

Repeated Treatments with Albendazole Enhance Th2 Responses to *Ascaris Lumbricoides*, but Not to Aeroallergens, in Children from Rural Communities in the Tropics

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Geohelminth infections are associated with modulation of immunity to parasite antigens and aeroallergens. To investigate the possibility that this modulation is affected by anthelmintic treatment, we compared cytokine responses in children who were treated with repeated doses of albendazole over 1 year versus those in children who had were not treated. Whole-blood samples were cultured with *Ascaris* antigen and house dust mite and cockroach allergens, and levels of interleukin (IL)–5, IL-13, interferon- γ , and IL-10 were measured. Anthelmintic treatment was associated with enhanced production of Th2 cytokines in response to parasite antigen but did not affect responses to aeroallergens. The data indicate that long-term treatment may be associated with increased Th2 antiparasite immunity.

Geohelminth parasites are estimated to infect 2 billion humans worldwide [1], and these infections induce strong immunological responses associated with the type 2 cytokines interleukin (IL)–4, IL-5, and IL-13 [2, 3]. Geohelminth infections may have major modulatory effects on host immunity [4, 5] and therefore may in part explain the low prevalence of allergic diseases reported in the rural Tropics [5].

Previous studies have shown that single or multiple doses of anthelmintic drugs have negligible effects on human cytokine responses to parasite antigens [6, 7] and that long-term anthelmintic treatments may increase allergen skin-test reactivity to aeroallergens [8, 9]. Because programs to control geohelminth infections administer anthelmintic drugs periodically over long periods, it is important to determine whether anthelmintic drugs administered over the long term have important immunologic effects. To investigate whether repeated anthelmintic treatments have important effects on immunity to parasite antigens and aeroallergens, we examined cytokine response and histamine release in whole-blood samples from children living in rural Ecuadorian communities who either were treated with albendazole every 2 months for 1 year or were not treated.

Subjects, materials, and methods. Children attending schools in Pichincha Province in Ecuador were sampled within a cluster-randomized study that examined the effect that treatment with albendazole has on the prevalence of allergy. The study design is described elsewhere [10]. In brief, children attending 68 rural schools were recruited. Schools were randomized to either be treated with albendazole (single doses of 400 mg every 2 months for 12 months, for a total of 7 treatments) or not be treated, and the treatments with albendazole were directly observed. The present study was a cross-sectional study nested within the intervention study and was performed at the end of the 12-month follow-up period. A total of 214 children from 42 schools were selected from 1632 children who completed the follow-up. Selection of children was blinded to school treatment status but was such that children whose allergen skin tests were positive were overrepresented in the sample (2:3 ratio of skin test–positive children to skin test–negative children). Neither tissue helminth infection nor malaria was endemic in these communities. Informed written consent was obtained from a parent of each child. The study protocol was approved by the ethics committees of Hospital Pedro Vicente Maldonado in Ecuador and St. George's Hospital in the United Kingdom.

Skin-prick testing for reactivity to house dust mite (*Dermatophagoides pteronyssinus*) (Greer Laboratories), American cockroach (*Periplaneta americana*) (Greer Laboratories), *Alternaria tenuis* (Greer Laboratories), cat (Greer Laboratories), grass/pollen mix (Greer Laboratories), fungi pollen (Greer Laboratories), and histamine (ALK-Abello) and to saline (ALK-Abello) controls was performed as described elsewhere [10]. Reactions were considered to be positive if the mean wheal diameter was at least 3 mm greater than that for the saline controls. Stool samples were collected from children both at the beginning of the study

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(i.e., before they had received the first dose of albendazole) and at 12 months (i.e., before receiving the seventh dose of albendazole) and were examined by the modified Kato-Katz and formol-ethyl acetate–concentration methods [11]. Seven days after the treated children had received the seventh dose of albendazole, blood samples (7 mL) from the treated children and the untreated children were drawn into Vacutainers (Becton Dickinson) containing sodium heparin; the blood samples then were transported, in insulated boxes at ambient temperature, and were analyzed <5 h after being collected.

Whole-blood samples were diluted 1:4 in RPMI 1640 (Bio-Whittaker) containing L-glutamine, gentamicin at 80 mg/mL, and 1% HEPES. Samples (0.5 mL) of the diluted whole blood were then cultured either alone or in the presence of *A. lumbricoides* adult-worm antigen [2], lipopolysaccharide (Sigma-Aldrich), tuberculin (purified protein derivative) (Statens Serum Institute), or *Staphylococcus enterotoxin B* (SEB) (Sigma-Aldrich), all at 10 µg/mL; *D. pteronyssinus* (Greer Laboratories), at 100 AU/mL; or *P. americana*–allergen extract (Greer Laboratories), 1:50 dilution). Cultures were incubated in a humidified atmosphere of 5% CO₂ at 37°C.

Supernatant fluids were collected from cultures at 24 h (for determination of levels of IL-10) and 5 days (for determination of levels of interferon [IFN]–γ, IL-5, and IL-13) and were stored in liquid nitrogen. Cytokine levels were measured by sandwich ELISA using antibody pairs (BD Biosciences), according to the manufacturers' instructions. The lower detection limits for IL-5, IL-13, IL-10, and IFN-γ were 7.8, 58.6, 19.5, and 19.5 pg/mL, respectively. Total IgE levels were measured as described elsewhere [11]. Histamine-release assays of reactivity to *Ascaris* adult and larval-stage (L2/L3 and L3/L4) antigens [2], aeroallergens (*D. pteronyssinus* and *P. americana* [Greer Laboratories], and purified Der p1 and recombinant Der p2 [Indoor Biotechnologies]), all at concentrations of 0.03 µg/mL, were performed as described elsewhere [11], by a commercial assay (Immuno-tech).

The rank-sum test was used to compare the cytokine levels and the percentage of histamine release in the treated children versus those in the untreated children; the proportions were compared by χ^2 test. Associations between cytokines were assessed by calculation of Spearman's rank-correlation coefficients. Significant findings of a treatment effect were assessed by either multivariate linear regression (using log_e-transformed cytokine levels) or logistic regression (cytokine responders vs. nonresponders); the analyses controlled for a priori confounders, relevant baseline factors, and clustering by school. To assess the effect that the interaction between atopy and treatment status had on cytokine levels, an interaction term was added to the linear-regression models for the cytokines. For bivariate analyses, statistical significance was inferred when $P \leq .01$, to minimize type 1 statistical errors. Analyses were performed by use of Stata 7 (Stata).

Results. A total of 214 children were investigated, of whom 107 received anthelmintic treatment and 107 did not receive such treatment. Baseline characteristics of the children are shown in table 1; the baseline characteristics of the treated children were not significantly different from those of the untreated children.

Treatment was associated with greater production of IL-5 and IL-13, both by *A. lumbricoides*–stimulated cultures (IL-5, $P < .001$ in bivariate analysis and $P = .005$ in multivariate analysis; IL-13, $P = .01$ in bivariate analysis and $P = .02$ in multivariate analysis) and by SEB-stimulated cultures (IL-5, $P < .001$ in bivariate analysis and $P = .03$ in multivariate analysis; IL-13, $P = .01$ in bivariate analysis and $P = .13$ in multivariate analysis) (table 2). SEB-stimulated and *A. lumbricoides*–stimulated IL-5 ($\rho = 0.52$; $P < .001$) and IL-13 ($\rho = 0.40$; $P < .001$) were strongly correlated. The treatment effect for parasite-antigen and SEB-induced IL-5 and IL-13 was not modified by allergen skin-test status. Treatment was associated with a reduction both in the production of IL-10 ($P = .004$ in bivariate analysis and $P = .007$ in multivariate analysis) by *A. lumbricoides*–stimulated cultures and in the proportion of children ($P = .004$ in univariate analysis and $P = .02$ in multivariate analysis) producing detectable levels of IL-10. Levels of *A. lumbricoides*–induced IL-10 were not associated with levels of IL-5 or IL-13. The treated group and the untreated group were not different in either the levels of IFN-γ or the proportions of subjects producing detectable levels of IFN-γ in response to any of the stimuli. Cytokine levels in cultures stimulated by *D. pteronyssinus* and *P. americana* antigens did not differ significantly between the treated group and the untreated group. There were no deficiencies, in cytokine production in antigen- and SEB-stimulated cultures, between geohelminth-infected and -uninfected children (data not shown). Histamine release in response to parasite antigens and aeroallergens did not differ significantly between the treated group and the untreated group (table 2); histamine release of >10% was strongly associated with skin-test reactivity for *D. pteronyssinus* ($\chi^2 = 13.5$; $P < .001$) and *P. americana* ($\chi^2 = 11.5$; $P = .001$).

Discussion. The results of the present study, which are based on a cross-sectional analysis of schoolchildren living in rural Ecuadorian communities in which ascariasis and trichuriasis are endemic, suggest that repeated anthelmintic treatments cause significant increases in production of the Th2 cytokines IL-5 and IL-13, by peripheral blood leukocytes (PBLs) stimulated with *A. lumbricoides* antigen or the superantigen SEB. Because levels of *A. lumbricoides*–induced Th2 cytokines were strongly positively associated with levels of SEB-induced Th2 cytokines, it is likely that the enhanced superantigen response is at least partly attributable to the elevated antiparasite cytokine response. There was no strong evidence that, with regard to host cytokine or IgE-mediated inflammatory responses (measured by histamine release) to aeroallergens (*D. pteronyssinus* and *P.*

Table 1. Characteristics of 107 children treated with albendazole and of 107 untreated children.

Variable	Untreated <i>n</i> = 107	Treated
	<i>n</i> = 107	<i>n</i> = 107
Age, mean (SD), years	9.7 (1.8)	9.3 (1.9)
Sex, male/female, %	54/53	54/53
Socioeconomic level, mean (SD), index	2.1 (1.0)	1.9 (0.9)
Crowding, mean (SD), persons/room	2.6 (1.2)	2.4 (1.1)
BMI, mean (SD)	17.3 (2.5)	16.7 (2.1)
Total IgE, IU/mL		
GM at <i>t</i> = 0	1208	953
GM at <i>t</i> = 12	1110	687
White-cell count, mean (SD), ×10 ⁶ cells/L	7599 (1578)	7705 (1664)
Allergen skin-test reactivity at <i>t</i> = 12, %		
Any	37.4	37.4
<i>Dermatophagoides pteronyssinus</i>	18.7	14.0
<i>Periplaneta americana</i>	29.9	30.8
Other ^a	0.9	1.9
Geohelminth infections at <i>t</i> = 0	<i>n</i> = 106	
Any, %	75.5	74.8
<i>Ascaris lumbricoides</i>		
Frequency, %	56.6	57.9
Intensity, GM (range), epg ^b	5500 (71–294,211)	6819 (71–213,641)
<i>Trichuris trichiura</i> , %	57.1	57.9
Intensity, GM (range), epg ^b	437 (71–23,031)	573 (71–64,681)
Hookworm, %	5.7	9.4
<i>Strongyloides stercoralis</i> , %	0	4.7
Geohelminth infections at <i>t</i> = 12	(<i>n</i> = 99)	(<i>n</i> = 102)
Any, %	60.6	21.6
<i>A. lumbricoides</i> , %	34.3	5.9
Intensity, GM (range) epg ^b	7621 (211–268,661)	997 (71–14,001)
<i>T. trichiura</i>		
Frequency, %	51.5	19.6
Intensity, GM (range), epg ^b	463 (71–36,751)	99 (71–3991)
Hookworm frequency, %	3.0	0
<i>S. stercoralis</i> frequency, %	0	0
Treatment frequency, % ^c		
No treatment	100	0
1–3 treatments	0	1.9
4–6 treatments	0	8.4
7 treatments	0	89.7

BMI, body-mass index; epg, eggs per gram of stool; GM, geometric mean; *t*, time when observed (*t* = 0 is the value at baseline or the pretreatment value; *t* = 12 is the value at 12 months of follow-up).

^a Includes allergen skin tests positive for *Alternaria tenuis*, cat, grass pollen mix, and fungi mix.

^b Infection intensity shown excludes noninfected children.

^c Directly observed treatments with single doses of 400 mg of albendazole.

americana) or to other heterologous immunological stimuli (tuberculin and lipopolysaccharide), the treated children were different from the untreated children. The data provide evidence that long-term anthelmintic treatment has important effects on antiparasite immunity, but not on aeroallergen-associated immunity, in peripheral blood.

Two strengths of the present study are that selection into the study was blinded to treatment status and that the treated group and the untreated group were similar with respect to important baseline factors. A relatively large sample of children was investigated, optimizing the study's ability to detect important differences. Children were selected from a large number of schools,

Table 2. Cytokine levels and histamine release in 107 children treated with albendazole and in 107 untreated children, at the end of the 12-month follow-up period.

Immunologic variable	Untreated			Treated		
	Subjects, no.	Median (IQR)	R, %	Subjects, no.	Median (IQR)	R, %
Cytokine						
Interleukin-10						
Medium	102	0 (0–0)	20	95	0 (0–0)	13
SEB	102	131 (86–217)	92	95	116 (55–212)	97
<i>Ascaris</i>	102	0 (0–8)*	27*	95	0 (0–0)	9
Tuberculin (purified protein derivative)	102	0 (0–18)*	34	95	0 (0–0)	20
Lipopolysaccharide	102	1503 (1016–2035)	100	95	1284 (817–1949)	100
Cockroach	102	14(0–50)	64	95	22(0–122)	61
<i>Dermatophagoides pteronyssinus</i>	102	0 (0–221)	50	95	0 (0–278)	43
Interferon-γ						
Medium	107	0 (0–0)	15	107	0 (0–0)	11
SEB	107	9961 (9160–9961)	100	107	9961 (9257–9961)	100
<i>Ascaris</i>	107	0 (0–2)	28	107	0 (0–28)	32
Tuberculin (purified protein derivative)	107	72 (4–389)	77	107	107 (3–521)	79
Lipopolysaccharide	107	68 (0–407)	74	107	71 (0–402)	69
Cockroach	107	0 (0–8)	29	107	0 (0–0)	20
<i>Dermatophagoides pteronyssinus</i>	107	0 (0–90)	59	107	35 (0–172)	61
Interleukin-5						
Medium	107	0 (0–0)	18**	92	0 (0–0)	2
SEB	107	1374 (850–1902)**	100	92	1992 (1274–1992)	100
<i>Ascaris</i>	107	11 (0–69)*	60	92	43 (0–173)	74
Tuberculin (purified protein derivative)	107	4 (0–33)	56	92	3 (0–38)	55
Lipopolysaccharide	107	0 (0–0)	15	92	0 (0–0)	5
Cockroach	107	0 (0–0)	16	92	0 (0–0)	12
<i>Dermatophagoides pteronyssinus</i>	107	0 (0–0)	8	92	0 (0–0)	5
Interleukin-13						
Medium	104	0 (0–0)	22	106	0 (0–8)	30
SEB	104	4274 (2929–6991)*	100	106	5919 (3651–7440)	100
<i>Ascaris</i>	104	18 (0–141)*	65	106	84 (0–1279)	75
Tuberculin (purified protein derivative)	104	10 (0–124)	59	106	10 (0–92)	58
Lipopolysaccharide	104	0 (0–2)	28	106	0 (0–3)	28
Cockroach	104	0 (0–1)	26	106	0 (0–2)	30
<i>Dermatophagoides pteronyssinus</i>	104	0 (0–0)	23	106	0 (0–4)	35
Histamine release						
<i>Ascaris</i> antigen						
<i>Ascaris</i> adult	32	39 (3–69)	69	19	47 (9–94)	74
Larval stage L2/L3	32	28 (1–100)	94	19	75 (42–108)	84
Larval stage L3/L4	32	95 (62–100)	91	19	100 (71–100)	89
Aeroallergen						
<i>Dermatophagoides pteronyssinus</i>	32	1(0–3)	15	19	0 (0–2)	6
Der p1	32	0 (0–2)	7	19	0 (0–6)	11
Der p2	32	0 (0–3)	3	19	0 (0–1)	0
<i>Periplaneta americana</i>	32	3 (1–10)	27	19	2 (2–8)	17

NOTE. IQR, interquartile range; R, proportion of subjects with either detectable levels of cytokine or >10% histamine release; SEB, *Staphylococcus enterotoxin B*.

* $P \leq .01$ (from bivariate analysis).

** $P \leq .001$ (from bivariate analysis).

thereby minimizing any systematic biases that could occur if only a few schools were considered. The data collected were from a population in which ascariasis and trichuriasis have high prevalence (defined as $\geq 50\%$ [12]) and light intensity (defined as $>90\%$ of subjects with stool egg counts of $<50,000$ eggs per gram [epg] for *A. lumbricoides* and $<10,000$ epg for *Trichuris trichiura* [12]); the immunomodulating effects in populations with high intensities of infection may be greater, or the effects may be different for other geohelminth parasites, such as hookworm. Because of widespread access to anthelmintic drugs and mass-treatment programs, few populations in Latin America have high-prevalence heavy-intensity ascariasis and trichuriasis infections, and the findings of the present study may be generalizable to most endemic areas where these parasites predominate. After 12 months, repeated treatments with albendazole had not cured all geohelminth infections in the treated group, and the prevalence of geohelminth infections had declined slightly in the untreated group—suboptimal treatments in the treated group and possible treatment contamination in the untreated group may have attenuated the immunological differences between the 2 groups. Blood samples were analyzed 7 days after the treated children had received the final dose of albendazole; however, it is unlikely that recent treatment would have affected cytokine responses, because most treated children were infection free at this time, and we have shown elsewhere that antiparasite cytokine responses do not change for as long as 35 days after treatment [6].

Enhanced Th2 responses to *A. lumbricoides* antigen were not associated with changes in Th2 immune responses to the aeroallergens *D. pteronyssinus* and *P. americana*, which are the important allergens associated with allergen skin-test reactivity in this population [10, 11]. Previous intervention studies have provided evidence of increased allergen skin-test reactivity after repeated anthelmintic treatments [8, 9]. A mechanism by which this could occur is the reversal of geohelminth-mediated suppression of allergic reactivity, accompanied by increased production of aeroallergen-induced Th2 cytokines and associated allergic inflammation. It has been suggested that such suppression occurs via the enhanced production of parasite antigen-induced IL-10 [13]. In the present study, we did not observe anthelmintic treatment as having an effect on Th2 cytokine production or IgE-mediated inflammation in response to aeroallergens. Overall, the data provided by the present study give biological support to the clinical observation in the randomized-intervention study in which the current study was nested—that is, repeated anthelmintic treatment does not affect the prevalence of allergen skin-test reactivity or clinical allergy [10].

Suppression of Th2 cytokine responses in infected subjects may be an important survival mechanism for geohelminths; several studies have provided evidence that Th2 cytokines mediate protective immunity against geohelminths in humans [3, 14, 15]. The data provided by the present study indicate that con-

tinuous exposure to geohelminths (i.e., as in the untreated group) may suppress parasite-specific Th2 cytokine production—and that repeated treatments with albendazole for 1 year may reverse this effect. Interestingly, IgE-mediated inflammatory responses (measured by histamine release) to parasite antigens were not affected by long-term treatment. Previous studies examining the short-term effects that 2 or more doses of anthelmintic drugs have on the immune response to ascariasis [6] and hookworm [7] showed that such treatment had negligible effects on cytokine production by parasite antigen-stimulated PBLs. There are 3 possible explanations for the differences between the short- and long-term immunologic effects of anthelmintic drugs. First, a prolonged infection-free period may be necessary to reverse the suppression of Th2 cytokines. Second, geohelminth larvae may be the primary target of host immunity and immune regulation [6, 15]. In the present study, all family members of children in the treated group were treated also with albendazole every 2 months. If suppression of Th2 responses were linked to larval infections with *A. lumbricoides*, then the reversal of suppression could be delayed if the infection reservoir in families was gradually eliminated. Finally, albendazole per se may stimulate Th2 cytokine responses in the long term, although there is no evidence to support this.

In conclusion, the data provided by the present study indicate that, in schoolchildren living in communities where there is a high prevalence of ascariasis and trichuriasis, repeated treatments with albendazole enhance Th2 cytokine production by PBLs stimulated with *A. lumbricoides* antigen. The data do not support the idea that geohelminths play a role in mediating important systemic effects on immunity to aeroallergens or other heterologous antigens—or at least on those that may be altered by anthelmintic treatment. These findings do not preclude, however, the existence of either subtle systemic immunological effects or effects localized to particular tissues such as the lungs or intestine.

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