

Ascaris lumbricoides–Induced Interleukin-10 Is Not Associated with Atopy in Schoolchildren in a Rural Area of the Tropics

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Background. In areas where intestinal helminth infections are endemic, infections by these parasites may protect against skin test–measured reactivity to allergens, and it has been suggested that interleukin (IL)–10 may mediate this effect. This study investigated whether IL-10 and populations of IL-10⁺ T cells may modulate atopy in children living in an area where intestinal helminth infections are endemic.

Methods. Ecuadorian schoolchildren from rural communities were assessed for skin test–measured reactivity to *Periplaneta americana* allergen and *Dermatophagoides pteronyssinus* allergen. Blood samples were collected from 39 skin test–positive and 41 skin test–negative children, and peripheral-blood leukocytes were cultured in the presence of *Ascaris lumbricoides* antigen, to measure IL-10 protein and the frequency of T cell populations expressing intracellular IL-10. We also investigated whether these immunological factors affected the association between allergen-specific IgE and skin test–measured reactivity to aeroallergens.

Results. There was no evidence of association between the level of *A. lumbricoides*–induced IL-10 protein or IL-10⁺ T cells and skin test–measured reactivity to allergens. The association between allergen-specific IgE and skin test–measured reactivity was not affected by the level of IL-10 protein or the frequency of IL-10⁺ T cells.

Conclusions. The results of this study do not support the notion that IL-10 plays a role in modulating atopy in children living in a tropical area where intestinal helminth infections are endemic.

Atopy can be defined as the presence of either skin test–measured reactivity to allergens or allergen-specific IgE in serum. Previous studies have found that, although skin test–measured reactivity to allergens and the presence of allergen-specific IgE are strongly associated in industrialized countries [1, 2], the association is weak or nonexistent in tropical rural regions of Africa [3, 4] and Latin America [5], such that the proportion of individuals who have elevated levels of allergen-specific IgE are present in a much higher proportion of individuals than have skin test–measured reactivity for the same allergen.

The disassociation between the results of skin testing for allergens and allergen-specific IgE in tropical regions may be caused by regulation of allergic effector responses. Previous studies that have examined the determinants of skin test–measured reactivity to allergens have implicated a modulatory role for the cytokine interleukin (IL)–10 [6]. IL-10 has important antiallergic effects [7, 8], is able to inhibit IgE-induced activation of human mast cells [9], and has been associated with reduced risk of skin test–measured reactivity to allergens [6, 10].

Populations living in rural areas of the tropics, where the prevalence of allergic disease tends to be low [11, 12], have been hypothesized to have a highly regulated immune system that is more capable of controlling allergic inflammatory responses [13, 14] than is that of populations living in urban areas or in industrialized countries where the prevalence of allergic disease may be much greater [12, 15]. Chronic exposures to infectious diseases, particularly helminth

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parasites, may contribute to such more highly developed immune regulation [13, 14].

Allergic inflammation may be regulated by regulatory T cells, among which IL-10⁺ T cells are considered to be important [16]. In the present study, we sought to test the hypothesis that intestinal helminth-induced IL-10 and IL-10⁺ regulatory T cells attenuate skin test-measured responses to allergens in children living in an area where there is a high prevalence of intestinal helminth infection. Furthermore, we investigated whether helminth-induced IL-10 and IL-10⁺ regulatory T cells affect the association between skin test-measured responses to allergens and the level of allergen-specific IgE. To do this, we examined the relationship between in vivo (i.e., skin-prick testing for allergens) and in vitro (i.e., the level of allergen-specific IgE in plasma) atopic responses to aeroallergens and the production and expression of IL-10 by leukocytes stimulated with *Ascaris lumbricoides* antigen in blood from schoolchildren living in an area of Ecuador in which the parasite is endemic. The study was designed to investigate possible IL-10-associated immunological mechanisms underpinning the inverse association between allergy and intestinal helminth infection, and we recruited an atopy-enriched sample from a population in which an inverse association between intestinal helminth infections and skin test-measured reactivity to allergens has been demonstrated consistently [11, 14].

METHODS

Study design and population. Children in this cross-sectional were recruited from 5 small rural schools in a subtropical area of Pichincha Province in Ecuador. Children were recruited on the basis of skin prick-test results that were either (1) positive for *Dermatophagoides pteronyssinus* and/or *Periplaneta americana* or (2) negative for 6 relevant aeroallergens that included *D. pteronyssinus* and *P. americana*. Skin testing for allergens was performed on 2 occasions that were 1 month apart, and children were excluded from the study if the results of these 2 skin tests were inconsistent. Skin-test reactions to *D. pteronyssinus* and *P. americana* account for ~90% of skin test-positive reactions in the study area [11]. The study protocol was approved by the ethics committees of the Hospital Pedro Vicente Maldonado (Ecuador) and St. George's Hospital (United Kingdom).

Collection and analysis of blood and stool samples. Immediately after the second skin test, 10 mL of blood was collected, by venipuncture, into plastic tubes (BD Vacutainer) containing sodium heparin as an anticoagulant. White-cell and differential counts were calculated for each individual by standard methods. Stool samples were collected on the same days as the skin tests for allergens were performed, and the modified Kato-Katz method and formol-ethyl acetate-concentration methods were used to examine the samples for the presence of helminth eggs and larvae [17].

The sensitivity of the Kato-Katz method for the quantification of infection intensities was 70 eggs per gram.

Skin-prick testing for allergens. Skin-prick testing for responses to house dust-mite extract (*D. pteronyssinus*; Greer Laboratories), *Alternaria tenuis* (Greer Laboratories), American cockroach (*P. americana*; Greer Laboratories), cat (Greer Laboratories), grass/pollen mix (Greer Laboratories), tree pollen (Greer Laboratories), histamine (ALK-Abello), and a saline control (ALK-Abello) was performed. Allergens were pricked onto the volar surface of the forearm, and reactions were recorded 15 min later. Reactions were considered to be positive if the mean diameter was ≥ 3 mm greater than that caused by the saline control. Skin testing was performed by a single observer (M.E.C.).

Measurement of antibodies. The levels of total IgE and of IgE specific for *D. pteronyssinus*, *A. lumbricoides*, and *P. americana* were determined by the CAP system (Pharmacia Diagnostics AB). The levels of *A. lumbricoides*-specific IgG and IgG4 were measured as described elsewhere [5], and the cutoff for positivity was defined as >3 SDs above the mean values of 10 control sera from subjects living in Quito who did not have a history of intestinal helminth infection.

Histamine release. Histamine release by heparinized whole blood incubated with *Ascaris* antigens (*A. lumbricoides* adult, *A. suum* L2/L3, and *A. suum* L3/L4) at a concentration of 0.03 $\mu\text{g/mL}$ was measured in 29 individuals (12 were skin-test negative for allergen and 17 were skin-test positive) by a commercial assay (histamine enzyme immunoassay kit; Immunotech) used according to the manufacturer's instructions.

Whole-blood culture. Heparinized venous blood was collected and diluted 1:4 in RPMI 1640 medium (BioWhittaker) containing L-glutamine (BioWhittaker), 80 mg/mL gentamicin, and 1% HEPES (GIBCO BRL). Diluted whole blood (0.5 mL) was cultured either alone or with either 10 $\mu\text{g/mL}$ *A. lumbricoides* adult-worm antigen (prepared as described by Cooper et al. [5]), 100 AU/mL *D. pteronyssinus* (Greer Laboratories), a 1:50 dilution of *P. americana* allergen extract (Greer Laboratories), or 10 $\mu\text{g/mL}$ staphylococcus enterotoxin B (SEB; Sigma-Aldrich). Cultures were incubated for 24 h at 37°C in a humidified atmosphere of 5% CO₂. Supernatant fluids were collected and were stored in liquid nitrogen.

Analysis of IL-10 in supernatant fluids. Levels of IL-10 were measured in supernatant fluids from whole-blood cultures by use of a commercially available antibody pair (Pharmingen) by sandwich ELISA performed according to the manufacturer's instructions. The detection limit for the assay was 19.5 pg/mL; values below this limit were assigned the value of the detection limit.

Preparation and preservation of cells for flow cytometry. Blood samples were diluted with an equal volume of supplemented RPMI 1640 (as described above for whole-blood cultures) and were cultured in microplate wells, alone or with either *A. lumbricoides* adult-worm antigen, *D. pteronyssinus*, *P. americana*, or SEB, at the

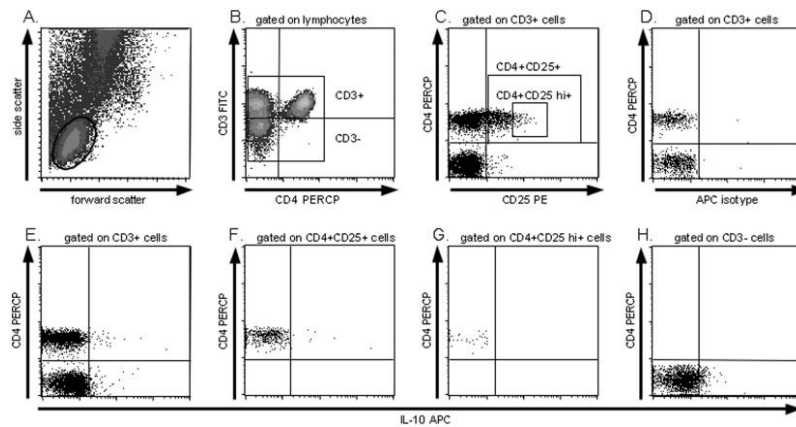


Figure 1. Schematic of detection of interleukin (IL)-10 by flow cytometry. *A*, Identification of lymphocytes by forward and side scatter. *B*, CD3⁺ and CD3⁻ cells identified by CD3–fluorescein isothiocyanate staining. *C*, CD3⁺ gated cells analyzed for positivity for CD4 and CD25, enabling identification of CD4⁺CD25⁺ and CD4⁺CD25^{hi} subsets. *D*, Positivity for IL-10, determined by isotype staining. *E–H*, Plots of IL-10 staining for CD4⁺ cells (*E*, upper panels), CD4⁺CD25⁺ cells (*F*), CD4⁺CD25^{hi} cells (*G*), and CD3⁻ cells (*H*). APC, allophycocyanin; PE, phycoerythrin; PERCP, peridin chlorophylla protein conjugated.

concentrations described above. Cultures were maintained at 37°C in 5% CO₂. After 2 h, Brefeldin A (Sigma-Aldrich) was added to each culture well (final concentration, 20 µg/mL), and the cultures were incubated for an additional 12 h. After incubation, stimulated whole-blood samples were resuspended in BD lysing solution (Pharmingen) for 10 min. Cells were then washed once in cold PBS, centrifuged at 1200 rpm for 10 min, and fixed by resuspending the cell pellets in 4% paraformaldehyde (Sigma-Aldrich) in PBS for 5 min. After the cells were washed in a solution of 1% bovine serum albumin (BSA) and PBS, they were centrifuged, and the resulting pellet was resuspended in a solution of PBS and 10% dimethylsulfoxide, was placed in a freezer overnight at –20°C, and then was stored in liquid nitrogen.

Flow cytometry. Fixed cells were thawed at room temperature and were washed in a solution of PBS and 0.1% BSA and then were centrifuged, and the resulting pellet was resuspended and permeabilized in a solution of PBS, 1% BSA, and 0.1% saponin (Calbiochem) for 1 h at 4°C. Cells were then stained, for 30 min at 4°C, with fluorescein isothiocyanate (FITC)–conjugated mouse anti-human CD3 (Immunotech), peridin chlorophylla protein–conjugated (PerCP) mouse anti-human CD4 (Pharmingen), R-phycoerythrin (PE)–conjugated mouse anti-human CD25 (Pharmingen), and allophycocyanin (APC)–conjugated rat anti-human IL-10 APC (Pharmingen). FITC-conjugated mouse IgG₁ (Pharmingen), PE-conjugated mouse IgG₁, PerCP-conjugated mouse IgG₁k (Pharmingen), and rat IgG_{2a}APC (Pharmingen) were used as isotype controls. After staining, cells were washed twice in a solution of PBS and 0.1% saponin and were resuspended in PBS. Data were collected by a FACSCalibur flow cytometer (BD Biosciences) and were analyzed by CellQuest software (BD Biosciences). A schematic diagram to illustrate how IL-10⁺ PBL populations in a single individual were identified by flow cytometry is provided in figure 1.

Statistical analysis. The production of IL-10 by peripheral blood leukocytes (PBLs) and the frequencies of IL-10⁺ PBLs stimulated in vitro were normalized by subtraction of the relevant medium control values. We wanted to test the hypothesis that, in whole blood stimulated with *A. lumbricoides* antigen, skin test–measured reactivity to allergen is associated both with a lower level of IL-10 and a lower frequency of specific populations of putative regulatory T cells (defined as CD3⁺CD4⁺ IL-10⁺, CD3⁺CD4⁺CD25⁺ IL-10⁺, and CD3⁺CD4⁺CD25^{high} IL-10⁺). We examined also the association between the frequency of putative regulatory T cells and the presence of allergen-specific IgE (≥0.7 IU/mL) for *D. pteronyssinus* or *P. americana*. The associations between atopy and immunological parameters were analyzed by logistic regression. The effects of the variables total IgE, age, sex, crowding (persons per sleeping room), and socioeconomic level on the estimates of the effect for atopy were assessed by multiple logistic regression, but, because none of these factors was significantly associated with atopy, they were not included in the final models. The interaction between recent anthelmintic treatment and active intestinal helminth infection on skin-test responses was assessed by addition of an interaction term to the models. For these analyses, active intestinal helminth infection was defined as the presence of eggs in fecal samples. The presence of antibodies (IgG, IgG4, and IgE) to *A. lumbricoides* was taken to indicate past infection with or exposure to *A. lumbricoides* or to other intestinal helminth infections that share extensive immunologic cross-reactivity with *A. lumbricoides* [18]. To assess whether putative regulatory T cells might affect the association between allergen-specific IgE (to *P. americana* or *D. pteronyssinus*) and skin test–measured reactivity (to *P. americana* or *D. pteronyssinus*), we added the variables for these cell populations to logistic regression models of the association between allergen-specific IgE and skin test–measured reactivity for each of *P. americana* and *D. pteronyssinus*. Comparisons of data on skin

Table 1. Baseline characteristics of study population.

Variable	Skin-test negative (n = 41)	Skin-test positive (n = 39)
Age, mean (range), years	9.2 (7–13)	9.3 (7–13)
Sex, % male:female	61:39	54:46
Socioeconomic level, mean (range)	2.1 (1–4)	2.1 (1–4)
Crowding, mean (range), persons/room	4.2 (1.3–9.0)	3.4 (1.3–8.0)
Skin test–measured reactivity to allergens, %		
Any ^a	0	100
HDM <i>Dermatophagoides pteronyssinus</i>	0	56.4
<i>Periplaneta americana</i>	0	69.2
Recent treatment with albendazole ^b , % treated:untreated	46.3:53.7	38.4:61.5
Intestinal helminths in stools		
Any, % of subjects	30.0	43.4
<i>Ascaris lumbricoides</i> , % of subjects	15.0	23.1
Intensity, geometric mean (range), eggs per gram	7,394 (71–162121)	822 (71–25201)
<i>Trichuris trichiura</i> , % of subjects	25.0	33.3
Intensity, geometric mean (range), eggs per gram	463 (70–2,590)	296 (70–3,920)
Hookworm, % of subjects	2.5	2.6
Total IgE, geometric mean (range), IU/mL	729 (10–5,253)	1,291 (190–8,898)
Anti- <i>A. lumbricoides</i> IgE, geometric mean (range), kU/L [% of subjects ^c]	2.8 (<0.35–100) [85.4]	4.4 (<0.35–100) [94.9]
Anti- <i>A. lumbricoides</i> IgG, % of subjects	100	100
Anti- <i>A. lumbricoides</i> IgG4, % of subjects	100	100
Histamine release >10% (n = 29), no. of subjects	12	17
<i>A. lumbricoides</i> ^d , % of subjects	100	100

^a *Dermatophagoides pteronyssinus*, *Alternaria tenuis*, *P. americana*, cat, grass-pollen mix, and tree-pollen mix.

^b Anthelmintic treatment with 400 mg of albendazole during the previous 2 months.

^c Proportion with >0.35 kU/L *A. lumbricoides*–specific IgE.

^d Histamine release to any adult worm and larval antigen preparation (stages L2/L3 and L3/L4).

test–reactive children versus skin test–nonreactive children, classified as continuous or binary variables, also were performed, by rank-sum or χ^2 test/Fisher's exact test, respectively. Socioeconomic level was calculated as a score based on paternal and maternal education and occupation and material goods in the household. Analyses were performed by Stata (version 7.0; Stata).

RESULTS

Characteristics of study population. Baseline characteristics of the study children, stratified by skin test–measured reactivity to allergens, are shown in table 1. Important confounding factors of age, sex, socioeconomic level, and level of household crowding were similar in skin test–positive and skin test–negative subjects. Although all children had evidence of prior exposure to intestinal helminths, as determined both by the presence of *A. lumbricoides*–specific antibodies (IgG, IgG4, and IgE) and by evidence of basophil histamine release (>10%) to *Ascaris* adult- and larval-stage antigens, the overall prevalence of active intestinal helminth infection as measured by stool microscopy was only 36.7%. This result is likely due, in part, to the high percentage of children who had received

anthelmintic treatment (42.5%), because a previous survey of the prevalence of active intestinal helminth infections in such a population had reported a prevalence of 63.4% for any intestinal helminth infection [11].

Relationship between skin test–measured reactivity to allergens and IL-10 protein and intracellular expression by PBLs. We observed no relationship between skin test–measured reactivity to any allergen and the level of IL-10 protein in whole-blood cultures stimulated with *A. lumbricoides* antigen (figure 2A, first row).

There was no association between skin test–measured reactivity to any allergen and the frequency of either CD3⁺CD4⁺IL-10⁺ (odds ratio [OR], 1.01 [95% CI, 0.98–1.05]; $P = .48$), CD3⁺CD4⁺CD25⁺IL-10⁺ (OR, 1.06 [95% CI, 0.81–1.38]; $P = .69$), or CD3⁺CD4⁺CD25^{high}IL-10⁺ (OR, 0.15 [95% CI, 0.02–1.38]; $P = .09$) T cells from *A. lumbricoides* antigen–stimulated cultures (figure 2A, rows 2–4). Skin test–measured reactivity to any allergen revealed no differences in the proportion of subjects with elevated frequency of IL-10⁺ PBLs: for CD3⁺CD4⁺IL-10⁺, there were 47.4% [18/38] skin test–positive subjects versus 39.0% [16/41] skin test–negative subjects

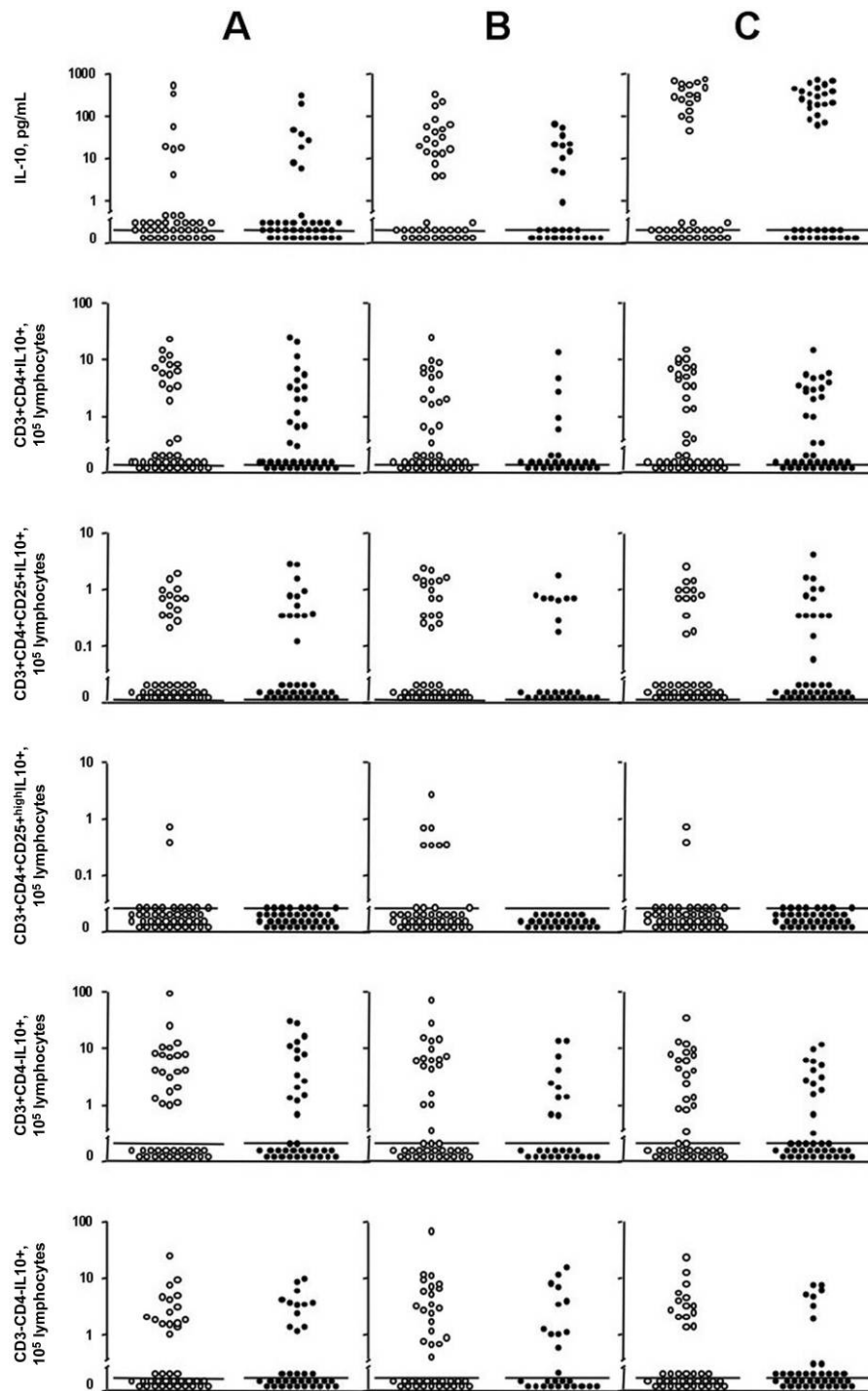


Figure 2. Interleukin (IL)–10 protein and frequency of IL-10⁺ peripheral-blood leukocytes (PBLs), as determined by skin test–measured reactivity to allergens. Shown are *Ascaris lumbricoides* antigen–induced IL-10 (graph A row 1) and IL-10⁺ PBLs (graphs A rows 2–6), as determined by skin test–measured reactivity to any allergen; *Periplaneta americana* allergen–induced IL-10 protein (graph B1) and IL-10⁺ PBLs (graphs B rows 2–6), as determined by skin test–measured reactivity to *P. americana*; and *Dermatophagoides pteronyssinus* allergen–induced IL-10 protein (graph C row 1) and IL-10⁺ PBLs (graphs C rows 2–6), as determined by skin test–measured by reactivity to *D. pteronyssinus*. Both skin test–positive subjects (blackened circles) and skin test–negative subjects (white circles) are shown. The horizontal bars indicate median values.

($P = .45$); for CD3⁺CD4⁺CD25⁺IL-10⁺, the corresponding proportions were 34.2% versus 34.2% ($P = .96$); for CD3⁺CD4⁺CD25^{high}IL-10⁺, they were 0% versus 4.9%

($P = .17$); for CD3⁺CD4[−]IL-10⁺, they were 42.1% versus 51.2% ($P = .42$); for CD3[−]CD4[−]IL-10⁺, 34.2% versus 41.7% ($P = .51$). There was no association between skin test–mea-

Table 2. Relationship between allergen-specific responses measured by skin-test reactivity and levels of *Dermatophagoides pteronyssinus*-specific and *Periplaneta americana*-specific IgE.

Skin-test reactivity	Allergen-specific IgE		
	<0.35 kU/L	0.35–0.69 kU/L	≥0.70 kU/L
<i>D. pteronyssinus</i>			
No (n = 58)	38 (65.5)	9 (15.5)	11 (19.0)
Yes (n = 22)	3 (13.6)	1 (4.6)	18 (81.8)
<i>P. americana</i>			
No (n = 53)	38 (71.7)	6 (11.3)	9 (17.0)
Yes (n = 27)	9 (33.3)	6 (22.2)	12 (44.4)

NOTE. Data are no. (%) of subjects tested (i.e., of n value shown).

sured reactivity to any allergen and the frequency of other IL-10⁺ PBL populations (figure 2A, rows 5 and 6). Active intestinal helminth infection was not associated with either the production of IL-10 by PBLs or the frequency of putative regulatory T cell populations expressing intracellular IL-10 in whole-blood cultures stimulated with *A. lumbricoides* antigen (data not shown). Neither the level of IL-10 protein in whole-blood cultures (figure 2B and 2C, row 1) nor the frequency of IL-10⁺ PBL populations (figure 2B and 2C, rows 2–6) from cultures stimulated with either *P. americana* or *D. pteronyssinus* was associated with skin test–measured reactivity to the respective allergens.

Relationship between allergen-specific IgE and IL-10 protein and intracellular expression by PBLs. Production of IL-10 protein by whole-blood cultures stimulated with specific allergen extracts (*P. americana* and *D. pteronyssinus*) was not associated with the presence of allergen-specific IgE (>0.7 kU/L) (data not shown). The frequency of the IL-10⁺ PBL populations in cultures stimulated with *P. americana* and *D. pteronyssinus* was not associated with the presence of allergen-specific IgE (>0.7 kU/L) (data not shown).

Association between skin test–measured reactivity to allergen and allergen-specific IgE. There was evidence of a disassociation between the results of skin testing for allergen and the level of allergen-specific IgE (table 2). The relationship between the results of skin-prick testing for *D. pteronyssinus* and *P. americana* allergen extracts and the level of IgE specific for the same allergen is shown in table 2. A total of 20 (34.5%) of the 58 children who were skin test negative for *D. pteronyssinus* had detectable levels (≥0.35 kU/L) of *D. pteronyssinus*-specific IgE, whereas 15 (28.3%) of the 53 children who were skin test negative for *P. americana* had detectable levels of allergen-specific IgE. When a more stringent cutoff value of ≥0.7 kU/L was used, relatively fewer skin test–negative children—19.0% and 17.0% for *D. pteronyssinus* and *P. americana*, respectively—had significant levels of allergen-specific IgE.

Effect of IL-10 and IL-10⁺ PBLs on the association between allergen-specific IgE and skin test–measured reactivity. The independent effects that IL-10 protein and IL-10⁺ populations

of putative regulatory T cells (CD3⁺CD4⁺L-10⁺, CD3⁺CD4⁺CD25⁺IL-10⁺, and CD3⁺CD4⁺CD25^{high}IL-10⁺) had on the association between allergen-specific IgE and skin test–measured reactivity to *D. pteronyssinus* and *P. americana* were assessed by adding these variables separately to models of this association. The findings, shown in table 3, indicate that neither IL-10 nor the putative regulatory T populations had any effect on this association, for either *P. americana* or *D. pteronyssinus*.

DISCUSSION

In the present study, we investigated the association between skin test–measured reactivity to allergens and the production of IL-10 protein in supernatant fluids from whole-blood PBLs stimulated with *A. lumbricoides* antigen or aeroallergen extracts and the expression of intracellular IL-10 by populations of PBLs, as defined by surface staining for CD3, CD4, and CD25. We investigated a particularly interesting population: schoolchildren living in a rural tropical area where the prevalence of allergic symptoms is extremely low [11]—and where, in a previous report, we have shown that there is a strong inverse association between skin test–measured responses to allergens and active intestinal helminth infection [11]. In such a population, regulatory effects to control allergic inflammation that are associated with either intestinal helminth infection or other potentially protective rural environmental exposures [14] are likely to be well developed. The study of such children may provide important insights into the immune regulation of allergic inflammation. The data of the present study do not support the hypothesis that intestinal helminth–induced IL-10–expressing regulatory T cells affect either (1) skin test–measured response to allergens or (2) the association between allergen-specific IgE in plasma and skin test–measured reactivity to the same allergen.

Strengths of the present study are that it recruited children with skin test–measured reactivity to allergens that was confirmed by repeated skin testing and by the study of a well-characterized population of skin test–reactive children and skin test–nonreactive children in whom previously identified [5] important confounding factors were balanced at baseline. One limitation of the present study is that a high proportion (42.5%) of children had received anthelmintic treatment within the 2 months before blood samples were collected, which was reflected in a lower-than-expected (36.7%) prevalence of active intestinal helminth infection in the children studied; however, all of the children had evidence of immune sensitization to *A. lumbricoides* infection, as measured by detection of specific antibodies and release of histamine against *A. lumbricoides* antigens, indicating past exposure to or infection with this and other intestinal helminth parasites. Furthermore, there was no evidence that anthelmintic treatment modified the association between skin test–measured reactivity to allergen and IL-10⁺ PBL populations. Another limitation of the present is that it had relatively low power to detect small effects, particularly for low-frequency T

Table 3. Association between allergen-specific IgE and skin test–measured reactivity.

Model	Skin test–measured reactivity to allergen		Putative regulatory T cells	
	Odds ratio (95% CI)	P	Odds ratio (95% CI)	P
Anti- <i>Periplaneta americana</i> IgE	1.16 (0.96–1.41)	.12	...	...
+ <i>Ascaris lumbricoides</i> IL-10 protein	1.15 (0.95–1.40)	.14	0.99 (0.99–1.01)	.54
+ <i>A. lumbricoides</i> CD3 ⁺ CD4 ⁺ IL-10 ⁺	1.15 (0.95–1.39)	.14	1.00 (0.96–1.03)	.83
+ <i>A. lumbricoides</i> CD3 ⁺ CD4 ⁺ CD25 ⁺ IL-10 ⁺	1.18 (0.97–1.42)	.10	1.27 (0.90–1.80)	.17
+ <i>A. lumbricoides</i> CD3 ⁺ CD4 ⁺ CD25 ^{high} IL-10 ⁺	1.14 (0.95–1.37)	.16	0.35 (0.05–2.39)	.29
+ <i>P. americana</i> IL-10 protein	1.16 (0.97–1.41)	.11	0.98 (0.96–1.01)	.19
+ <i>P. americana</i> CD3 ⁺ CD4 ⁺ IL-10 ⁺	1.18 (0.97–1.43)	.10	1.00 (1.00–1.00)	.44
+ <i>P. americana</i> CD3 ⁺ CD4 ⁺ CD25 ⁺ IL-10 ⁺	1.16 (0.96–1.41)	.13	0.95 (0.71–1.26)	.71
+ <i>P. americana</i> CD3 ⁺ CD4 ⁺ CD25 ^{high} IL-10 ⁺	1.15 (0.95–1.39)	.15	0.26 (0.05–1.37)	.11
Anti- <i>Dermatophagoides pteronyssinus</i> IgE	2.89 (1.29–6.49)	.01	...	...
+ <i>A. lumbricoides</i> IL-10 protein	3.01 (1.31–6.91)	.01	1.00 (1.00–1.01)	.29
+ <i>A. lumbricoides</i> CD3 ⁺ CD4 ⁺ IL-10 ⁺	2.72 (1.21–6.10)	.02	1.02 (0.96–1.08)	.54
+ <i>A. lumbricoides</i> CD3 ⁺ CD4 ⁺ CD25 ⁺ IL-10 ⁺	3.02 (1.31–6.70)	.02	0.79 (0.57–1.10)	.16
+ <i>A. lumbricoides</i> CD3 ⁺ CD4 ⁺ CD25 ^{high} IL-10 ⁺	2.83 (1.31–6.70)	.01	0.79 (0.57–1.10)	.16
+ <i>D. pteronyssinus</i> IL-10 protein	3.01 (1.29–6.97)	.01	1.00 (1.00–1.00)	.38
+ <i>D. pteronyssinus</i> CD3 ⁺ CD4 ⁺ IL-10 ⁺	2.80 (1.25–6.28)	.01	1.02 (0.96–1.09)	.55
+ <i>D. pteronyssinus</i> CD3 ⁺ CD4 ⁺ CD25 ⁺ IL-10 ⁺	3.08 (1.32–7.15)	.01	0.85 (0.63–1.15)	.30
+ <i>D. pteronyssinus</i> CD3 ⁺ CD4 ⁺ CD25 ^{high} IL-10 ⁺	2.87 (1.28–6.43)	.01	0.74 (0.14–4.04)	.73

NOTE. Data are effects of simultaneously controlling for putative regulatory T cell populations and allergen-specific IgE, on the basis of risk of skin test–measured reactivity, in logistic regression models. CI, confidence interval; IL, interleukin.

cell populations (i.e., CD3⁺CD4⁺CD25^{high}IL-10⁺). The cost and time that are necessary to perform these labor-intensive immunological assays limit the number of subjects who can be studied, and it may be preferable to restrict the number of analyses performed (e.g., by focusing on specific populations of regulatory T cells), so that a greater number of subjects can be investigated in future studies. An important regulatory marker is the transcription-factor fork-head box P3 (FOXP3), which is considered to identify natural regulatory cells [19] among CD4⁺CD25⁺ T cells. Because a reagent that would allow FOXP3 to be detected by flow cytometry was not available when the analyses were performed, we were unable to assess the expression of this marker.

A previous study, of an African population living in an area where intestinal helminth infection and *Schistosoma mansoni* infection are endemic, has described an inverse association between the production of IL-10 by peripheral-blood mononuclear cells stimulated with *S. mansoni* antigen and the risk of skin test–measured reactivity to *D. pteronyssinus* [6]. The same study also showed that increasing levels of IL-10 appeared to reduce the risk, at a given level of allergen-specific IgE, of skin test–measured positivity for *D. pteronyssinus* [6]. There are several possible explanations for these observations: (1) peripheral-blood responses may reflect a generalized anti-inflammatory systemic effect that infection has on host allergic responses, including those occurring in the skin; (2) parasite-specific IL-10⁺ T cells are present in the skin, the site of entry by schistosome

cercariae, and affect mast-cell function by releasing IL-10; (3) parasite antigen–induced IL-10 is a marker for other immunologic factors that directly mediate these effects; and (4) parasite antigen–induced IL-10 is a marker for nonimmunologic causal factors (e.g., those associated with poverty and that contribute to the risk of chronic schistosomiasis). In the present study, performed in an area where intestinal helminth infection is endemic, we were unable to show a similar inverse association between such skin test–measured positivity and either IL-10 or the frequency of IL-10⁺ PBLs. Our data, therefore, suggest that factors not associated with IL-10 may play a role in mediating the observed effects.

IL-10 does not appear to play a prominent role in the human immune response to *A. lumbricoides* and *Trichuris trichiura* infections [20, 21], the intestinal helminth parasites that are most prevalent in the population in the present study (table 1; also see reference [11]). This finding is in contrast to what has been observed for tissue helminth infections, such as filarial and schistosome infections, which, in humans, appear to have strong regulatory effects that are mediated, at least in part, by IL-10 [22, 23]. The possible allergy-modulating effects of helminth parasites that are largely confined to the intestinal tract (i.e., intestinal helminths) are likely to be subtler than those of helminth parasites that are present in the systemic circulation (e.g., adult schistosomes and some filarial adults and larvae), at least when immune responses in peripheral blood are measured, and it is probable that current assays using peripheral blood are too in-

sensitive to detect such effects. The data reported by the present study do not support the notion that intestinal helminth infections play an important role in inducing populations of IL-10⁺ putative regulatory T cells that may be capable of regulating allergic inflammatory responses to aeroallergens and that may play a role in suppressing allergic inflammation in experimental murine models of intestinal helminth infection [24].

A high proportion of children in the present study had detectable levels (≥ 0.35 kU/L) of allergen-specific IgE in the absence of skin test-measured positivity for *D. pteronyssinus* and *P. americana*, but fewer skin test-negative children had higher titers (≥ 0.7 kU/L) of allergen-specific IgE. It is possible that the low prevalence of allergic disease in this population [11] may be caused by inhibition of allergic effector responses (e.g., immediate hypersensitivity reactions causing skin test-measured reactivity to allergens), rather than by inhibition of allergic sensitization. However, caution should be exercised in the interpretation of low IgE titers, because the disassociation also could be due to false-positive serological reactions caused by factors such as immunological cross-reactivity between intestinal helminth antigens and inhalant allergens [25]. A previous study in a rural village in Kenya showed that serum IgE antibodies (≥ 0.7 kU/L) to cat allergen were present in 47% of the children studied, even though none had either skin test-measured positivity or a history of significant exposure to cats [4]; furthermore, the IgE antibodies to cat allergen could not be inhibited by cat extract [4].

In conclusion, we have examined the association between markers of atopy and the production and expression of IL-10 by PBLs in children living in a rural tropical area where intestinal helminths are endemic. The data reported by the present study do not support the notion that intestinal helminth-induced IL-10 or IL-10⁺ T cell populations play an important regulatory role in the modulation of skin test-measured reactivity to allergens in this population.

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