

Early life IgE responses in children living in the tropics: A prospective analysis

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Abstract

Background: There are few birth cohort studies analyzing IgE sensitization in the tropics.

Objectives: We aimed to describe the evolution of total IgE and specific IgE responses to house-dust mite (HDM) allergens and *Ascaris* in a birth cohort (Risk Factors for Asthma and Allergy in the Tropics, FRAAT), analyzing their relationships with wheezing.

Methods: Total and specific IgE were measured by ImmunoCap in mothers and children at four different time points (S1–S4) between 0 and 42 months. Parasite infection was evaluated by stool examination.

Results: Maternal total IgE (aOR: 2.43, 95% CI: 1.09–5.43; $p = 0.03$) and socio-demographic factors were associated with high cord blood (CB) total IgE. High CB total IgE was positively associated with higher *Blomia tropicalis* and *Ascaris*-specific IgE values during lifetime, but protected from recurrent wheezing (aOR: 0.26, 95% CI: 0.08–0.88, $p = 0.03$). Prevalence rates of IgE sensitization were high; at around 3 yr old, they were 33.3, 18.6, and 26.5% for *B. tropicalis*, *Dermatophagoides pteronyssinus*, and *Ascaris*, respectively. Indicators of unhygienic conditions were risk factors for HDM and *Ascaris* sensitization in children. A weak statistical association between *B. tropicalis*-specific IgE and ever wheezing was found (aOR: 1.47 95% CI: 1.00–2.28, $p = 0.05$).

Conclusions: In a socioeconomically deprived community from the tropics, sensitization to HDM allergens was very frequent at early life, especially to *B. tropicalis*. In contrast to expected according to the hygiene hypothesis, unhygienic/poverty conditions were risk factors for allergen sensitization. High CB total IgE levels were a risk factor for allergen sensitization but protected from recurrent wheezing.

The patterns and evolution of IgE antibody responses in early life provide important information about the natural history of atopy and allergic diseases. As the process is influenced by the environment and the genetic background of the population, birth cohort studies are valuable strategies to analyze early sensitization in humans. However, few of these investigations have been performed in the tropics (1–3), which is an important gap considering that about half of human beings live in this zone. The current concepts of diagnosis and management of allergic diseases are based on evidence collected in industrialized countries and temperate zones; then, knowledge of the evolution of IgE responses during infancy and its relationship

with particular environmental factors in the tropics is essential for obtaining a more realistic view of the pathogenesis of allergy.

Populations in the tropics are perennially exposed to house-dust mite (HDM), mainly *Blomia tropicalis* and *Dermatophagoides pteronyssinus* (4); besides, helminth infections such as ascariasis (caused by *Ascaris lumbricoides*) are still frequent health problems (5). Therefore, studies in the tropics also help to analyze the universality of conceptual frameworks such as the hygiene hypothesis and the role of mite and parasite exposure in the inception of allergy (6). In this region, the effects of conventionally atopy-associated phenotypes, such as

cord blood total IgE, and early allergen sensitization on wheezing deserve further investigation. In addition, the role of particular conditions such as poverty, usually considered protective from IgE sensitization (7), on the evolution of IgE responses in childhood is still controversial (8). We designed the Risk Factors for Asthma and Allergy in the Tropics (FRAAT) birth cohort of children living in Cartagena, a tropical city of Colombia (South America), to investigate several aspects of allergy inception (6). Here, we describe the evolution of total and specific IgE responses in this population and their relationships with wheezing.

Methods

Study design and characteristics of the population

The general design, objectives, and methods, including setting and eligibility criteria for participants of the FRAAT cohort, have been described in detail elsewhere (6). It was conducted in Cartagena de Indias in the north Caribbean coast of Colombia, which has 943,553 inhabitants; most of them are poor according to the governmental indexes (9). Socioeconomic stratification in Colombia ranges from 1 to 6, and the majority of the city population, as well as the study participants, belonged to the lowest strata, 1–3. This is a compounded index comprising single parameters such as type of housing, overcrowding (three or more people per bedroom), access to basic services, income, and school attendance. Stratum 1 ('low-low class') corresponds to the poorest inhabitants (non-brick building material of the houses, homes with critical overcrowding and no access to basic public services, strong economic dependence of the family on one provider member, and families with no assistance to any school-aged children 7–11 yr old) (9).

The genetic background of this population resulted from admixture between Native Americans, Africans, and Spaniards (10). The Ethic Committee of the Fundación Santa Fe de Bogotá, Colombia (CCEI-282-206) approved the study. Written informed consent detailing all procedures to be carried out on children was signed by parents. Three hundred and twenty-six mother–infants pairs were included at baseline, but serology data were obtained from 290 children.

Subjects and serology data

Serology data were obtained from 290 children (Fig. 1), 289 mothers, and 285 cord blood (CB) samples. Two hundred and thirty-four children (80.7%) had two or more determinations of total and/or allergen-specific IgE at different times between 6 and 42 months. Fifty-two children dropped out from the study (16%); exclusion reasons were described previously (6). Antecedents of allergic diseases were similar between excluded and non-excluded mothers and did not influence the willingness to participate in the study. Although remaining active during follow-up, for some participants, one or more samples could not be analyzed due to several reasons (Fig. 1). There were no significant differences in gender, socioeconomic status, access to sewage, tap water, and pet ownership between those where

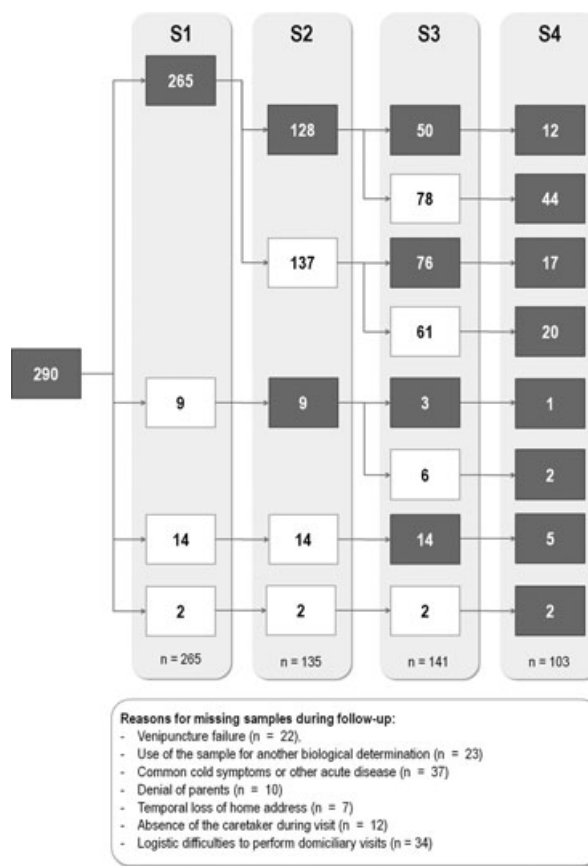


Figure 1 Distribution of children serum samples by time periods. This flow diagram depicts the number of serum samples taken from children during follow-up. Dark boxes indicate the number of collected samples in each time period. As the planned four samples (from S1 to S4) were not collected for all children, the number of missing samples appears as white boxes. Each box is split into two in the next time period to discriminate between collected and missing samples. Lower case n is the total number of children with a serum sample at that time period (sum of numbers inside dark boxes).

serum sample could be obtained and those without sample (excluded and temporarily absent cases) at any particular stage. The influence of prenatal factors on CB total IgE was evaluated in 277 children who had serology data and no evidence of maternal blood contamination. Immunoglobulin A was detected in five cord blood samples, which were excluded from the analysis. The effect of CB total IgE on recurrent wheezing was evaluated in a dataset of 261 children who had evaluations of clinical phenotypes up to 24 months. Risk factors for IgE sensitization at 2–3 yr old (range: 24–42 months.) were analyzed in 176 children who had serology data at this time. Furthermore, in this same database, IgE response to specific markers of HDM (Blo t 5) and *Ascaris* (Asc s 1) as well as to the cross-reactive allergen Asc l 3 (tropomyosin) was evaluated in children sensitized to *B. tropicalis* (40 of 47, 85.1%) or *Ascaris* (40 of 40, 100%) to identify cases of genuine sensitization. Clinical phenotypes and relevant

socio-demographic factors of all subgroups are described in Table S1.

Serum samples

During children's follow-up, serum samples were obtained at different ages, whenever possible, either during planned clinical evaluations at the outpatient service or during domiciliary visits. The samples were grouped into four categories according to the age of collection: S1 (6–11 months), S2 (12–19 months), S3 (20–29 months), and S4 (30–42 months). Six months was the minimum interval time for serial serum collection. When a child had two samples within the same age category ($n = 20$), only the older sample was included in the analysis.

Cord blood (CB) samples were obtained from the maternal portion immediately after delivery using a careful technique to avoid maternal blood contamination (6). Immunoglobulin A levels (IgA) were quantified in all CB samples by immunoturbidimetry (Roche Diagnostic Systems, Berne, Switzerland). Only those with IgA below 4 m/dl were included in the analysis. Blood samples from mothers were collected 12 hours after delivery to obtain sera for antibody determinations. Sera were stored at -20°C until use.

Quantification of total and specific IgE

IgE antibody levels were determined using ImmunoCAP system (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden) according to the manufacturer's instructions. A low-range total IgE assay was chosen for determination of total IgE. Total and specific IgE to *B. tropicalis*, *D. pteronyssinus*, and the nematode *Ascaris* spp. were measured in CB and at different time points between 6 and 42 months. The minimum detection limit for total and specific IgE was 0.01 kU/l.

In addition, specific IgE to molecular components of HDM and *Ascaris* was measured by ELISA as described previously (11). The following recombinant allergens were tested in mothers: Der p 2 (UniProt accession number: A6XEP9), Blo t 5 (O96870), Blo t 12.0101 (Q17282), and Asc s 1 (also named ABA-1, Q06811). In children, IgE ELISA testing included the following: Blo t 5, Asc s 1 and the *A. lumbricoides* tropomyosin: Asc l 3 (ACN32322.1). All samples were assayed in duplicate, and inter- and intra-assay variation coefficients were lower than 15% and 10%, respectively.

House-dust mite (HDM) recombinants were produced as His-tagged proteins and purified by affinity Ni-NTA chromatography. Asc s 1 and Asc l 3 were produced and purified as described previously (12, 13). Cutoffs to determine sensitization to the recombinant allergens were obtained using receiver-operator characteristic (ROC) curve analysis, defining as the state variable a positive result to *B. tropicalis* (for Blo t 5 and Blo t 12), *D. pteronyssinus* (for Der p 2), or *Ascaris* (for Asc s 1 and Asc l 3) extracts by ImmunoCAP.

Skin prick testing

Skin prick tests (SPT) were performed in children at 2–3 yr of age (range: 24–42 months) with a set of allergens containing

the following: HDM species (*B. tropicalis* and *D. pteronyssinus*), cockroach (*Periplaneta americana*), cat and dog dander, histamine phosphate 10 mg/ml as positive control, and allergen diluent as negative control (kindly supplied by Leti; Madrid, Spain). The test was considered positive if the mean diameter of the wheal at 15 min was >3 mm than the negative control. SPT and blood sampling was performed in parallel. Skin testing was not performed when the child was taking antihistamines, refused to collaborate, or had skin lesions.

Stool samples and parasitological examination

Parasitological analyses were performed using 0.85% saline solution and lugol staining; counting helminth eggs were carried out by the Kato-Katz method using a commercial kit (Copro Kit; C&M Medical, Campinas Brazil). The results were expressed as egg per gram of feces (e.p.g.). The presence of eggs from geohelminths or parasite visualization was considered diagnostics of active infection.

Mothers were instructed to collect stool samples from their children at different time points during follow-up. One positive exam was enough to consider a child as infected. Those with negative results in all the stool tests were considered as uninfected; however, to avoid the effect of potential false negatives, children with less than two stool examinations or without examination during the last year of follow-up were excluded from the analyses. Hence, data from 162 children were used for analyses when the effect of *Ascaris* infection was evaluated; 97% ($n = 157$) of them had three or more recorded stool examinations. The cumulative prevalence of a positive stool examination for *A. lumbricoides* was 10.5% and 1.8% for *Trichuris trichiura*. All cases of infection were informed to their parents and children received antihelminthic medication. Use of antihelminthic drugs before 2 yr old was previously reported (6); after this age (range: 24–42 months), 124 children had been treated.

Clinical phenotypes and other outcomes

Wheezing: Mothers were interrogated by a physician 'Have your child ever had episodes of wheezing at any time in the past?' For each affirmative response, additional validation questions were carried out about the episode, medications, and medical management (6). **Recurrent wheezing:** Three or more episodes of wheezing in 12 months. **Maternal asthma and other allergic diseases:** The diagnosis of asthma and other allergic diseases among mothers was performed using a questionnaire (14) and examination by a physician. **Sensitization:** A positive IgE value (>0.35 kU_A/l, ImmunoCAP) to any of the tested allergens. **HDM sensitization:** A positive IgE result to *B. tropicalis* or *D. pteronyssinus* extracts. **HDM components' sensitization:** A positive IgE result to at least one of the recombinant allergens (rBlo t 5, rBlo t 12, and rDer p 2) in the mother. Because these recombinant mite allergens do not have known close homologs among common intestinal helminths, this variable should be less liable to false-positive reactions induced by helminth infections. **High total IgE:** IgE value greater than the 70th percentile in mothers. For high CB total IgE, the 80th percentile was used as cutoff (15).

Statistical analysis

All analyses were performed using SPSS version 13.0 (SPSS, Chicago, IL, USA). Total and specific IgE values were not normally distributed; descriptive information about these data for each age category was reported as the median value and its interquartile range. Mann–Whitney *U* test was used for comparison of continuous variables. Differences between proportions were analyzed by Pearson chi-squared test. A linear by linear association chi-squared test was employed to compare frequencies of IgE sensitization among age categories in children with repeated data.

Univariate and multivariate binary logistic regressions were used to analyze the relationships of exposures and outcomes. The predictor/exposures included the following: poverty/dirt indicators (i.e., socioeconomic stratum, type of house, sewage, tap water, pests at home, and pig/poultry at home) and other socio-demographic conditions, high maternal total IgE, maternal HDM or *Ascaris* sensitization, maternal allergic phenotypes, high CB total IgE, and levels of child-specific IgE. The outcomes were as follows: high CB total IgE, *B. tropicalis* or *D. pteronyssinus* sensitization in children, *Ascaris* sensitization in children, and recurrent and ever wheezing. Factors associated with the outcome in the univariate analysis were included in subsequent multivariate models. Gender was included in all multivariate models as a covariate. Crude (OR) and adjusted odds ratios (aORs), 95% confidence intervals (95% CI), and *p*-values were calculated.

Linear mixed models (LMM) were used to assess whether serial determinations of total IgE (log-transformed values) and mite- or *Ascaris*-specific IgE in children were affected by binary variables such as high CB total IgE, recurrent wheezing, and *Ascaris* infection (treated as *fixed factors*). Dependent variables were analyzed as continuous variables, which imply that the statistics do not refer to sensitization status (determined by a specific cutoff). The indicator of a repeated measure was the variable *time* that grouped IgE serology data within the specified age ranges (S1–S4). The exact age in months for serology determination, gender, and socioeconomic status were added to the model as covariates. The obtained estimates (coefficient regression for each effect), 95% CI, and their *p*-values were reported. Positive or negative values of the estimates indicate the direction of the relationship between each predictor and the outcome.

Power was calculated assuming an independent case–control design and expressing the alternative hypothesis as odds ratio (ψ). For a given risk factor, the calculation is performed by setting type I error probability at $\alpha = 0.05$, number of wheezing cases (*n*), probability of exposure among non-wheezers (*p*0), and the ratio of controls/cases (*m*) (16).

Results

Maternal IgE and socio-demographic factors influence cord blood total IgE

Geometric mean of total serum IgE levels was 198.18 kU/l (IQR: 81.1–633.0) in mothers; half of them (59.4%) were above

a frequently used cutoff level to define atopy in other populations (150 kU/l) (17). The cutoff point to define high total IgE in mothers was 502 kU/l (15). Maternal sensitization to *Ascaris* spp., *B. tropicalis*, and *D. pteronyssinus* was 49.5%, 37.4%, and 38.8%, respectively. Regarding CB, only one case of possible sensitization to *D. pteronyssinus* (specific IgE 0.2 kU_A/l) was found. CB total IgE levels in 220 samples were below the limit of detection, but were included in the analysis with an assigned 0.01 kU/l value. The range of CB total IgE was between 0.01 and 26 kU/l (Fig. 2). The 80th percentile of CB total IgE (0.07 kU/l) was used as cutoff to define high CB total IgE (15).

The influence of maternal IgE and prenatal socio-demographic factors on high CB total IgE was analyzed by logistic regression. Crude and adjusted ORs are shown in Table 1. In a multivariate model including all predictors associated with high CB total IgE in the univariate analysis, only maternal high total IgE, cockroaches at home, and pet ownership remained significantly associated with this outcome. By stratified analysis, high maternal total IgE was significantly related to high CB total IgE in the subgroup of HDM-sensitized mothers, but it was independent of *Ascaris* sensitization. Furthermore, maternal high total IgE in interaction with sensitization to any of the non-cross-reactive recombinant allergens (Blo t 5, Blo t 12, or Der p 2) was also strongly associated with high CB total IgE after adjustment for covariates (aOR: 5.88 95% CI: 2.47–13.98, *p* < 0.001; data not shown).

Cord blood total IgE influence the evolution of IgE responses and wheezing in opposite directions

By LMM and after adjustment by gender, age and socioeconomic status, values of log₁₀ total IgE (adjusted estimate: 0.75, 95% CI: 0.57–0.93; *p* < 0.001) and specific IgE to *B. tropicalis* (adjusted estimate: 0.32, 95% CI: 0.11–0.52 *p* < 0.001) or *Ascaris* (adjusted estimate: 1.34, 95% CI: 0.25–2.42; *p* = 0.02) during lifetime were higher in children who had high CB total IgE. No differences were found for *D. pteronyssinus*-specific IgE (Table 2). Fig. 3 shows the dynamics of total and specific IgE over time according to CB total IgE levels. *Ascaris* infection also showed an influence on IgE levels, higher log₁₀ total IgE (adjusted estimate: 0.22, 95% CI: 0.11–0.43 *p* = 0.04)

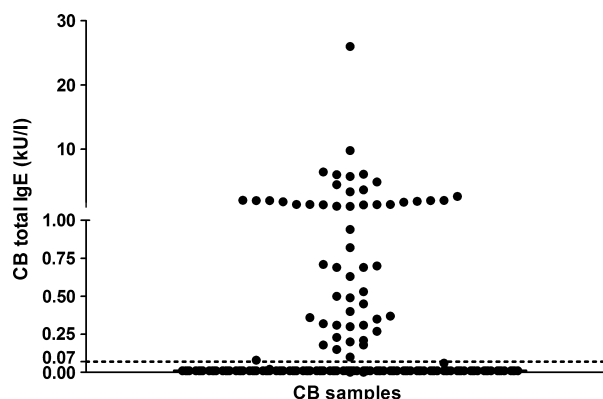


Figure 2 Cord blood (CB) total IgE levels in children. Dashed line represents the cutoff point to define high CB total IgE.

Table 1 Logistic regression analysis of factors influencing high cord blood total IgE

Predictors	Crude			Adjusted*		
	OR	95% CI (OR)	p	OR	95% CI (OR)	p
High maternal total IgE (>502 kU/l)†	3.83a	2.07–7.07	<0.001	2.43	1.09–5.43	0.03
House-dust mite (HDM)-sensitized mothers (n = 132)	3.13	1.37–7.18	0.007	2.84	1.15–7.01	0.02
Non-HDM-sensitized mothers (n = 145)	2.62	0.64–10.77	0.181	1.70	0.33–8.64	0.52
Ascaris-sensitized mothers (n = 136)	2.39	1.10–5.26	0.030	2.42	1.06–5.53	0.036
Non-Ascaris-sensitized mothers (n = 141)	4.58	1.02–20.5	0.047	6.75	1.20–37.89	0.03
HDM sensitization in the mother	2.75b	1.47–5.12	<0.001	1.51	0.69–3.30	0.30
Ascaris sensitization in the mother	3.14c	1.66–5.95	<0.001	1.57	0.69–3.51	0.28
Cockroaches at home	1.92d	0.98–3.72	0.054	2.08	1.01–4.23	0.048
Pet during pregnancy	2.33e	1.28–4.25	0.010	2.74	1.42–5.29	<0.001
Brick house (vs. wood)	0.41f	0.22–0.77	0.010	0.59	0.29–1.20	0.14

*All significant predictors in the univariate analysis were included in the multivariate model. †The effect of high maternal total IgE was evaluated in stratified analyses by maternal HDM or Ascaris sensitization. Powers to detect association between each predictor and the outcome in the univariate analysis: ^a0.99 (54.5% of exposed subjects in high CB total IgE vs. 23.9% in control group), ^b0.91 (67.3% vs. 42.8%), ^c0.96 (70.9% vs. 43.7%), ^d0.50 (69.1% vs. 81.1%), ^e0.79 (49.1% vs. 29%), and ^f0.79 (60% vs. 78.4%).

Table 2 Factors influencing the evolution of allergen-specific and total IgE

Factors	Log ₁₀ total IgE		<i>Blomia tropicalis</i> sIgE		Ascaris sIgE		<i>Dermatophagoides pteronyssinus</i> sIgE	
	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
Cord blood (CB) Total IgE								
Estimate	0.87	0.75	0.43	0.32	1.17	1.34	0.02	0.01
95% CI	0.44–1.30	0.57–0.93	0.01–0.67	0.11–0.52	0.03–2.37	0.25–2.42	–0.05 to 0.08	–0.05 to 0.08
p-value	<0.001	<0.001	0.001	<0.001	0.03	0.02	0.60	0.64
Ascaris infection								
Estimate	0.54	0.22	0.23	0.09	1.20	0.26	0.020	0.024
95% CI	0.05–1.02	0.11–0.43	–0.10 to 0.55	–0.01 to 0.028	0.04–2.37	0.12–0.40	–0.07 to 0.11	–0.06 to 0.118
p-value	0.03	0.04	0.17	0.30	0.04	0.001	0.66	0.60

The effects of these factors on child's total and specific IgE levels during follow-up (S1–S4) were analyzed by LMM. Adjusted data included gender, age, and socioeconomic status as covariates. sIgE, specific IgE.

and Ascaris-specific IgE values (adjusted estimate: –0.26, 95% CI: –0.40 to –0.12, $p < 0.001$) were observed in children with a positive *A. lumbricoides* stool examination (Table 2).

The effects of cord blood IgE on recurrent wheezing were explored by logistic regression. High CB total IgE was inversely associated with recurrent wheezing (aOR: 0.26, 95% CI: 0.08–0.88, $p = 0.03$), after adjustment by gender, maternal asthma, and socioeconomic status. Interestingly, in the LMM analysis, it was detected that recurrent wheezers had lower log₁₀ total IgE values during lifetime (adjusted estimate: 0.20, 95% CI: 0.02–0.37; $p = 0.02$).

HDM and Ascaris are sensitizers during the first years of life

The frequencies of allergen sensitization to *B. tropicalis*, *D. pteronyssinus*, and Ascaris were calculated for each age stage (S1 to S4), including all children irrespective of having repeated data or not (Table 3). A trend for increase in the sensitization rates was observed ($p < 0.001$ for each allergen) when a group of children with serology data ($n = 55$) at three time points (S1, S2, and S4) was analyzed. Remarkably, the

prevalence of IgE sensitization to *B. tropicalis* at S4 (median age: 35 months) was significantly higher than that to *D. pteronyssinus* ($p = 0.002$) and similar to that found in mothers (33.3% and 37.4%, respectively; $p = 0.44$).

SPT was performed in 203 children. Forty-five children ($n = 22.2\%$) responded to any allergen; 29 to any HDM (14.3%), 24 to *B. tropicalis* (11.8%), 9 to *D. pteronyssinus* (4.4%), 11 to cat (5.4%), 8 to dog (3.9%), and 4 to American cockroach (2.0%). In 140 children with available IgE serology between 24 and 42 months, the concordance to define child's sensitization was low (kappa 0.21 for *B. tropicalis* and 0.17 for *D. pteronyssinus*); however, SPT-positive cases were more frequent among those IgE positive (21.6% vs. 4.9% in non-sensitized; $p = 0.003$ for *B. tropicalis* and 15.0% vs. 2.5%, $p = 0.011$ for *D. pteronyssinus*).

Unhygienic conditions are risk factors for house-dust mite and Ascaris sensitization in children

In a subsample of 176 children, with data on IgE serology between 24 and 42 months, the effects of prenatal factors on

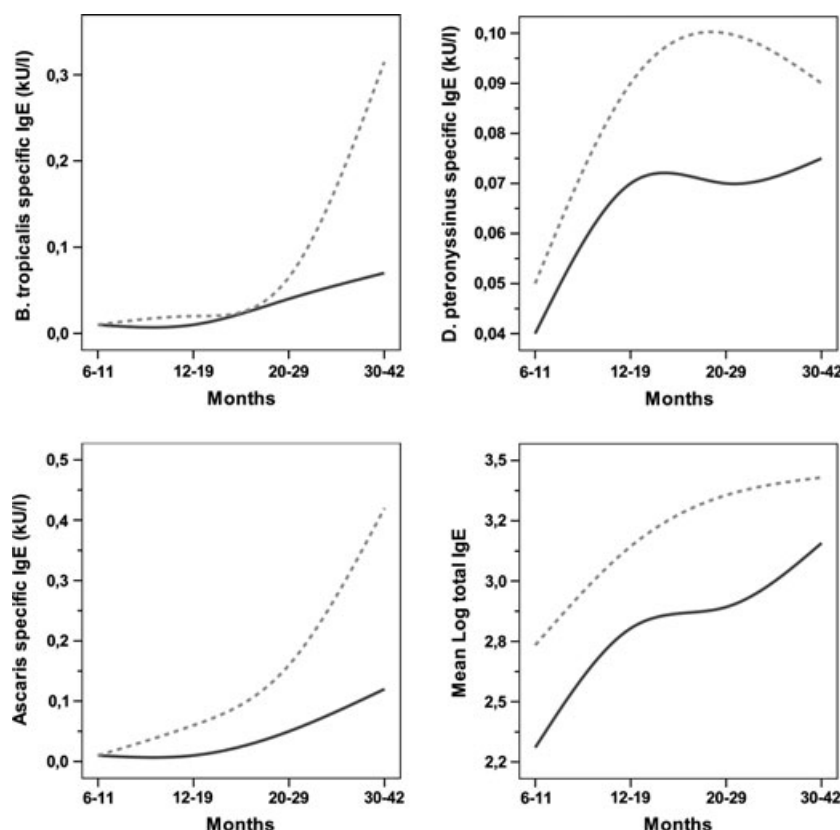


Figure 3 Difference in the dynamics of IgE response in children according to cord blood (CB) total IgE. Graph lines represent median IgE levels to *Blomia tropicalis*, *Dermatophagoides pteronyssinus*, *Ascaris spp.*, and mean Log total IgE at the different time points during child life and discriminated by high CB total IgE (dashed line) or not (solid line).

Table 3 Descriptive of specific serum IgE levels in children from 6 to 42 months

	S1 (6–11 months) n = 265		S2 (12–19 months) n = 137		S3 (20–29 months) n = 143*		S4 (30–42 months) n = 103‡	
	Median (IQR)	n (%)	Median (IQR)	n (%)	Median (IQR)	n (%)	Median (IQR)	n (%)
<i>Ascaris spp.</i>	–	0 (0)	0.82 (0.62–1.01)	10 (7.3)	0.79 (0.55–1.30)	25 (17.9)	1.06 (0.58–1.84)	27 (26.5)
<i>Blomia tropicalis</i>	0.57 (0.37–0.77)	2 (0.8)	0.52 (0.48–0.74)	9 (6.6)	1.47 (0.70–2.72)	24 (16.9)	0.97 (0.55–1.65)	34 (33.3)
<i>Dermatophagoides pteronyssinus</i>	0.69 (0.36–1.02)	2 (0.8)	0.67 (0.48–0.99)	7 (5.1)	0.69 (0.48–1.55)	10 (7.0)	0.81 (0.48–1.35)	19 (18.6)
Total IgE†	24.40 (8.73–66.3)	–	71.50 (25.2–200.5)	–	93.17 (34.6–254)	–	167.33 (61.1–375)	–

Median (IQR)-specific IgE levels to any antigen/allergen were calculated in the subgroup of sensitized children (n [%]) for each respective source.

**Ascaris spp.* (n = 140). *D. pteronyssinus* (n = 142). *B. tropicalis* (n = 142).

†Geometric mean (IQR) of total IgE is reported instead.

‡IgE to *Ascaris* was measured in 102 children.

IgE sensitization were analyzed. The frequency of sensitization to *Ascaris*, *B. tropicalis*, and *D. pteronyssinus* was 23.6%, 27.3%, and 14.2%, respectively. As frequencies of sensitization to *B. tropicalis* and *D. pteronyssinus* were markedly different, the risk factors for these outcomes were assessed separately. Sensitization to *B. tropicalis* was associated with various indicators of unhygienic conditions in the univariate analysis (Table 4). When covariates such as gender, age, and high CB total IgE were included, living in a wood house (aOR: 2.40

95% CI: 1.10–5.23, $p = 0.03$) and poultry/pigs at home during pregnancy (aOR: 2.78 95% CI: 1.08–7.14, $p = 0.03$) remained associated with *B. tropicalis* sensitization. In regard to *D. pteronyssinus*, after adjustment, only poultry/pigs at home during pregnancy (aOR: 3.00 95% CI: 0.98–9.23, $p = 0.05$) and high maternal total IgE maintained their significant associations (aOR: 2.74 95% CI: 1.04–7.19, $p = 0.04$). As expected, living under poverty and unhygienic conditions (as indicated by living in wood house) were positively associated with *Ascaris*

Table 4 Crude odds ratios for house-dust mite (HDM) and *Ascaris* sensitization in children as outcomes

Predictor*	Ascaris		<i>Dermatophagoides pteronyssinus</i>		<i>Blomia tropicalis</i>	
	OR	p	OR	p	OR	p
Socioeconomic stratum (being 1 the poorest)	0.20 (0.05–0.84)	0.03	0.77 (0.28–2.10)	0.61	0.66 (0.30–1.48)	0.31
Tap water	0.32 (0.12–0.84)	0.02	0.62 (0.19–2.04)	0.43	1.16 (0.42–3.23)	0.77
Sewage	0.35 (0.17–0.74)	0.01	0.53 (0.23–1.26)	0.15	0.66 (0.34–1.28)	0.22
Poultry/pigs at home	2.21 (0.89–5.52)	0.09	3.97 (1.48–10.66)	0.01	3.22 (1.33–7.79)	0.01
Wood house (vs. brick)	3.77 (1.80–7.88)	<0.001	0.74 (0.28–1.99)	0.55	2.24 (1.11–4.51)	0.02
<i>Ascaris</i> infection	3.71 (1.14–12.06)	0.03	0.00 (0.00–0.00)	1.00	2.19 (0.70–6.91)	0.18
Gender (female)	1.11 (0.55–2.23)	0.78	1.75 (0.73–4.22)	0.21	0.99 (0.51–1.92)	0.98
Child age (months)	1.05 (0.97–1.13)	0.22	1.10 (1.00–1.20)	0.04	1.07 (0.99–1.15)	0.05
High CB total IgE (>0.07 kU/l)	6.15 (2.61–14.46)	<0.001	1.47 (0.53–4.04)	0.46	2.76 (1.23–6.23)	0.01
High maternal total IgE (>502 kU/l)	4.50 (2.14–9.49)	<0.001	2.82 (1.18–6.72)	0.02	1.63 (0.80–3.33)	0.18
Maternal HDM sensitization defined by recombinants	2.54 (1.24–5.22)	0.01	1.07 (0.44–2.59)	0.88	1.34 (0.67–2.67)	0.40
Maternal HDM sensitization defined by ImmunoCAP	2.35 (1.14–4.85)	0.02	1.20 (0.63–2.29)	0.59	1.06 (0.55–2.06)	0.86
Maternal <i>B. tropicalis</i> sensitization (>0.35 kU/l)	2.84 (1.38–5.82)	<0.001	0.97 (0.40–2.35)	0.95	1.19 (0.60–2.36)	0.61
Maternal <i>D. pteronyssinus</i> sensitization (>0.35 kU/l)	2.47 (1.21–5.05)	0.01	1.06 (0.45–2.51)	0.90	1.18 (0.60–2.31)	0.64
Maternal <i>Ascaris</i> sensitization (>0.35 kU/l)	5.77 (2.48–13.46)	<0.001	1.05 (0.45–2.46)	0.90	1.70 (0.87–3.35)	0.12
Maternal rhinitis	1.12 (0.53–2.38)	0.77	0.28 (0.08–0.99)	0.05	0.73 (0.35–1.56)	0.42
Maternal asthma	1.33 (0.39–4.49)	0.65	1.01 (0.21–4.80)	0.99	0.71 (0.19–2.66)	0.61

*Predictors significantly associated were further evaluated in multivariate analysis. Adjusted odds ratios are shown in the main text. Significant associations are shown in bold.

sensitization in children (aOR: 4.15, 95% CI: 1.33–12.99, $p = 0.01$). High CB total IgE (aOR: 4.89 95% CI: 1.50–15.94, $p = 0.01$) and maternal sensitization to *Ascaris* (aOR: 4.01 95% CI: 1.28–12.62, $p = 0.02$) were the most important predictors of *Ascaris* sensitization in children.

Logistic regression analysis of the relationship of HDM or *Ascaris* sensitization with wheezing showed no association with recurrent wheezing. Atopy, determined by SPT, was neither associated with this phenotype. We only found a marginal association between *B. tropicalis*-specific IgE levels and ever wheezing (aOR: 1.47 95% CI: 1.00–2.28, $p = 0.05$) when adjusted by other known risk factors (maternal asthma) and potential confounders such as age, socioeconomic status, and gender.

There is high correlation between the IgE responses to HDM and *Ascaris*

Ascaris-specific IgE levels correlated with those to *B. tropicalis* (Spearman rho: 0.69 $p < 0.001$) and *D. pteronyssinus* (Spearman rho: 0.54 $p < 0.001$). *Ascaris* sensitization was associated with HDM sensitization when grouping *B. tropicalis* and *D. pteronyssinus* data in one category ($\chi^2 = 37.3$, $p < 0.001$) or analyzed separately ($\chi^2 = 40.1$, $p < 0.001$ for *B. tropicalis* and $\chi^2 = 13.4$, $p < 0.01$ for *D. pteronyssinus*). Fifty-two percent of *Ascaris*-sensitized children, but only 10% of non-sensitized, responded to any of the two HDM species. The odds ratio for responding to *Ascaris* in the presence of HDM co-sensitization was 9.4 (95% CI: 4.29–20.99, $p < 0.001$).

Considering the IgE cross-reactivity between *Ascaris* and HDM allergens (12) and to identify cases of genuine sensitization to *B. tropicalis* and *Ascaris*, IgE reactivity to the specific markers Blo t 5 and Asc s 1 as well as to the cross-reactive allergen Asc l 3 was tested in children sensitized to *B. tropicalis* or *Ascaris*. It was found that among *B. tropicalis*-sensitized children, 59.6% (28 of 40) were positive to Blo t 5. Among *Ascaris*-sensitized children 75% (30 of 40) responded to Asc s 1. Sensitization to the highly cross-reactive Asc l 3 was 48.9% and 62.5% in *B. tropicalis*- and *Ascaris*-sensitized children, respectively. *B. tropicalis*-sensitized subjects, with a negative result to Blo t 5, were neither sensitized to Asc l 3.

Discussion

Research focused on atopy, wheezing, and asthma in the tropics is scarce, in spite of that these conditions are as frequent as in other latitudes. Here, we describe the evolution of early life total and specific IgE response to common allergens and *Ascaris*. As expected, high serum maternal total IgE was associated with high CB total IgE (18), which in turn pre-disposed to stronger IgE responses during infancy. In agreement with other reports, we found that high CB total IgE was positively associated with allergen sensitization (15) but protected from wheezing (19). Although in developed countries, high total CB IgE has been found associated with atopy-related phenotypes, such as recurrent wheezing and eczema (20), our opposite finding replicates the results obtained by Sunyer et al. (19) in Tanzania, a tropical underdeveloped country. Why high CB total IgE protects from wheezing is unknown, but before speculating on it, some comments about CB IgE are pertinent. The source of CB IgE is controversial, and both fetal and maternal productions have been described (30) and may influence IgE detection. We excluded maternal blood contamination and think that CB IgE was mainly a fetal product because an important role of placental transfer is not supported by our results. First, in spite of that in the tropics, responses to HDM are strong, only one case of possible CB sensitization to *D. pteronyssinus* was found, being the mother negative for that allergen. Second, although total IgE values detected in mothers were higher than those reported in studies from developed countries (15), the corresponding CB IgE titers were lower (21–22).

In regard to mechanisms, we think it is possible that total IgE does not directly mediate protection but may be associated with an ongoing protective process, probably induced *in utero* by parasite infections in mothers. This process could be extended during the first months (longitudinal total IgE values were lower in recurrent wheezers), modulating the pulmonary inflammation induced by viral or other agents and reducing the rate of wheezing. It is unlikely that this association is due to poor hygiene conditions influencing wheezing because significance remains after adjustment by socioeconomic status and other potential confounding factors. But, independently of possible mechanisms, our results confirm previous findings in a tropical country (19), suggesting that in these environments, high levels of total IgE in CB are not risk factor for atopy-related phenotypes. This finding should be further evaluated in

this and other cohorts from the tropics because it might be a particular characteristic with potential clinical relevance.

In this cohort, as in other studies from socioeconomically deprived populations living in the tropics, allergen sensitization rates are high (2, 3), compared with other latitudes (23, 24). Alcantara-Neves et al. reported similar sensitization rates in Brazilian preschool-aged children, especially a high frequency of response to *B. tropicalis* (36.1%). Contrastingly, in the MAS study, at 5 yr of age, airborne allergen sensitization in German children was lower than 15% (23). One general explanation for high sensitization in unhygienic conditions is the presence of helminth infections. Our cohort has a low intensity burden of *Ascaris* infection (6), a condition that may potentiate Th2 responses through various mechanisms (25). However, this point had never been evaluated in cohort studies. We found that 52% of *Ascaris*-sensitized children, but only 10% of non-sensitized, have positive IgE to any of the two HDM species. Genetic background of the host and unspecific boosting of the Th2 responses by the parasite may influence this finding, but cross-reactivity may also play a role; it is possible that some cases of HDM sensitization correspond to cross-reactive *Ascaris*-induced IgE antibodies rather than genuine sensitization from HDM (12, 25). Purified components are very helpful to evaluate the role of cross-reactivity and are progressively used in children (26). In the FRAAT cohort, specific IgE to the highly cross-reactive *Ascaris* tropomyosin was found in almost half of *B. tropicalis*-sensitized children at 2–3 yr old. However, IgE serology to specific markers from both sources suggests that genuine sensitization to *B. tropicalis*, as well as to *Ascaris*, is also common in this population and starts at early age. In addition to the potential clinical effects of HDM sensitization, specific IgE response to *Ascaris* may have biologic impact and act on different directions. For example, *Ascaris*-specific IgE can induce allergic symptoms after recognition of its own antigens and also those derived from HDM; furthermore, it can boost the responses to cross-reactive HDM antigens, to which population is perennially exposed.

Although previous analysis of this cohort has shown that poverty-associated conditions, such as wood houses and poultry/pigs at home, are not associated with wheezing (6); in this study, their effects on IgE responses were remarkable. Some indicators of poverty and unhygienic living conditions were identified as risk factors for IgE sensitization. The association with HDM sensitization could be due to the cross-reactivity with *Ascaris* antigens; however, it is important to mention that at this age, sensitization to *B. tropicalis* was more frequent than that to *Ascaris*, so, it is possible that unhygienic conditions have a direct effect on this outcome.

In this cohort, frequency of sensitization to *B. tropicalis* was markedly higher than to *D. pteronyssinus*. As the samples were analyzed at the same time for both allergens, technical errors can be ruled out. Limitation of *D. pteronyssinus* extract to detect sensitization in our population (e.g., different isoforms) was also ruled out because in mothers, the expected results were found. In a work performed in wheezing children from central Taiwan, it was also found higher sensitization rates to *B. tropicalis* than to *D. pteronyssinus* (27). These data differ from those obtained in adults where frequencies of sensitiza-

tion to these mites are similar (28), a finding that was also shown in mothers of the present study. Interestingly, frequency of *B. tropicalis* sensitization, but not to *D. pteronyssinus*, at 3 yr of age was similar to that in mothers. These HDM species are in similar proportions in homes of Cartagena (29); therefore, other origins of this finding are possible. The sensitization process to each species may be independently controlled. Differences in the gene expression profile among these species, especially of 'pro-allergenic' molecules (30), and their interactions with the genetic background of exposed individuals can shape the specific IgE responses. Supporting this idea, individual genetic associations have been found with sensitization to each species. Although it is not known how some polymorphisms modulate IgE sensitization, in addition to HLA alleles, other genetic variants may influence the response to *B. tropicalis* (31) or *D. pteronyssinus* sensitization (32). SPT also indicated that *B. tropicalis* is the most frequent sensitizer at early age; however, as in other studies, we obtained lower prevalence of reactivity compared with IgE serology (3, 33), but similar to those found in some affluent countries (34).

Total IgE in children increased rapidly reaching adult levels at around 3 yr, much earlier than that observed in children from industrialized countries (35). The reported levels are markedly high compared with other populations, such as children from the United States (especially Caucasians) (36) and Sweden (37), but similar to those found in tropical regions (3). This may be explained by several reasons. High levels of total IgE have been consistently described in populations exposed to parasites, especially helminths (38). In addition, in our population and others, African ancestry has been strongly associated with higher total IgE phenotype (10, 39). Therefore, it is possible that the genetic background also supports the tendency to have high levels of total IgE in early infancy. In this regard, our data support the idea that total IgE in children of low income communities living in the tropics has not the same diagnostic value for atopy as in industrialized parasite-free countries.

Several limitations of this study should be discussed. A birth cohort has advantages when evaluating the evolution of immune responses, but, in general, technical drawbacks are expected. Our study was hampered by particular socio-demographic conditions, such as frequent address changes, social conflicts, poverty, and low education level (6). Sample size was too small to accurately define some associations, affected by its *a priori* calculation, the response rate, and missing data from active participants. *Ascaris* infection rate was lower than its *a priori*

estimation, even though it was based on epidemiological information of near communities (40). Bias can result from a high rate of loss to follow-up. Even though at 2 yr of follow-up, response rate was 86%, there were missing data from active patients, for at least one time period. However, having longitudinal data about IgE responses during 3 yr of life was advantageous and helped to overcome some of these difficulties. LMM are helpful statistic methods for longitudinal analysis in the presence of missing data, also used in allergy research due to its robustness, flexibility, and capability of handling a variety of repeated measure problems. The concordance of the results derived from LMM with those obtained by cross-sectional analysis supports the validity of our findings.

Results of this study support several previous findings and contradict others. For example, contrary to observed in industrialized countries, high total IgE levels in CB protects against wheezing. In addition, contrary to expected according to the hygiene hypothesis, in this socioeconomically deprived, tropical population, the frequency of sensitization to any HDM during the first 3 yr of age is high. The more evident reason to explain this dynamic toward high levels of total and specific IgE (in addition to the perennial exposure to HDM allergens) is low burden helminth infections, whose stimulant effects on the Th2 response seem to overcome potential Th2 inhibitory mechanisms induced by poor hygiene conditions. However, other factors, such as air pollution, microbiota composition, and genetic background, deserve more investigation.

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Conflict of interest

No conflict of interest declared.

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Supporting Information

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

Table S1. Descriptive information about demographic factors, prenatal exposures and clinical phenotypes in the different data sub-sets analyzed in the study.

Online repository material

Table S1 Descriptive information about demographic factors, prenatal exposures and clinical phenotypes in the different data sub-sets analyzed in the study

Factor	Baseline (n=326)	Influence of prenatal factors on CB total IgE (n=277)	Influence of prenatal factors on wheezing (n=261)	Risk factors for sensitization at 2-3 years (n=176)
Gender [male, n (%)]	161 (49.3)	140 (50.5)	134 (51.3)	92 (52.3)
Child age (months). Mean $\pm$ SD	--	--	24 $\pm$ 0	31.5 $\pm$ 4.8
Ever wheezing [n (%)]	--	--	103 (39.5)	77 (43.8)
Recurrent wheezing [n (%)]	--	--	46 (17.6)	27 (15.3)
Maternal age at child birth (years. Mean $\pm$ SD)	23.2 $\pm$ 5.83	23.3 $\pm$ 5.9	23.2 $\pm$ 5.9	23.2 $\pm$ 5.9
Maternal Asthma [n (%)]	21 (6.44)	20 (7.2)	19 (7.3)	14 (8.0)
Maternal Rhinitis [n (%)]	99 (30.3)	83 (30)	78 (29.9)	52 (29.5)
Wood house [n (%)]*	83 (25.5)	70 (25.3)	68 (26.1)	51 (29)
Tap water	209 (89)	246 (88.8)	229 (87.7)	156 (88.6)
Sewage [n (%)]	191 (58.6)	164 (59.2)	150 (57.5)	94 (53.4)
Cockroaches at home [n (%)]	255 (78.2)	218 (78.7)	206 (78.9)	141 (80.1)
Dog at home [n (%)]	143 (43.9)	127 (45.8)	121 (46.4)	74 (42)
A pet (cat/dog) during pregnancy [n (%)]	107 (32.8)	92 (33.2)	89 (34.1)	53 (30.1)
Poultry/pigs during pregnancy [n (%)]	44 (13.5)	39 (14.1)	36 (13.8)	24 (13.6)

*Proportion in relation to brick house.