein component of *Ascaris lumbricoides*, specific serum IgE to this molecule positively correlating with the IgE levels to mite and cockroach [27]. We hypothesize that cross-reacting allergens of *Ascaris* and mites are involved in the reported association between IgE sensitization to *Ascaris* and asthma. In this study, we aimed first to evaluate, in a large and well-characterized population, whether IgE sensitization to *Ascaris* is associated with asthma; secondly, to determine whether the IgE response to purified allergens of *Ascaris* and HDM is associated with asthma; and thirdly, to evaluate whether past or current ascariasis (as detected by sensitization to a nematode-specific marker) influences the IgE responses to mite allergens.

Methods

Subjects

The general design and methods, including setting and eligibility criteria, for participants are described in detail elsewhere [1]. Briefly, we performed a cross-sectional population-based survey with an ancillary nested case-control study in six Colombian cities. The questionnaires were based on the International Study of Asthma and Allergies in Childhood initiative (ISAAC) [28] and validated for ages 7–59 years on a previous study [2]. A 'case' was defined as any subjects who answered yes to having current or past asthma symptoms. Asthma symptoms in the last year: Have you (or your child) had wheezing or whistling in the chest in the past 12 months? Past (lifetime) asthma symptoms: Have you (or your child) ever had wheezing, or whistling in the chest at any time in the past? 'Control' was defined as any subject who (i) reported not having had symptoms of asthma, allergic rhinitis or atopic eczema in the last year and (ii) reported no previous medical diagnosis of asthma, allergic rhinitis or atopic eczema. The Colombian population is divided into six socio-economic strata considering urban characteristics such as population density, quality of public areas and housing characteristics. Stratum one corresponds to the lowest and stratum six to the highest. From a total of 5978 participants in the 'Prevalence of asthma and other allergic conditions in Colombia' study, 855 (cases and controls) were frequency-matched by age group, gender and city [1]. Additional consent to donate a blood sample was requested from all members of this group. Serum samples were stored at -70°C until use. In this study, only subjects aged 7 to 59 years old were included, considering that in children below 7 years, asthma may be transient and the relation with an established phenotype of asthma is less clear (Fig. 1). Therefore, serology analyses were performed on 791 blood samples distributed as follows: Barranquilla ($n = 95$), Bogotá ($n = 325$), Bucaramanga ($n = 80$), Cali ($n = 111$), Medellín ($n = 121$) and San Andrés Island ($n = 59$). Ethical approval was obtained from the Clinical Research Ethics Committee at Fundación Cardio-Infantil-Instituto de Cardiología in Bogotá, Colombia (IORG0006438). Signed informed consent was obtained from all subjects or their parents.

Total and specific IgE

Total IgE and specific IgE antibody levels to *D. pteronyssinus*, *B. tropicalis* and *Ascaris spp.* extracts were measured using ImmunoCAP system (Thermo Fisher Scientific), following the technical instructions of the manufacturer. Total IgE was

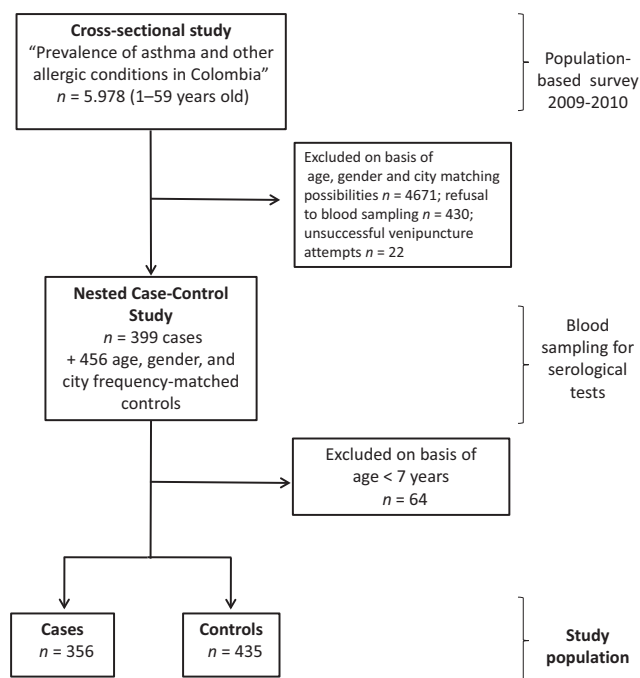


Fig. 1. Flowchart for the individuals included in this study. A total of 5978 subjects were identified, and 855 were case–control frequency matched. Subjects below 7 years of age were excluded because an established phenotype of asthma is less clear in that group. The final sample size was 791 subjects (356 cases and 435 controls).

analysed using 100 kU/L as cut-off. Specific IgE levels equal to or greater than 0.35 kU_A/L were considered positive. Specific serum IgE levels to the recombinant allergens rAsc l 3, rBlo t 10, rDer p 10, rAsc s 1 and rGSTA were detected in duplicate using indirect enzyme-linked immunosorbent assay (ELISA) as described previously [10, 27, 29]. rGSTA is the GST1 from *Ascaris suum*; IgE levels to this molecule highly correlate with those to Asc l 13. Each well was coated with 0.75 µg of rGSTA diluted in PBS (pH 7.4) and 1 µg of all other recombinants diluted in sodium carbonate/bicarbonate buffer (pH 9.6). Microtiter plates (IMMULON 4HBX, Thermo Fisher Scientific) were incubated overnight at 4°C in humid chamber, washed five times (PBS pH 7.4, 0.1% Tween 20) and blocked with 1% bovine serum albumin. The plates were washed again, and serum samples (diluted 1 : 5) were added and incubated overnight at room temperature in a humid chamber, then washed and incubated for 2 h with anti-human IgE–alkaline phosphatase conjugate (Sigma) diluted 1 : 500 and developed with *p*-nitrophenylphosphate substrate (1 mg/mL; Sigma) diluted in 10% diethanolamine, 0.5 mM MgCl₂. The reaction was stopped with 100 µL of 3N NaOH, and absorbance was measured at 405 nm in a spectrophotometer (Spectra Max 250; Molecular Devices, Sunnyvale, CA, USA). Positive and negative control sera were used in each experiment and PBS

as a control for non-specific binding of anti-human IgE–alkaline phosphatase conjugate. Cut-off values to define positive or negative IgE responses to the recombinant allergens were calculated as the mean optical density (OD) of 11 negative, non-mite/*Ascaris*-sensitized controls + 3 SD. The cut-off values were rAsc l 3: 0.116, rBlo t 10: 0.120, rDer p 10: 0.121, rAsc s 1: 0.124 and rGSTA: 0.150. Sensitization was defined as a positive specific IgE result to any extract or recombinant allergen.

Expression and purification of recombinant allergens

rAsc s 1 (ABA-1; GenBank Accession Number L03211) was cloned into pGEX-1λT vector and expressed as a glutathione transferase fusion protein in *Escherichia coli* BL21 (Invitrogen Corporation, Carlsbad, CA, USA). Expression was induced with isopropyl β-D-thiogalactopyranoside (IPTG) 0.1 mM, 4 h at 37°C. Lysates were incubated with glutathione–Sepharose beads, the eluates were digested with thrombin, and rAsc s 1 was purified as a non-tagged protein [23]. rAsc l 3 (accession number FJ655903.1) and rGSTA, accession number X75502.1, were cloned into pQE-30 vector and expressed in *E. coli* M15 cells, purified with Ni-NTA agarose as a 6xHis-tagged protein, dialysed extensively and lyophilized as previously described [4, 27]. rBlo t 10 (accession number ABU97466.1) and rDer p 10 (accession number AAB69424.1) were cloned into pET-45b(+) vector. Competent *E. coli* BL21 (DE3) cells were transformed and selected on Luria broth agar plates containing ampicillin, 100 µg/mL. Expression was induced as mentioned, and pellets from induced cultures were re-suspended in native buffer (50 mM NaH₂PO₄, 300 mM NaCl), incubated with lysozyme (1 mg/mL, 30 min on ice) and sonicated. Lysates were incubated with Ni-NTA resin (Invitrogen) for 1 h and eluted with native buffer plus 250 mM imidazole as 6xHis-tagged proteins and dialysed extensively.

Statistical analysis

Analyses were carried out using SPSS version 17.0 (SPSS, Chicago, IL, USA) and GRAPHPAD PRISM version 5.01 for Windows (GraphPad Software, San Diego, CA, USA). IgE values were not normally distributed, and transformations for normalizing were unsuccessful; they were therefore reported as median values and interquartile ranges. Differences between proportions of subjects sensitized were analysed by Pearson chi-square test. Mann–Whitney *U*-test was used for comparison of continuous variables, and correlations were analysed with Spearman test. Univariate and multivariate binary logistic regression models were used to analyse the relationships

of exposures and outcomes. Predictor/exposures were: sensitization to *Ascaris* or HDM extracts and sensitization to recombinant allergens. The outcomes were asthma and sensitization to HDM allergens. *A priori* adjusting variables were age, gender, socio-economic stratum and city. In addition, as cross-reactivity between *Ascaris* and mites may act as a potential confounder of the associations with asthma, other covariates (e.g. IgE sensitization to any of the extracts or recombinants) were included in the models, as needed. Crude (OR), adjusted odd ratios (aORs), 95% confidence intervals (95% CI) and *P* values were calculated. Population attributable fractions (PAFs) were calculated using the formula $P \times (OR-1)/OR$, where *P* is the prevalence of sensitization among asthmatics. In addition to logistic regression models, the analyses were performed using mixed-effects logistic regression with two-level data structure (we specified random effects at the level identified by group variable city, and the covariates mentioned above were treated as fixed effects). There were not important differences between the two procedures (data not shown). Power was calculated assuming an independent case-control design. Setting type I error level = 0.05, the number of asthma cases *n* = 356 and a prevalence of *Ascaris* sensitization of 29%, the study power is 84.8% for detecting an effect on asthma symptoms of OR = 2.17. For all analyses, *P* values < 0.05 (two tailed) were considered significant.

Results

Characteristics of the study population

To assess potential bias and representativeness of cases and controls from the population from which they were derived, the distribution of possible risk factors for which data were available was analysed as shown in Table 1. The final study population is representative of the original case-control subpopulation. Frequency matching by age, gender and city was not significantly altered by excluding children under 7 years old. Mean age and standard deviation (SD) was 29.0 ± 15.8 for cases and 31.5 ± 15.6 for controls (*P* = 0.02). The gender ratio was similar for cases and controls (females 66.0% and 61.1%, *P* = 0.16). All cases of past asthma also reported current asthma. Serology tests were performed for 791 subjects, 356 cases and 435 controls. Median of total serum IgE levels was higher in cases than controls (123 vs. 47.2 kU/L, *P* < 0.001); also, specific IgE to *Ascaris*, *B. tropicalis* and *D. pteronyssinus* and the recombinant allergens rAsc l 3, rBlo t 10, rDer p 10 and rAsc s 1 was significantly greater in cases than controls, but there were no differences regarding rGSTA. In addition, for rAsc s 1 and rGSTA, the frequency of sensitization was almost the same in patients and controls (Fig. 2, Table 2).

Table 1. Descriptive statistics of study population*

Variables	Subjects with asthma symptoms					Subjects without asthma symptoms				
	Entire sample, <i>n</i> = 635		Subsample, <i>n</i> = 356		<i>P</i> value	Entire sample, <i>n</i> = 4705		Subsample, <i>n</i> = 435		<i>P</i> value
	<i>n</i>	%	<i>n</i>	%		<i>n</i>	%	<i>n</i>	%	
Sex										
Male	240	37.8	121	34.0	0.232	1822	38.7	169	38.9	0.959
Female	395	62.2	235	66.0		2883	61.3	266	61.1	
City										
Barranquilla	92	14.5	48	13.5	0.293	540	11.5	47	10.8	0.047
Bogotá	244	38.4	148	41.6		1896	40.3	177	40.7	
Cali	110	17.3	44	12.3		781	16.6	67	15.4	
Bucaramanga	66	10.4	34	9.5		333	7.1	46	10.6	
San Andrés	36	5.7	22	6.2		330	7.0	37	8.5	
Medellín	87	13.7	60	16.9		825	17.5	61	14.0	
Socio-economic stratum										
I-II	306	48.1	173	48.6	0.919	2344	49.8	219	50.3	0.559
III-IV	314	49.5	176	49.4		2221	47.2	207	47.6	
V-VI	15	2.4	7	2.0		140	3.0	9	2.1	
Age										
7-18	266	41.9	124	34.8	0.078	1241	26.4	118	27.1	0.860
19-38	185	29.1	122	34.3		1727	36.7	154	35.4	
39-59	184	29.0	110	30.9		1737	36.9	163	37.5	

*Subjects under 7 yrs were not included; then, only 5340 of 5978 were analysed.

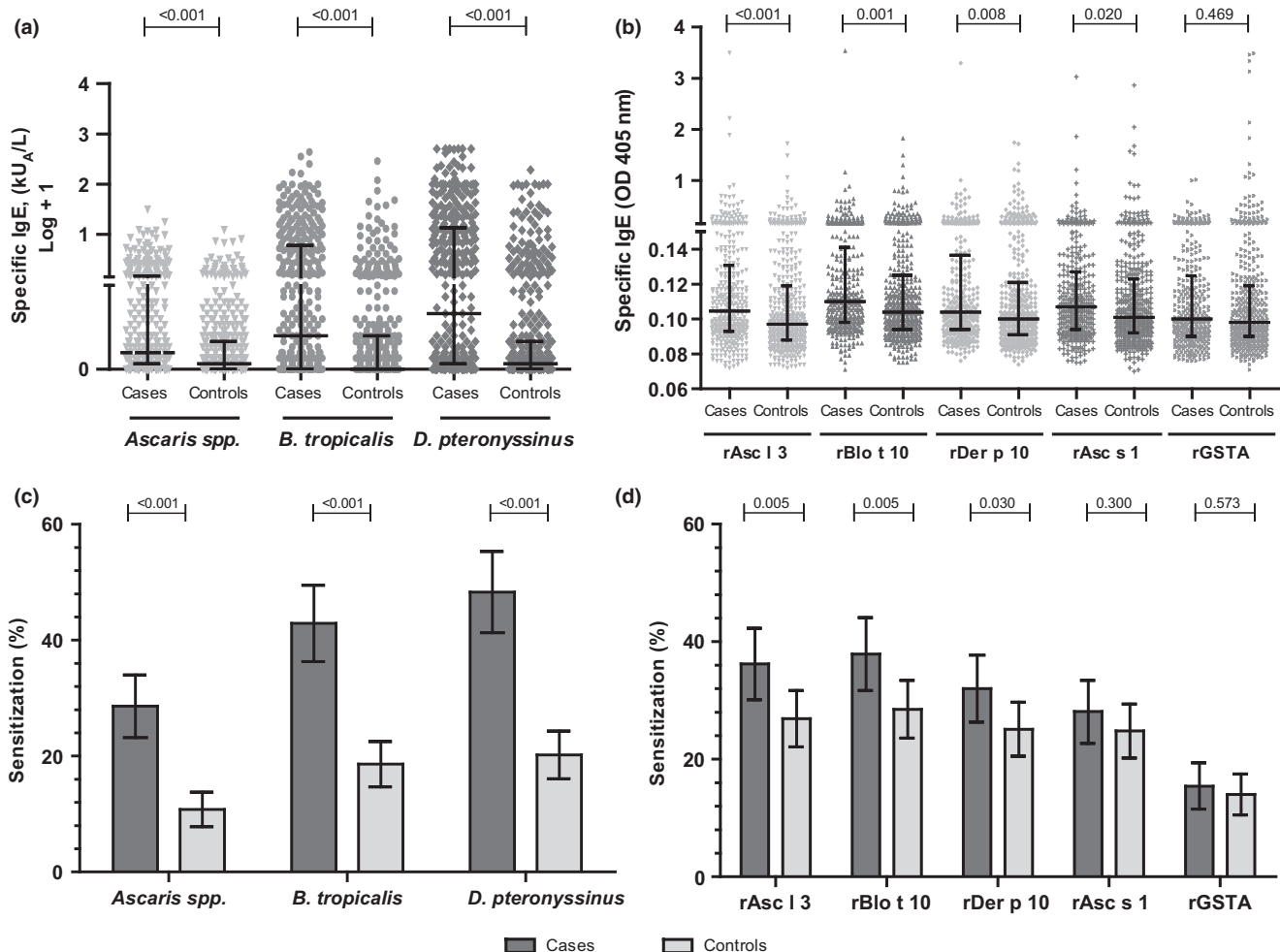


Fig. 2. Strength and frequency of the IgE reactivity to *Ascaris*, mites or recombinant protein allergen components. (a) Log scale IgE levels to *Ascaris*, *B. tropicalis* and *D. pteronyssinus*. (b) IgE levels to rAsc l 3, rBlo t 10, rDer p 10, rAsc s 1 (ABA-1) and rGSTA. Each dot indicates an individual serum from a case or control (356/435). Error bars indicate 95% CI of the median IgE levels. *P* values were calculated using the Mann-Whitney *U*-test. (c) Bar graphs summarizing frequencies of sensitization to *Ascaris*, *B. tropicalis*, *D. pteronyssinus*, (d) rAsc l 3, rBlo t 10, rDer p 10, rAsc s 1 and rGSTA. *P* values were calculated using the chi-square test.

Sensitization to *Ascaris* or *D. pteronyssinus* is associated with asthma

Univariate analysis showed that sensitization to *Ascaris*, *B. tropicalis* or *D. pteronyssinus* was associated with asthma; in multivariate analysis (Table 2), sensitization to *Ascaris* and *D. pteronyssinus* remained associated (aORs: 2.17, 95% CI: 1.37–3.42, *P* = 0.001 and 2.46, 95% CI: 1.54–3.92, *P* < 0.001, respectively), suggesting that both *Ascaris* and *D. pteronyssinus* sensitization are independent risk factors for asthma in this population. In the case of *B. tropicalis*, when sensitization to *Ascaris* and *D. pteronyssinus* was included as covariates, the association disappeared (aOR: 1.33, 95% CI: 0.80–2.21, *P* = 0.28). The population attributable fractions for asthma associated with sensitization to *Ascaris* and *B. tropicalis* were 15.4% and 10.6%, respectively, while that for *D. pteronyssinus* was 28.7%.

Sensitization to *Ascaris* or mite tropomyosins is associated with asthma

We found that the IgE response to any of the highly allergenic and cross-reactive tropomyosins rAsc l 3, rBlo t 10 or rDer p 10 is associated with asthma (Table 2). Moreover, when the analysis included as a variable a positive result to at least one of them, the significance of the association increased (aOR 1.81, 95% CI: 1.26–2.59, *P* = 0.001). Population attributable fractions for asthma associated with sensitization to tropomyosins were higher than those for Asc s 1 or GSTA. In addition, specific IgE levels to mite tropomyosins were significantly higher than those to Asc l 3 (Wilcoxon matched-pairs signed-rank tests *P* < 0.001). We also analysed the IgE levels to tropomyosins for each quartile using logistic regression models. We found a significant, positive dose–response association

Table 2. Association of sensitization with *Ascaris*, mites or recombinant molecular components and asthma

Sensitization to	Cases (n = 356)	Controls (n = 435)	Crude OR (95% C.I.)	P value	Adjusted OR* (95% C.I.)	P value	PAF (%)
<i>Ascaris</i> spp.	102 (28.6%)	47 (10.8%)	3.32 (2.27–4.85)	< 0.001	2.17 (1.37–3.42) ¹	0.001	15.4
<i>Blomia tropicalis</i>	153 (42.9%)	81 (18.6%)	3.29 (2.39–4.54)	< 0.001	1.33 (0.80–2.21) ²	0.279	10.6
<i>Dermatophagoides pteronyssinus</i>	172 (48.3%)	88 (20.2%)	3.69 (2.69–5.04)	< 0.001	2.46 (1.54–3.92) ³	< 0.001	28.7
rAsc l 3	129 (36.2%)	117 (26.9%)	1.55 (1.14–2.09)	0.005	1.76 (1.21–2.57) ⁴	0.003	15.6
rBlo t 10	135 (37.9%)	124 (28.5%)	1.53 (1.14–2.07)	0.005	1.64 (1.14–2.35) ⁴	0.008	14.8
rDer p 10	114 (32.0%)	109 (25.1%)	1.41 (1.03–1.92)	0.031	1.51 (1.02–2.24) ⁴	0.040	10.8
rBlot 10, rDer p 10 or rAsc l 3	145 (40.7%)	131 (30.1%)	1.60 (1.19–2.14)	0.002	1.81 (1.26–2.59) ⁴	0.001	18.2
rAsc s 1	100 (28.1%)	108 (24.8%)	1.18 (0.86–1.63)	0.300	0.98 (0.66–1.45) ⁵	0.917	–0.6
rGSTA	55 (15.4%)	61 (14.0%)	1.12 (0.76–1.66)	0.573	0.73 (0.44–1.21) ⁶	0.224	–5.7

OR, odds ratio; PAF, population attributable fractions.

*All 9 models were adjusted by age, gender, socio-economic stratum, city plus one of the following groups of variables:

¹Sensitization to *B. tropicalis* or *D. pteronyssinus*.

²Sensitization to *Ascaris* or *D. pteronyssinus*.

³Sensitization to *Ascaris* or *B. tropicalis*.

⁴Sensitization to rAsc s 1 or rGSTA.

⁵Sensitization to rAsc l 3 or rGSTA.

⁶Sensitization to rAsc s 1 or rAsc l 3.

with asthma (Table S1) among those at higher quartiles. Inclusion of tropomyosins sensitization as three independent variables in the model for the association between *Ascaris* sensitization and asthma (data not shown) resulted in a small change of OR from 3.32 (95% CI: 2.27–4.85, $P < 0.001$) without tropomyosins to 3.16 (95% CI: 2.15–4.64, $P < 0.001$) with tropomyosins, showing no evidence of effect mediation by tropomyosin. However, this analysis might be limited by collinearity between IgE variables.

The effect of sensitization on asthma: analysis by age and gender

To examine whether there were age-dependent differences in associations with asthma, we divided the population into three age groups, namely 7–18, 19–38 and 39–59 years with a similar number of subjects in each group (Table S2). In all groups examined, sensitization to *Ascaris* or *D. pteronyssinus* was a strong risk factor for asthma. As in the general population, the effect of *B. tropicalis* sensitization on asthma disappeared when adjusted by *Ascaris* and *D. pteronyssinus* sensitization in all age groups (data not shown). In the groups 19–38 years and 39–59 years, sensitization to any of the three tropomyosins was associated with asthma (aORs: 1.67, 95% CI: 0.99–2.84, $P = 0.05$ and 1.82, 95% CI: 1.05–3.17, $P = 0.03$, respectively). Total IgE levels above 100 kU/L were associated with asthma in individuals aged 7–18 years and 19–38 years (Table S2). We also examined whether the effects of sensitization on asthma differed by gender, and we found that in

females sensitization to Der p 10 and Blo t 10 was not associated with asthma (Table S3).

IgE levels and frequencies of sensitization to mite allergens are greater in subjects sensitized to Asc s 1

IgE levels to HDM allergens and *Ascaris* tropomyosin were significantly higher in subjects sensitized to Asc s 1 (Table 3). In addition, the frequencies of sensitization to any allergenic source or purified allergen were significantly higher in Asc s 1-sensitized individuals (Table S4). In fact, these individuals were at least twice the odds of being sensitized to HDM. Also, significant correlations between IgE antibodies to rAsc s 1 and tropomyosins were found (Fig. 3a).

Specific IgE to tropomyosins from *Ascaris* and mites strongly correlate

Ascaris IgE antibody levels correlated with those to *B. tropicalis* (Spearman rho: 0.70 $P < 0.001$) and *D. pteronyssinus* (Spearman rho: 0.59 $P < 0.001$). We also analysed the relationship between the IgE responses to *Ascaris* and mite tropomyosins in the whole population ($N = 791$) and found that *Ascaris* tropomyosin IgE levels correlated with those to Blo t 10 (Spearman rho: 0.69, $P < 0.001$) and Der p 10 (Spearman rho: 0.70, $P < 0.001$). Furthermore, there was a correlation between IgE levels to Blo t 10 and Der p 10 (Spearman rho: 0.87 $P < 0.001$) (Fig. 3b). The frequencies of sensitization to each tropomyosin between individuals sensitized to their corresponding source (*Ascaris*, *B. tropicalis* or *D. pteronyssinus*) were Asc l 3, 47.7%

Table 3. Serum IgE levels to mites and tropomyosins stratified by IgE reactivity to rAsc s 1

IgE levels to	Negative anti-rAsc s 1 IgE (N = 583)	Positive anti-rAsc s 1 IgE (N = 208)	P*
	Medians (interquartile range 25th–75th percentile)		
<i>B. tropicalis</i>	0.02 (0.00–0.27)	0.18 (0.01–4.90)	< 0.001
<i>D. pteronyssinus</i>	0.03 (0.01–0.89)	0.11 (0.02–9.99)	< 0.001
rAsc l 3	0.096 (0.088–0.109)	0.135 (0.109–0.206)	< 0.001
rBlo t 10	0.102 (0.094–0.115)	0.145 (0.110–0.236)	< 0.001
rDer p 10	0.099 (0.090–0.111)	0.146 (0.104–0.231)	< 0.001

IgE levels to extracts are in kU/L and recombinant allergens in optical density (OD).

*Mann–Whitney U-test.

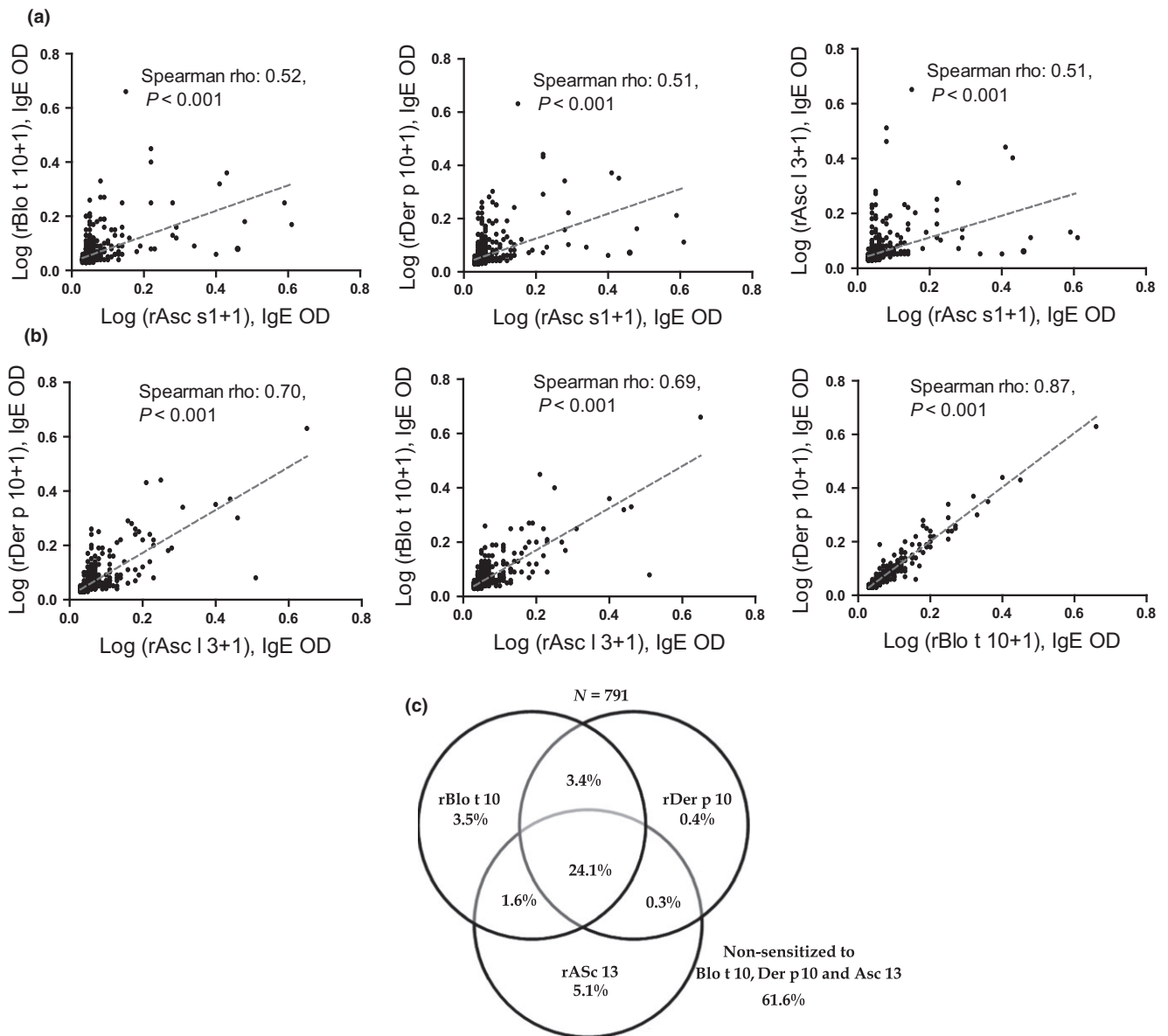


Fig. 3. IgE responses (Log scale) to the recombinant tropomyosins from *Ascaris* and mites. (a) Correlation between specific IgE levels to rAsc s 1, rAsc l 3, rBlo t 10 and rDer p 10. (b) Correlation between specific IgE levels to rAsc l 3, rBlo t 10 and rDer p 10. (N = 791); each dot represents an individual serum value. (c) A Venn diagram illustrating the overlapping sensitization to *Ascaris* or mite tropomyosins. The circles show percentages of individuals positive to rBlo t 10, rDer p 10 or rAsc l 3. Areas within the diagram but outside the circles represent the proportions of non-sensitized individuals.

(95% CI: 36.9–58.5); Blo t 10, 41.0% (33.0–49.0); and Der p 10, 34.6% (27.7–41.6). In addition, 5.1% (40/791) of sera were positive exclusively to Asc l 3, 3.5% (28/791) to Blo t 10 and 0.4% (3/791) to Der p 10 (Fig. 3c).

Discussion

Helminthiasis are recognized modifiers of allergic responses, but a full appreciation of their impacts on human allergic diseases remains to be achieved. Here, we found that the IgE response induced by ascariasis is an independent risk factor for asthma in the general population. Among other possible explanations for this relationship, three merit consideration (Fig. 4) because they have some immunoepidemiological support [11, 22, 30]: firstly, co-exposure to both HDM and *A. lumbricoide*s induces an enhanced Th2-biased immune response causing symptoms in susceptible individuals; secondly, cross-reactivity among components of both sources intensifies the Th2 (mainly IgE) response to particular disease-associated allergens such as tropomyosins; thirdly, the helminth *A. lumbricoide*s has components that during infection are able to boost the IgE responses against environmental allergens from mites.

Our data suggest that the IgE responses to particular allergens (i.e. tropomyosins) underlie the association between ascariasis and asthma. Whether this reactivity is directed against cross-reacting, species-specific or disease-specific epitopes of tropomyosins is currently under study in our laboratory. We also observed that having an IgE response to Asc s 1, a marker for current or past ascariasis, is associated with more reactivity to mite allergens, supporting the idea that ascariasis may be a non-specific booster of the Th2

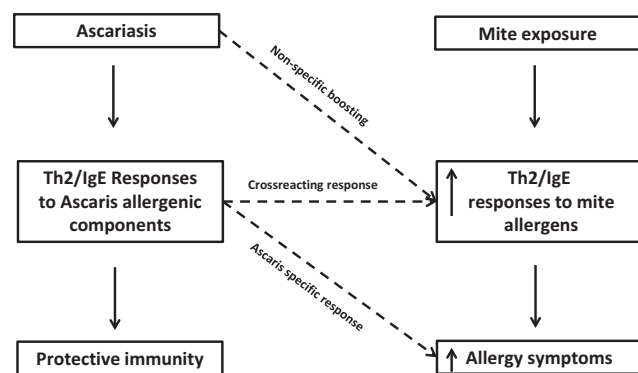


Fig. 4. A hypothetical model showing interactions between the IgE responses to *Ascaris* (during and after ascariasis, left) and the IgE responses to mite allergens (right). Dashed arrows indicate a stimulant effect. Co-exposure to both sources generates conditions resulting in the increase of allergy symptoms, explaining some of the findings in this study. The model applies to tropical conditions where house dust mite exposure is permanent and ascariasis is commonly present. In this study, only the IgE responses were evaluated.

responses, a process that has been described in experimental animal infection [19–21] and in humans infected at moderate intensities [31]. Further studies are needed to define more accurately this boosting effect on the IgE responses to mite species-specific allergens and to evaluate whether there is a common genetic predisposition for reacting to Asc s 1 and mite allergens. But in general, our finding suggests that at least for mite sensitization and under particular conditions of co-exposure, ascariasis increases the IgE responses with clinical relevance at the population level.

The relationship between IgE responses to *Ascaris* and asthma or asthma-related phenotypes has been explored, but two limitations should be considered. First, previous studies have employed the whole extracts only [13, 14, 32, 33]. In contrast, our work supports some of these findings using organism-specific protein targets in a large and well-characterized population from a countrywide survey for asthma prevalence [1]. The predictor/exposure was specific IgE responses, an indirect way for establishing past or present contact with *Ascaris* or HDM. For detecting nematode and trematode infections, several studies indicate that serodiagnosis can be more sensitive than stool examination [34–36]. In addition, as *Ascaris* shares cross-reactive molecules with other invertebrates [4, 27], we used the specific nematode marker Asc s 1 to identify, among subjects reacting to the whole extract, those more likely to have been in contact with the parasite. However, as happens with other antigens and allergens, antibody responses to ABA-1 is probably MHC restricted [37–39]; it is therefore possible that some infected subjects could be missed, thereby lowering the sensitivity of the test. Second, some of these studies did not adjust for HDM sensitization, which is an important risk factor for asthma in the tropics and may confound the results. Now we demonstrate that sensitization to *Ascaris* is an independent risk factor for asthma in all age groups and more importantly that the IgE reactivity to HDM and *Ascaris* tropomyosins probably partially underlies this association, a finding that was already suggested when we evaluated a smaller population sample [4]. It can be speculated that inhaled exposure to cross-reacting tropomyosins in sensitized patients may trigger and maintain asthmatic symptoms, although other cellular/cytokine-mediated mechanisms may also be involved [31]. The possibility that the IgE responses to species-specific *Ascaris* components have an additional influence on asthma pathogenesis in tropical countries is theoretically possible as several IgE-binding components of *Ascaris* are not cross-reactive with HDM allergens [11, 23].

This investigation adds evidence showing that ascariasis does not always dampen allergic symptoms, a

situation that has been described for allergen skin tests in infected populations living in endemic rural areas [16–18]. Also, the results suggest that although helminths produce down-regulatory molecules, in some circumstances, such as low-intensity infections, their effects may be surpassed by the parasite immunostimulant components. Therefore, our results and additional data from developing tropical countries such as Ecuador, Brazil and Venezuela [12, 32, 33] might be expected in countries with similar hygiene conditions and urbanization. So far, the intensity of the infection has explained the different outcomes of the relationship between ascariasis and allergy [22, 40, 41], although other factors requiring clarification may play a role.

The PAF value for asthma associated with sensitization to *Ascaris* (15.4%) was lower than that calculated in a previous study (45.9%) in Ecuador [12]. This may reflect differences in the study population and environmental factors, particularly those related to rural vs. urban settings. In this regard, our data show that although *Ascaris* sensitization is an independent risk factor for asthma, mite sensitization (especially to *D. pteronyssinus*) also plays an important role in asthma pathogenesis.

Among the recombinant allergens utilized here, only sensitization to tropomyosins seems to have clinical relevance. The risk of asthma increased with the IgE levels to these allergens. There was no association with rGSTA sensitization, in agreement with the low strength of the IgE response to this recombinant found in a previous study [27]. Also there was no association between sensitization to rAsc s 1 and asthma, supporting the idea that *Ascaris* has IgE-binding components mainly associated with resistance (i.e. Asc s 1) and others associated with allergy [11]. Tropomyosins are a family of proteins that comprise a large number of reported allergens, hence their designation as so-called pan-allergens. The high amino acid sequence similarity among these molecules provides structural support for immunological cross-reactivity [23, 26] that could lead to the enhancement of sensitization to tropomyosins from both HDM and *Ascaris*. The capability of tropomyosins from these sources to induce allergic reactions has been evaluated *in vivo* and *in vitro* in small samples [4, 26, 42, 43]. Here, for the first time in a nationwide nested case-control study, we show that IgE sensitization to HDM or *Ascaris* tropomyosins has clinical relevance.

IgE-binding frequencies of Der p 10 in studies from China [44], Italy [45], Germany [46], Spain [42], Austria [47], Sweden [48] and France [49] range from 2 to 28%. There is little information on the prevalence of sensitization to Blo t 10 in atopic subjects. One report from Singapore [43] shows that 29% of patients with rhinitis have IgE to Blo t 10. These

results contrast with the high prevalence (55%) of IgE response to Der p 10 reported in Africa [50]. A possible explanation may be exposure to specific and cross-reacting epitopes of helminths. Moreover, in a previous study, we found that 74.9% of *Ascaris*-sensitized asthmatic patients were positive to Asc l 3 [4], and in the present work, 59% of asthmatics with a positive IgE response to the specific nematode marker Asc s 1 were sensitized to Der p 10 (data not shown). Also, a high correlation between the IgE responses to the three tropomyosins was observed (Fig. 3b). Furthermore, Santiago et al. [51] found in an experimental study that cross-reactive anti-tropomyosin antibodies induced by filarial infection bind to mite tropomyosin, which is in agreement with findings in humans. These data could help to define a need for the inclusion of *Ascaris* or HDM tropomyosins in arrays for component-resolved diagnosis in the tropics, where a simplification by combining these cross-reactive allergens in serological assays may be advantageous.

The fraction of asthma attributable to sensitization with any of the tropomyosins was higher than that for sensitization to *Ascaris* (18.2% vs. 15.4%), suggesting that the IgE responses to few key molecules belonging to the same family have an important contribution to the risk of asthma, higher than environmental factors associated with asthma in other populations [52]. As there are no previous studies analysing the association between mite tropomyosins and asthma in a large population, our results also provide a new view on the role of HDM tropomyosins in asthma. The IgE response to these allergens was higher than that to Asc l 3, suggesting that the primary sensitizer was Blo t 10 or Der p 10. In addition, the patterns of IgE responses to the three tropomyosins indicate that although most of the epitopes recognized are cross-reactive, species-specific epitopes may exist.

Some limitations of this study merit consideration. First, we did not investigate current *Ascaris* infection by stool analysis. However, the results of other studies from our group suggest that the frequency and intensity of ascariasis in urban settings are low [9, 10]. Second, although questionnaires for asthma were consistently validated, the lack of a complete clinical diagnosis of asthma could influence case definition, based mainly on wheezing. Third, as mentioned above, the screening of the IgE responses to recombinant HDM species-specific allergens would have provided a more precise picture of the IgE boosting effect of ascariasis. However, the availability of this panel of allergens is still limited, and further work is necessary to characterize them. Finally, in the definition of control, we excluded subjects with allergic rhinitis and atopic eczema because these diseases are associated

with asthma; however, this might lead to a selection bias of the controls because it was not applied to cases.

In summary, we have shown that, in a tropical environment at least, IgE responses to *Ascaris* are a risk factor for asthma in all ages and identified tropomyosins as allergens partially underlying this association. We could not rule out sensitization to other *Ascaris*-specific allergenic components participating as risk factors, so further investigation may reveal a larger set of *Ascaris* components potentially modifying the evolution of asthma. We also found an additional, probably non-specific boosting effect arising from a history of ascariasis on the IgE responses to HDM.

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Conflict of interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Odds ratios of asthma among quartile categories of IgE to tropomyosins.

Table S2. Association of sensitization to *Ascaris*, mites or recombinant molecular components and asthma: analysis by age groups.

Table S3. Association of sensitization to *Ascaris*, mites or recombinant molecular components and asthma: analysis by gender.

Table S4. Frequencies of sensitization to mites and tropomyosins according to IgE reactivity to rAsc s 1.