

Atopic Phenotype Is an Important Determinant of Immunoglobulin E–Mediated Inflammation and Expression of T Helper Cell Type 2 Cytokines to *Ascaris* Antigens in Children Exposed to Ascariasis

Philip J. Cooper,^{1,2,3} Martha E. Chico,¹ Carlos Sandoval,¹ and Thomas B. Nutman²

¹Laboratorio de Investigaciones, Hospital Pedro Vicente Maldonado, Pichincha Province, Ecuador; ²Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; ³Department of Infectious Diseases, St. George's Hospital Medical School, London, United Kingdom

Studies have shown a strong inverse relationship between atopy and geohelminth infection, indicating that atopy may protect against geohelminth infection. Resistance to ascariasis in atopic individuals may occur through greater immunoglobulin E–mediated responses and expression of T helper cell type 2 (Th2) cytokines to parasite antigens. To investigate the effect that atopy has on the immune response to *Ascaris* antigens, school-age children were recruited from rural schools in Ecuador. Immunologic variables were compared between children stratified by atopic and/or *A. lumbricoides*–infection status; the variables included cytokine expression by peripheral-blood mononuclear cells (PBMCs) and histamine release in response to *Ascaris* antigens. Atopic children had both greater frequencies of PBMCs expressing interleukin (IL)–4 and IL-5 and enhanced histamine release, compared with those in nonatopic children. Stratification by atopic and *A. lumbricoides*–infection status revealed the greatest histamine and Th2 cytokine responses in the stratum of atopic, noninfected children. Multivariate regression analyses showed significant effects for atopic status but not for infection status on Th2 cytokine expression and histamine release.

Ascaris lumbricoides is the most prevalent of intestinal helminth or geohelminth parasites and is estimated to infect one-quarter of the world's population [1]. In areas where the parasite is endemic, first exposures to infection with *A. lumbricoides* occur in early childhood and may persist into adulthood through repeated re-infections. Chronic, high-intensity infections with *A. lumbricoides* are associated with significant childhood morbidity [1, 2].

The immune response to geohelminth parasites has close parallels with that associated with allergic sensitization and allergic disease. Both allergy and helminth infections are associated with tissue mastocytosis, ele-

vated numbers of peripheral-blood eosinophils, high levels of polyclonal and specific IgE, and CD4⁺ T cells that preferentially secrete the Th2 cytokines interleukin (IL)–4, IL-5, and IL-13 [3, 4]. The high expression of Th2 cytokines that is typical of both human geohelminth infection [5] and experimental infections in animal models [6, 7] may mediate protective immunity against these parasites [7].

Several epidemiological studies have reported an inverse relationship between atopy and infection with geohelminth parasites, including ascariasis [8–10], and have shown reduced parasite burdens for hookworm [11] in atopic humans, compared with those in nonatopic humans. Such data could be interpreted to suggest that the atopic phenotype provides protection against geohelminth infection and could have afforded an evolutionary advantage to our ancestors, who may have been exposed to high levels of geohelminth infection [12].

Mast cell activation by specific IgE plays a central role in the initiation of allergic disease [3] and may

Received 28 February 2004; accepted 10 April 2004; electronically published 30 August 2004.

Financial support: Wellcome Trust (grant 060120/Z/99/C).

Reprints or correspondence: Dr. Philip J. Cooper, Hospital Pedro Vicente Maldonado, Casilla 17-14-30, Carcelen, Quito, Ecuador (pcooper@ecnet.ec).

The Journal of Infectious Diseases 2004;190:1338–46

© 2004 by the Infectious Diseases Society of America. All rights reserved.
0022-1899/2004/19007-0019\$15.00

also play a role in the initiation of inflammatory reactions that kill or expel helminth parasites [5, 6, 13]. Atopic individuals have a propensity to produce higher levels of specific IgE to environmental aeroallergens [14] and to *A. lumbricoides* [12] and appear to be more sensitive to allergen-induced activation of mast cells and basophils [15, 16].

Because the induction of protective immune responses to ascariasis may require the activation of mast cells by parasite-specific IgE, causing either inflammatory killing reactions directed against migratory larvae or the expulsion of juvenile and adult worms from the intestinal lumen, we hypothesized that, in response to parasite antigens, atopic children living in an area where ascariasis is endemic would show evidence of enhanced basophil histamine release and would express Th2 cytokines with greater frequencies than would nonatopic children.

POPULATION, MATERIALS, AND METHODS

Study population. The present study was conducted in a tropical/subtropical region of the districts of Pedro Vicente Maldonado and Puerto Quito, Pichincha Province, Ecuador, at altitudes between 200 m and 600 m. Study subjects were school-age children attending schools visited as part of a cross-sectional study of geohelminth infection and atopy [10]. Informed consent was obtained from parents or guardians, and the human-experimentation guidelines of the Department of Health and Human Services and St. George's Hospital Medical School were followed. The protocol was approved by the institutional review board of the National Institute of Allergy and Infectious Diseases and the ethics committee of the health foundation SALUDESA.

Allergen skin testing. Skin-prick testing was performed with the following allergens and controls: glycerol/saline control (Greer Laboratories), *Dermatophagoides pteronyssinus* (ALK-Abello), *D. farinae* (ALK-Abello), grass mix (Greer Laboratories), tree mix (Greer Laboratories), American cockroach (Greer Laboratories), *Alternaria tenuis* (Greer Laboratories), cat (Greer Laboratories), and histamine control (10 g/L; ALK-Abello). Allergens were scratched onto the volar side of the forearm by use of ALK-Abello plastic bifurcated lancets, and reaction sizes were recorded after 15 min by measurement of the wheal sizes. Reactions were considered to be positive if the wheal diameter was at least 3 mm greater than that produced by the saline control.

Sampling of subjects. Blood samples (10 mL) were collected into Vacutainer tubes (Becton Dickinson) containing sodium heparin. A single stool sample was collected from each subject and was examined for the presence of geohelminth eggs by use of the modified Kato-Katz technique [17]. Kato-Katz slides were examined within 90 min of preparation. All subjects were offered treatment with 400 mg of albendazole.

Antigens. Antigens used included purified protein derivative (PPD; Statens Serum Institute) and the following *Ascaris* antigens: a PBS-soluble extract of adult *A. lumbricoides* worms

(ASC) and excretory/secretory antigens from *A. suum* larvae developing from L2 to L3 (L2/L3) and from L3 to L4 (L3/L4). The preparation of the *Ascaris* antigens has been described elsewhere [18]. All *Ascaris* antigen preparations were analyzed for the presence of endotoxin by use of the limulus lysate assay (BioWhittaker); endotoxin levels were below the limits of detection in all samples.

Measurement of antibodies. Levels of *A. lumbricoides*-specific IgG1 and IgG4 were measured as markers of exposure to geohelminth infection (including ascariasis [18]) and infection chronicity [10], respectively, and were determined in plasma samples, as described elsewhere [18], with ASC as the coating antigen. Levels of IgE specific to *D. pteronyssinus*, cat, *Ascaris*, and American cockroach allergen were determined by use of the CAP system (Pharmacia Diagnostics). The assay used for detection of total IgE has been described elsewhere [10].

Histamine-release assays. Histamine release by heparinized whole blood in response to parasite antigens, at a concentration of 0.03 $\mu\text{g/mL}$, was performed by use of commercial assays (Histamine Enzyme Immunoassay Kit; Immunotech), according to the manufacturer's instructions.

Cytokine enzyme-linked immunospot (ELISPOT) assays. Peripheral-blood mononuclear cells (PBMCs) were isolated from whole blood by centrifugation on lymphocyte separation medium (Organon Teknika). After washing, the cells were plated at a concentration of 2.5×10^6 cells/mL (200 μL /well) in tissue culture medium (RPMI 1640; BioWhittaker) supplemented with 10% fetal calf serum (Atlanta Biologicals), 0.08 mg/mL gentamicin (Life Technologies, Gibco BRL), and 2 mmol/L L-glutamine (Biofluids). Antigen was added to the cell suspension at the following concentrations: PPD, 10 $\mu\text{g/mL}$; ASC, 10 $\mu\text{g/mL}$; L2/L3, 5 $\mu\text{g/mL}$; and L3/L4, 5 $\mu\text{g/mL}$. ELISPOT assays were performed for interferon (IFN)- γ , IL-4, and IL-5, as described elsewhere [18]. ELISPOT plates were read blind to *A. lumbricoides* infection and allergen skin-test status.

Statistical analysis. Because of a shortage of blood, not all assays were performed on all individuals. For the purposes of analysis, the study population was stratified according to the presence or absence of *A. lumbricoides* infection (*Ascaris*⁺ or *Ascaris*⁻, as determined by stool examination) or allergen skin-test reactivity (SPT⁺ or SPT⁻) and into 4 groups on the basis of *A. lumbricoides* infection and the presence or absence of allergen skin-test reactivity: *Ascaris*⁻/SPT⁻, *Ascaris*⁺/SPT⁻, *Ascaris*⁻/SPT⁺, and *Ascaris*⁺/SPT⁺. Differences between immunologic variables stratified according to binary variables (SPT⁺ vs. SPT⁻ and *Ascaris*⁻ vs. *Ascaris*⁺) were assessed by use of the Mann-Whitney *U* test. Differences between immunologic variables for the 4 groups were assessed either by use of the Kruskal-Wallis test (for comparisons among the 4 groups) or the Mann-Whitney *U* test (for comparisons between 2 of the groups); 2 groups were compared only when there was evidence of significant intergroup

heterogeneity among the 4 groups. Comparison of proportions was performed by use of the χ^2 test. The effects that *A. lumbricoides*-infection and/or SPT status had on log_e-transformed immunologic variables were evaluated by use of univariate and multivariate linear regression analyses. The effects that interactions between *A. lumbricoides*-infection and SPT status had on the magnitude of immunologic variables were assessed by use of likelihood ratio tests. Statistical analyses were performed by use of Intercooled Stata 7 software (Stata Corporation). Statistical significance was inferred by $P < .05$.

RESULTS

Characteristics of subjects. Ninety-nine (75.0%) of the 132 subjects had positive stool examinations for *A. lumbricoides* infection; 25 (18.9%) had evidence of skin-test reactivity to ≥ 1 of the 7 allergens tested. Positive reactions to individual allergens were as follows: *D. pteronyssinus*, 9.1%; *D. farinae*, 5.3%; grass mix, 3.0%; tree mix, 5.3%; American cockroach, 11.4%; *A. tenuis*, 0.8%; and cat, 1.5%. All of the subjects had positive skin-test reactions to the positive histamine control. Age, sex, and parasitologic characteristics of the study population stratified into the 4 groups according to *A. lumbricoides*-infection and SPT status are shown in table 1. No other geohelminth species was detected in stool samples. Almost all of the subjects had detectable levels of *A. lumbricoides*-specific IgG1 (only 2 of the children did not), indicating universal exposure to geo-

helminths in the study population. Because of potential antigenic cross-reactivity between *A. lumbricoides* and *Trichuris trichiura*, both of which are highly endemic in the study area, specific antibodies may reflect exposure to or infection with either parasite. SPT status was not associated with differences in levels of allergen-specific IgE (American cockroach, *D. pteronyssinus*, and cat; data not shown), and no significant differences in levels of allergen-specific IgE among the 4 groups stratified by *A. lumbricoides*-infection and SPT status were found (table 1)—median levels were negligible in all 4 groups.

Association between *A. lumbricoides* infection and the *Ascaris*-specific immune response. Histamine release induced by ASC was elevated in the *Ascaris*⁺ group ($P = .04$), but trends toward reduced responsiveness were observed for larval-stage antigens in the *Ascaris*⁺ group (figure 1A). Frequencies of cytokine-expressing PBMCs stimulated with *Ascaris* antigens were significantly reduced in the *Ascaris*⁺ group, compared with those in the *Ascaris*⁻ group, for both IL-4 ($P = .003$, for L2/L3; $P = .02$, for L3/L4) and IL-5 ($P = .002$, for ASC; $P = .01$, for L2/L3; $P = .03$, for L3/L4) (figure 2A and 2B). In the *Ascaris*⁺ subjects, evidence was also found for reduced frequencies of IFN- γ -expressing PBMCs stimulated with parasite antigens (ASC, $P = .03$; L3/L4, $P = .006$), although IFN- γ -expressing PBMCs were of low frequency overall (figure 2C). Therefore, active *A. lumbricoides* infection was associated with diminished Th2 cytokine responses, and perhaps also with diminished Th1

Table 1. Age, sex, parasitologic characteristics, and antibody levels, in children stratified into 4 groups according to *Ascaris lumbricoides*-infection status (*Ascaris*⁺ or *Ascaris*⁻) and allergen skin-test status (SPT⁺ or SPT⁻).

Category, parameter	SPT ⁻ / <i>Ascaris</i> ⁻ (n = 23)	SPT ⁻ / <i>Ascaris</i> ⁺ (n = 84)	SPT ⁺ / <i>Ascaris</i> ⁻ (n = 10)	SPT ⁺ / <i>Ascaris</i> ⁺ (n = 15)
Age, median (range), years	10 (5–17)	11 (5–19)	10 (6–14)	11 (7–15)
Sex, male:female	6:17	49:35	5:5	7:8
Geohelminths				
<i>A. lumbricoides</i>				
Prevalence	0	100	0	100
Intensity, GM (range), epG	0	13,161 (71–211,083)	0	10,729 (213–112,748)
<i>Trichuris trichiura</i>				
Prevalence	39.1	79.2	50.0	73.3
Intensity, GM (range), epG	6.8 (0–355)	143.5 (0–390,500)	10.4 (0–1948)	103.7 (0–4757)
<i>A. duodenale</i>				
Prevalence	0	13.1	0	20.0
Intensity, GM (range), epG	0	0.8 (0–497)	0	1.1 (0–639)
Any geohelminth, prevalence	39.1	100	50.0	100
Antibodies, median (range)				
Total IgE, IU/mL	863 (60–18,603)	4940 (53–31,250)	475 (290–14,469)	1890 (51–30,050)
Anti- <i>Ascaris</i> IgG1 ^a	46 (0–257)	91 (1–434)	76 (0–172)	95 (5–668)
Anti- <i>Ascaris</i> IgG4 ^a	9 (0–72)	17 (0–1000)	0 (0–88)	6 (0–936)
Anti- <i>Ascaris</i> IgE, kU/L	4.2 (0–33.4)	15.6 (0–100)	2.4 (0–94.1)	12.6 (0–8.6)
Anti- <i>D. pteronyssinus</i> IgE, kU/L	0 (0–3.6)	0 (0–8.4)	0.2 (0–1.3)	0 (0–5.9)
Anti-American cockroach IgE, kU/L	0 (0–8.4)	0 (0–4.7)	0 (0–9.3)	0 (0–3.7)
Anti-cat IgE, kU/L	0 (0–13.8)	0.8 (0–25.1)	0 (0–9.6)	0 (0–7.9)

NOTE. epG, No. of eggs per gram of stool; GM, geometric mean.

^a Units are arbitrary.

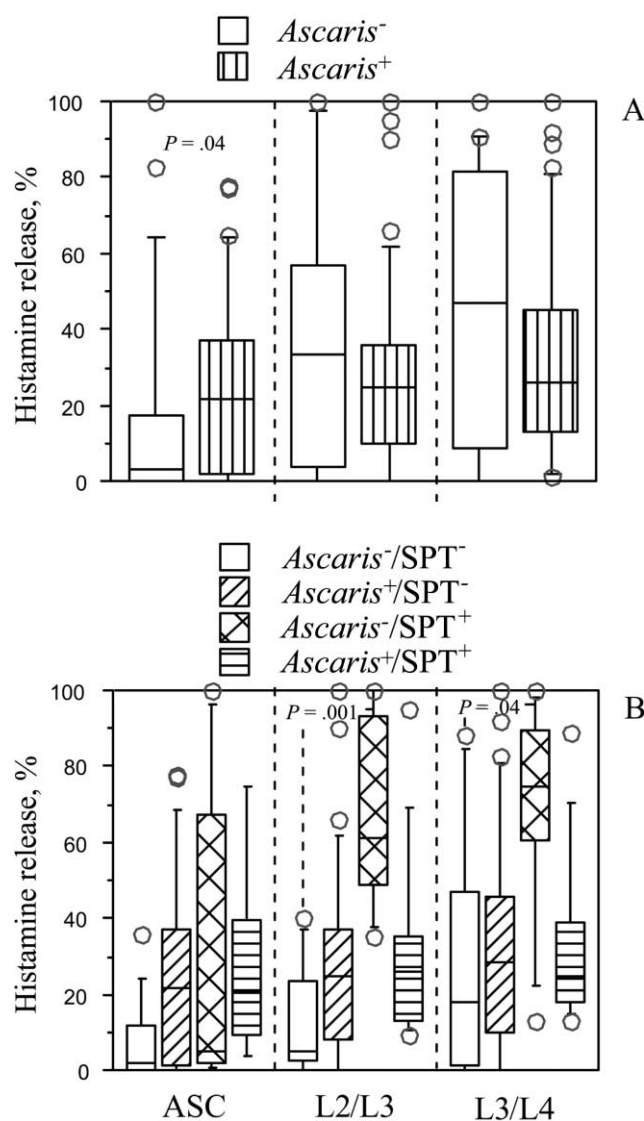


Figure 1. Histamine release by whole blood incubated with parasite antigens, in children stratified according to *Ascaris lumbricoides*-infection status (*Ascaris*⁺ or *Ascaris*⁻) (A) or into 4 groups according to *A. lumbricoides*-infection and allergen skin-test status (SPT⁺ or SPT⁻) (B). Box plots represent median values (central lines), interquartile ranges (top and bottom box margins), 95% confidence intervals (bars), and outlying values (circles). Antigens are adult *Ascaris* antigen (ASC) and larval-stage *Ascaris* antigens (L2/L3 and L3/L4).

cytokine responses, to *Ascaris* antigens (figure 2) in a population with universal exposure to geohelminth parasites (table 1).

Association between SPT status and the *Ascaris*-specific immune response. Evidence was found for enhanced histamine release induced by larval-stage antigens ($P = .006$, for L2/L3) in the SPT⁺ subjects, but evidence was not found for such inducement by adult antigens. Frequencies of Th2 cytokine-expressing PBMCs stimulated with parasite and nonparasite antigen were significantly elevated in the SPT⁺ subjects, compared with those in the SPT⁻ subjects (IL-4: $P = .003$, for PPD;

$P = .002$, for L2/L3; $P = .01$, for L3/L4) (IL-5: $P = .01$, for PPD; $P = .009$, for ASC; $P = .003$, for L2/L3; $P = .01$, for L3/L4). No significant differences in the expression of IFN- γ were observed between the SPT⁺ and SPT⁻ subjects.

Relationship between *A. lumbricoides*-infection and SPT status in the determination of the *Ascaris*-specific immune response. Our data show that both *A. lumbricoides*-infection and SPT status may influence the magnitude of anti-*Ascaris* immune responses. To examine the relative effects that *A. lumbricoides*-infection and SPT status have on *Ascaris*-specific immunity, the subjects were stratified into 4 groups according to *A. lumbricoides*-infection and SPT status, as described above. The 4-group stratification showed negligible intergroup differences in levels of polyclonal IgE and *A. lumbricoides*-specific IgG1 and IgE (table 1); however, there were significant intergroup differences in levels of *A. lumbricoides*-specific IgG4 ($P = .003$) that seemed to be largely accounted for by negligible antibody levels in the *Ascaris*⁻/SPT⁺ group ($P = .007$, for *Ascaris*⁻/SPT⁻ vs. *Ascaris*⁻/SPT⁺).

Significant intergroup differences were observed for histamine release by whole blood stimulated with larval antigens ($P = .001$, for L2/L3; $P = .05$, for L3/L4), with the greatest histamine release being observed in the *Ascaris*⁻/SPT⁺ group (*Ascaris*⁻/SPT⁻ vs. *Ascaris*⁻/SPT⁺: $P = .001$, for L2/L3; $P = .04$, L3/L4) (figure 1B). Histamine release appeared to be similar in the *Ascaris*⁺ subjects, irrespective of SPT status (figure 1B).

The 4-group stratification of the data for Th2 cytokine expression (figures 3A and 3B) showed significant intergroup differences for PBMCs stimulated with ASC ($P = .002$, for IL-5) and L2/L3 antigens ($P = .002$, for IL-4). Again, many of the intergroup differences appeared to be associated with enhanced Th2 cytokine secretion by the *Ascaris*⁻/SPT⁺ subjects (*Ascaris*⁻/SPT⁻ vs. *Ascaris*⁻/SPT⁺: $P = .03$, for IL-4 to L2/L3; $P = .05$, for IL-5 to ASC), and the same trend was observed for the other *Ascaris* antigens. Increased Th2 cytokine secretion was observed in the *Ascaris*⁺/SPT⁺ subjects, compared with that in the *Ascaris*⁺/SPT⁻ subjects, in response to the nonparasite antigen, PPD ($P = .003$, for IL-4; $P = .02$, for IL-5). No differences among the 4 groups in the expression of IFN- γ to parasite antigens were found (figure 3C).

Because the relationship between *A. lumbricoides*-infection and SPT status as determinants of the magnitude of histamine release and Th2 cytokine expression appears to be complex and may be confounded by other factors (e.g., age, sex, and infections with other geohelminth parasites), the relationship between these factors as determinants of these immunologic variables was examined by use of univariate and multivariate linear regression analyses (table 2). Univariate analyses showed that *A. lumbricoides*-infection status had significant effects on the frequencies of IL-4- and IL-5-expressing PBMCs stimulated with adult or larval antigens, but none of these remained significant

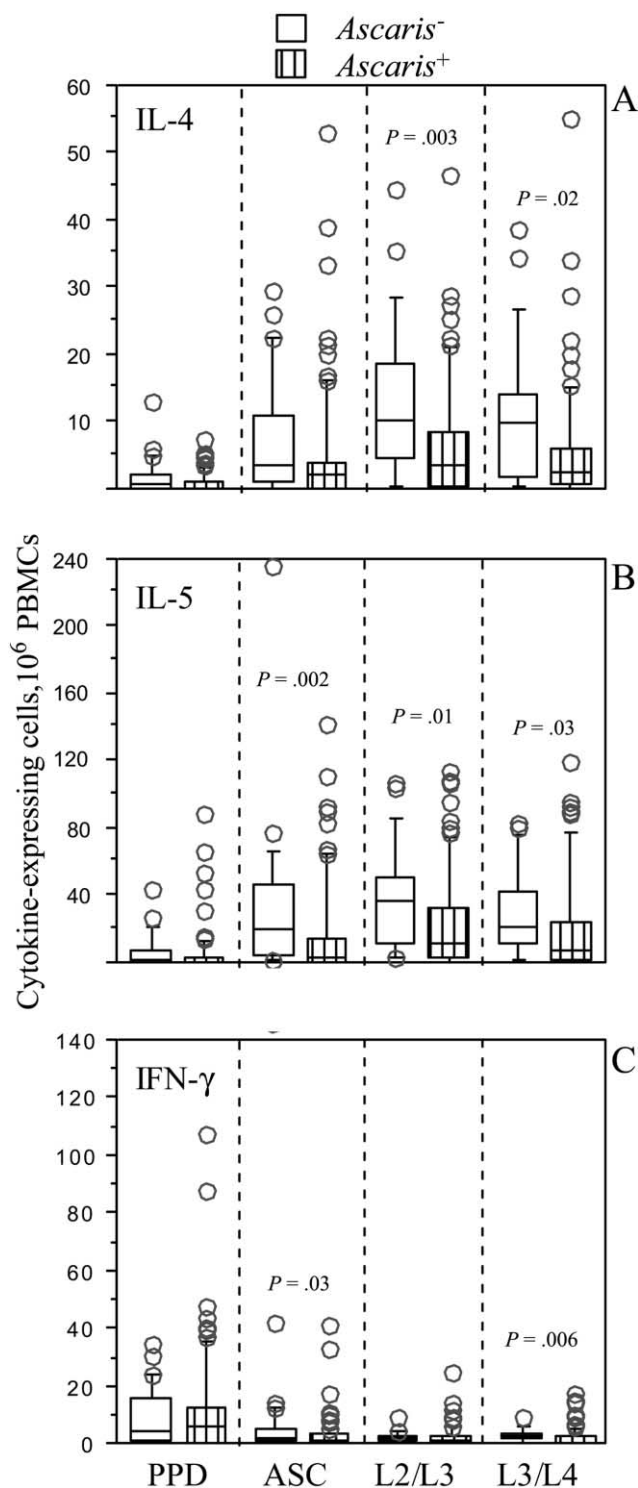


Figure 2. Frequencies of parasite antigen–stimulated peripheral-blood mononuclear cells (PBMCs) expressing interleukin (IL)–4, IL–5, and interferon (IFN)– γ , in children stratified according to *Ascaris lumbricoides*–infection status (*Ascaris*[–] or *Ascaris*⁺). Box plots represent median values (central lines), interquartile ranges (top and bottom box margins), 95% confidence intervals (bars), and outlying values (circles). Antigens are purified protein derivative (PPD), adult *Ascaris* antigen (ASC), and larval-stage *Ascaris* antigens (L2/L3 and L3/L4).

in multivariate models. In contrast, SPT status was highly significant in both univariate and multivariate analyses of Th2 cytokine expression induced by parasite antigens (multivariate analyses: $P = .002$, for IL–4 to ASC; $P = .004$, for IL–4 to L2/L3; $P = .009$, for IL–4 to L3/L4; $P = .001$, for IL–5 to ASC; $P = .02$, for IL–5 to L2/L3). Evidence was found for an interaction between *A. lumbricoides*–infection status and SPT status in the determination of the magnitude of histamine release induced by larval-stage antigens ($P = .001$, for histamine to L2/L3; $P = .03$, for histamine to L3/L4) and frequencies of adult antigen–stimulated PBMCs that expressed IL–5 ($P = .01$), indicating that the effect that SPT status had on these immunologic variables depended on *A. lumbricoides*–infection status.

DISCUSSION

We have investigated the relationship between measures of the anti-*Ascaris* immune response and allergen skin-test reactivity in children living in an area of Ecuador where geohelminth infection is endemic and where we have previously demonstrated a strong inverse relationship between allergen skin-test reactivity and *A. lumbricoides* infection [10]. As determined by measurement of *A. lumbricoides*–specific antibodies, almost all of the children in the present study had evidence of exposure to geohelminth parasites irrespective of allergen skin-test reactivity, and a high proportion of the children were infected at the time of sampling. Our data have shown that children with evidence of skin-test reactivity to aeroallergens have enhanced allergic responses to *Ascaris* antigens, as measured by basophil histamine release induced by parasite antigens and elevated frequencies of PBMCs expressing the Th2 cytokines IL–4 and IL–5.

The human immune system is likely to have evolved in the context of heavy helminth infestation and intense exposure to helminth antigens; allergic inflammation may, therefore, have developed as an important protective host mechanism for preventing the establishment of helminth infection and controlling parasite numbers [19]. Different components of the allergic Th2 response (e.g., IgE, eosinophils, and mast cells), either singly or in combination, appear to mediate protective immunity against different helminth infections in experimental animal models [7]. Although we were not able to demonstrate statistically significant differences in the prevalence of intensity of infection with *A. lumbricoides* or other geohelminth parasites between atopic and nonatopic individuals in the present small study, there were trends toward reduced prevalence and intensity of ascariasis and trichuriasis in the nonatopic subjects; moreover, a larger study of 2865 schoolchildren, of which our study population was a subset, showed strong inverse correlations between the presence of infection with *A. lumbricoides*, *T. trichiura*, and *A. duodenale* and allergen skin-test reactivity [10]. Furthermore, the data from the larger study showed that risk for atopy became lower as infection intensity increased, indicating that chil-

dren with evidence of allergen skin-test reactivity had reduced infection intensities of *A. lumbricoides* and *T. trichiura*. Together, these data suggest that children with evidence of allergen skin-test reactivity are less likely to be infected with *A. lumbricoides* and other geohelminth parasites and that this resistance to parasite infection is associated with enhanced Th2 cytokine and IgE-mediated inflammatory responses to parasite antigens.

Previous studies have provided evidence for resistance to reinfection with *A. lumbricoides* following anthelmintic treatment in a subgroup of individuals living in communities in which infection is endemic [20]. Resistance to reinfection with *A. lumbricoides* has been associated with high levels of anti-parasite IgE [21], IgE to the principal *Ascaris* allergen, ABA-1 [20], and elevated levels of eosinophilic cationic protein and other acute inflammatory markers [20]. Elevated levels of anti-parasite IgE and circulating eosinophilic degranulation products are markers of a polarized Th2 cytokine response, and, although we did not specifically examine resistance to infection in the present study, our findings for the atopic subjects (who appeared to be relatively resistant to infection with *A. lumbricoides*, compared with the nonatopic subjects) support a potential role for Th2 cytokine responses in mediating protective immunity against ascariasis and other geohelminth infections in humans. A recent study that examined Th2 cytokine production by *Ascaris* antigen-stimulated whole blood in children living in a community in which endemicity is high provided evidence for an inverse relationship between Th2 cytokine production and infection intensity in older children [22]. The strongest evidence for a protective role played by Th2 cytokine responses against helminths in humans derives from studies of reinfection with schistosomes after chemotherapy. These studies have demonstrated associations between individual components of the Th2 cytokine response (e.g., peripheral eosinophilia [23], levels of anti-parasite IgE [24, 25], and production of IL-5 in lymphocyte cultures stimulated with parasite antigen [26]) and resistance to reinfection. Genetic studies have suggested that resistance to infection with schistosomes is linked to a region on chromosome 5q31-q33 that contains several Th2-response genes [27].

We have shown previously that anti-parasite cytokine responses in young adults infected with *A. lumbricoides* and living in communities in which endemicity is high are strongly Th2-polarized, compared with those in uninfected control subjects from a nearby town where exposure to infection was likely to be limited [18]. In this study, we compared infected and uninfected children residing in the same communities (in which helminth infection was endemic) and showed that Th2 (IL-4 and IL-5) and perhaps Th1 (IFN- γ) cytokine responses appeared to be modulated in infected children. Similar findings have been reported from a region of Brazil where helminth infection is endemic [28]. The explanation for this may be

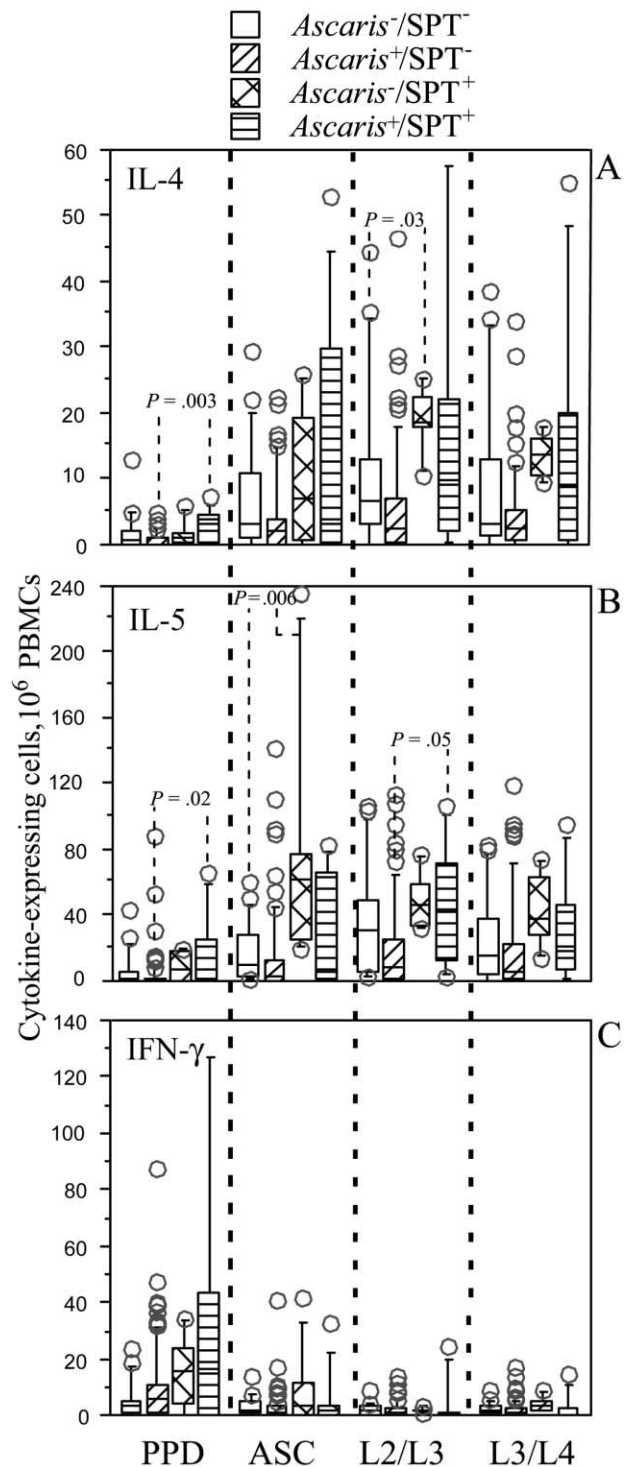


Figure 3. Frequencies of parasite antigen-stimulated peripheral-blood mononuclear cells (PBMCs) expressing interleukin (IL)-4, IL-5, and interferon (IFN)- γ , in children stratified into 4 groups according to *Ascaris lumbricoides*-infection status (*Ascaris*⁻ or *Ascaris*⁺) and allergen skin-test status (SPT⁻ or SPT⁺). Box plots represent median values (central lines), interquartile ranges (top and bottom box margins), 95% confidence intervals (bars), and outlying values (circles). Antigens are purified protein derivative (PPD), adult *Ascaris* antigen (ASC), and larval-stage *Ascaris* antigens (L2/L3 and L3/L4).

Table 2. Associations between *A. lumbricoides*–infection status (*Ascaris*) and allergen skin-test status (SPT) and magnitude of *Ascaris*-specific immunologic variables.

Immunologic variable	<i>Ascaris</i>		SPT		Interaction ^b
	Univariate analysis	Multivariate analysis ^a	Univariate analysis	Multivariate analysis ^a	
Histamine release induced by ASC	.16	.77	.44	.67	.17
Histamine release induced by L2/L3	.26	.46	.008	.03	.001
Histamine release induced by L3/L4	.08	.19	.08	.15	.03
IL-4 to ASC	.85	.57	.001	.002	.05
IL-4 to L2/L3	.02	.18	.001	.004	.57
IL-4 to L3/L4	.03	.18	.003	.009	.25
IL-5 to ASC	.04	.20	<.001	.001	.01
IL-5 to L2/L3	.04	.16	.009	.02	.45
IL-5 to L3/L4	.17	.41	.06	.08	.90

NOTE. Data are *P* values for coefficients for *Ascaris* or SPT in linear regression models. *P* values <.05 are shown in bold type. Immunologic variables were log_e transformed for linear regression. Antigens are purified protein derivative (PPD), adult *Ascaris* antigen (ASC), and larval-stage *Ascaris* antigens (L2/L3 and L3/L4). IL, interleukin.

^a Multivariate analyses were controlled for the effects of the other variable (SPT or *Ascaris*, as appropriate), age, sex, and *Trichuris trichiura* and *A. duodenale* infection.

^b *P* values determined by likelihood ratio tests.

differences in anti-parasite responses between control subjects living in areas where helminth infection is not endemic and those living in areas where helminth infection is endemic: the latter may be free of infection because of a greater degree of protective immunity that may be associated with a greater tendency to produce Th2 cytokine responses on exposure to the parasite. Moreover, our data from the present study show that the greatest expression of Th2 cytokines by PBMCs stimulated with parasite antigens was observed in children who were free of infection and had positive allergen skin tests. Multivariate analyses indicated that (1) allergen skin-test reactivity and not *A. lumbricoides*–infection status was the primary determinant of the magnitude of Th2 cytokine expression induced by parasite antigens and (2) the effect of allergen skin-test reactivity on basophil histamine release induced by larval antigens and on IL-5 expression by PBMCs stimulated with adult-derived antigen was dependent on *A. lumbricoides*–infection status (i.e., was greatest in the *Ascaris*[−] subjects).

Infected individuals living in areas where the parasite is endemic are likely to harbor chronic infections and may develop down-regulatory mechanisms that limit allergic inflammatory responses to ascariasis. Down-regulatory mechanisms that may be important in limiting both Th1 and Th2 inflammatory responses to helminth parasites include the production of cytokines such as IL-10 [29–32]. Our observations of reduced Th1 and Th2 cytokine responses to parasite antigens in infected individuals is consistent with a modified Th2 response [33], an immune phenotype that was first described for individuals with little or no allergic responsiveness to cat allergen despite high levels of exposure [34] and that has been extended to explain nonresponsiveness in helminth-infected populations [33]. The down-regulatory mechanisms that modulate allergic responses to par-

asite antigens during chronic helminth infection may also modulate immune responses to aeroallergens [35].

Several epidemiological studies have shown strong inverse associations between allergen skin-test reactivity and geohelminth infection [8–10]. Because most studies have been cross-sectional, the direction of this causal relationship is not clear—geohelminth infection may protect against allergen skin-test reactivity or allergen skin-test reactivity may protect against geohelminth infection. An intervention study provided evidence that anthelmintic treatment of geohelminth-infected children resulted in an increase in the prevalence of atopy, indicating that geohelminths are the antecedent exposure [12]. Our data have provided evidence that children who are atopic (or allergen skin-test reactive) have stronger allergic responses to parasite antigens than do nonatopic children. It is possible that the expression of atopy may vary over time in a single individual and may depend on the balance of environmental exposures (protective vs. risk exposures). Individuals with strong genetic traits associated with atopy may be more likely to have positive allergen skin tests to environmental allergens and may be more resistant to geohelminth infection but, given adequate exposure to geohelminths, may develop chronic infections that, in turn, may cause a down-regulation of allergic inflammation and result in negative allergen skin tests. Here, this tendency was observed as reduced Th1 and Th2 cytokine expression in the *Ascaris*⁺/SPT⁺ subjects, compared with that in the *Ascaris*[−]/SPT⁺ subjects.

In the present study, we used allergen skin-test reactivity as a marker for the presence of atopy. Atopy is an immune phenomenon that can be defined as the presence of either a positive skin test to extracts of inhalant allergens or the presence of allergen-specific IgE in serum. In studies conducted in indus-

trialized countries, positive allergen skin tests and the presence of allergen-specific IgE are strongly associated [36, 37], but, in rural regions of Africa, this relationship appears to be weak or nonexistent [38, 39]. In the present study, the presence of allergen skin-test reactivity and allergen-specific IgE in serum did not appear to be associated (table 1). Possible explanations for the disassociation between the 2 markers of atopy are potentiation of preexisting IgE responses to inhalant allergens by geohelminth infection [13] and false-positive serologic reactions caused by cross-reactivity between geohelminth antigens and inhalant allergens [40].

In conclusion, our findings have indicated that there are important differences in the immune responses to *Ascaris* antigens among children exposed to ascariasis according to the presence or absence of skin-test reactivity to common environmental allergens. Our data have shown that (1) children with positive allergen skin tests have greater frequencies of PBMCs that express Th2 cytokines (IL-4 and IL-5) on stimulation with *Ascaris* antigens and release more histamine from peripheral-blood basophils in response to larval-stage antigens than do children with negative allergen skin tests and (2) responses are greatest in children not infected with *A. lumbricoides*. Because atopic individuals may be more resistant to geohelminth infection and, when infected, harbor smaller parasite burdens than do nonatopic individuals, high Th2 cytokine expression and enhanced IgE-mediated inflammation (allergen-induced release of histamine by basophils) may be correlates of protective immunity against ascariasis. The data suggest that allergen skin-test reactivity or the atopic phenotype, not infection status, is the primary determinant of the allergic inflammatory response to ascariasis. Moreover, our data demonstrate that a modified antigen-specific T helper response occurs during patent intestinal human helminth infection (figure 2), a modification that may reflect ongoing regulatory networks of antigen-specific and antigen-nonspecific T cells [33].

Acknowledgments

We thank the children, parents, and teachers in the study communities, for their cooperation, and Brenda Rae Marshall, for editorial assistance.

References

- Chan MS, Medley GF, Jamison D, Bundy DAP. The evaluation of potential global mortality attributable to intestinal nematode infections. *Parasitology* **1994**; 109:373–87.
- de Silva NR, Chan MS, Bundy DAP. Morbidity and mortality due to ascariasis: re-estimation and sensitivity analysis of global numbers at risk. *Trop Med Int Health* **1997**; 2:519–28.
- Holgate ST. The epidemic of allergy and asthma. *Nature* **1999**; 402: B2–4.
- Cooper PJ. Can intestinal helminth infections (geohelminths) affect the development of and expression of asthma and allergic disease? *Clin Exp Immunol* **2002**; 128:398–404.
- Cooper PJ, Schwartz L, Awadzi K, Ottesen EA, Nutman TB. Plasma tryptase, a marker of mast cell degranulation, is associated with the development of adverse reactions following the treatment of onchocerciasis with ivermectin. *J Infect Dis* **2002**; 186:1307–13.
- Finkelman FD, Wynn TA, Donaldson DD, Urban JF Jr. The role of IL-13 in helminth-induced inflammation and protective immunity against nematode infections. *Curr Opin Immunol* **1999**; 11:420–6.
- Gause WC, Urban JF Jr, Stadecker MJ. The immune response to parasitic helminths: insights from murine models. *Trends Immunol* **2003**; 24:269–77.
- Hagel I, Lynch NR, Perez M, Di Prisco MC, Lopez R, Rojas E. Modulation of the allergic reactivity of slum children by helminthic infection. *Parasite Immunol* **1993**; 15:311–5.
- Nyan OA, Walraven GEL, Banya WAS, et al. Atopy, intestinal helminth infection and total serum IgE in rural and urban adult Gambian communities. *Clin Exp Allergy* **2001**; 31:1672–8.
- Cooper PJ, Chico ME, Rodrigues LC, et al. Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. *J Allergy Clin Immunol* **2003**; 111:995–1000.
- Grove DI, Forbes IJ. Increased resistance to helminth infestation in an atopic population. *Med J Aust* **1975**; 1:336–8.
- Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R, Alvarez N. Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. *J Allergy Clin Immunol* **1993**; 92:404–11.
- Jarrett EEE, Miller HRP. Production and activities of IgE in helminth infection. *Prog Allergy* **1982**; 31:178–233.
- Aalberse RC. The IgE response and atopy. *Eur Respir J* **1991**; 4:S78–84.
- Plaut M, Kazimierzczak W, Lichtenstein LM. Abnormalities of “basophil” releasability in atopic and asthmatic individuals. *J Allergy Clin Immunol* **1986**; 78:968–73.
- Boyce JA. Mast cells: beyond IgE. *J Allergy Clin Immunol* **2003**; 111: 24–32.
- World Health Organization (WHO). Diagnostic techniques for intestinal parasitic infections (IPI) applicable to primary health care (PHC) services [WHO/PDP/85.2, 1211]. Geneva: WHO, **1985**.
- Cooper PJ, Chico ME, Sandoval C, et al. Human infection with *Ascaris lumbricoides* is associated with a polarized cytokine response. *J Infect Dis* **2000**; 182:1207–13.
- Le Souef PN, Goldblatt J, Lynch NR. Evolutionary adaptation of inflammatory immune responses in human beings. *Lancet* **2000**; 356: 242–4.
- McSharry C, Xia Y, Holland CV, Kennedy MW. Natural immunity to *Ascaris lumbricoides* associated with immunoglobulin E antibody to ABA-1 allergen and inflammation indicators in children. *Infect Immun* **1999**; 67:484–9.
- Hagel I, Lynch NR, Di Prisco MC, Rojas E, Perez M, Alvarez N. *Ascaris* reinfection of slum children: relation with the IgE response. *Clin Exp Immunol* **1993**; 94:80–3.
- Turner JD, Faulkner H, Kamgno J, et al. Th2 cytokines are associated with reduced worm burdens in a human intestinal helminth infection. *J Infect Dis* **2003**; 188:1768–75.
- Hagan P, Blumenthal UJ, Chaudri M, et al. Resistance to reinfection with *Schistosoma haematobium* in Gambian children: analysis of their immune responses. *Trans R Soc Trop Med Hyg* **1987**; 81:938–46.
- Hagan P, Blumenthal UJ, Dunn D, Simpson AJ, Wilkins HA. Human IgE, IgG4, and resistance to reinfection with *Schistosoma haematobium*. *Nature* **1991**; 349:243–5.
- Dunne DW, Butterworth AE, Fulford AJ, et al. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur J Immunol* **1992**; 22:1483–94.
- Roberts M, Butterworth AE, Kimani G, et al. Immunity after treatment of human schistosomiasis: association between cellular responses and resistance to reinfection. *Infect Immun* **1993**; 61:4984–93.
- Marquet S, Abel L, Hillaire D, et al. Genetic localization of a locus controlling the intensity of infection by *Schistosoma mansoni* on chromosome 5q31-q33. *Nat Genet* **1996**; 14:181–4.

28. Geiger SM, Massara CL, Bethony J, Soboslay PT, Carvalho OS, Correa-Oliveira R. Cellular responses and cytokine profiles in *Ascaris lumbricoides* and *Trichuris trichiura* infected patients. *Parasite Immunol* **2002**; 24:499–509.
29. Mahanty S, Nutman TB. Immunoregulation in human lymphatic filariasis: the role of interleukin 10. *Parasite Immunol* **1995**; 17:385–92.
30. King CL, Medhat A, Malhotra I, et al. Cytokine control of parasite-specific anergy in human urinary schistosomiasis: IL-10 modulates lymphocyte reactivity. *J Immunol* **1996**; 156:4715–21.
31. Cooper PJ, Mancero T, Sandoval C, Lovato R, Guderian RH, Nutman TB. Early human infection with *Onchocerca volvulus* is associated with an enhanced parasite-specific cellular immune response. *J Infect Dis* **2001**; 183:1662–8.
32. Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. *Science* **2002**; 296:490–4.
33. Maizels RM, Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nat Rev Immunol* **2003**; 3:733–44.
34. Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitization, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* **2001**; 357:752–6.
35. van den Biggelaar AH, van Ree R, Rodrigues LC, et al. Decreased atopy in children infected with *Schistosoma hematobium*: a role for parasite-induced interleukin-10. *Lancet* **2000**; 356:1723–7.
36. Ali M, Ramanarayanan MP. Correlation of a micro-ELISA assay with the skin test and the RAST assay in inhalant allergy. *Ann Allergy* **1984**; 53:143–50.
37. van der Zee JS, de Groot H, van Swieten P, Jansen HM, Aalberse RC. Discrepancies between the skin test and IgE antibody assays: study of histamine release, complement activation in vitro, and occurrence of allergen-specific IgG. *J Allergy Clin Immunol* **1988**; 82:270–81.
38. van den Biggelaar AH, Lopuhaa C, van Ree R, et al. The prevalence of parasite infestation and house dust mite sensitization in Gabonese schoolchildren. *Int Arch Allergy Immunol* **2001**; 126:231–8.
39. Perzanowski MS, Ng'ang'a LW, Carter MC, et al. Atopy, asthma, and antibodies to *Ascaris* among rural and urban children in Kenya. *J Pediatr* **2002**; 140:582–8.
40. Thomas WR, Smith W. Towards defining the full spectrum of important house dust mite allergens. *Clin Exp Allergy* **1999**; 29:1583–7.