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Chronic Intestinal Helminth Infections Are Associated with Immune Hyporesponsiveness and Induction of a Regulatory Network▽†‡

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Helminth infections have been associated with protection against allergy and autoimmune diseases. We investigated the effects of chronic infections with *Ascaris lumbricoides* and *Trichuris trichiura* (measured twice over a 5-year period) on cytokine and antibody responses. We collected blood from 1,060 children aged 4 to 11 years living in a poor urban area of Brazil and measured Th1 (gamma interferon [IFN-γ]) and Th2 (interleukin-5 [IL-5] and IL-13) cytokines and the regulatory cytokine IL-10 in unstimulated and stimulated (with mitogen or *A. lumbricoides* antigens) cultures of peripheral blood leukocytes and levels of total IgE and anti-*A. lumbricoides* IgG4 and IgE in serum. Intestinal helminth infections were associated with an increased proportion of children producing IL-5 in response to *A. lumbricoides* and producing IL-10 spontaneously, especially among coinfected and chronically infected children. Helminth infections were associated with a generalized suppression of cytokine responses to mitogen. Levels of total IgE and anti-*A. lumbricoides* IgG4 and IgE were especially elevated in chronically infected children. In conclusion, intestinal helminth infections were associated with a typical Th2 immune response profile and with the induction of immune hyporesponsiveness that was associated with greater frequencies of the production of spontaneous IL-10.

Among infectious agents, helminth parasites are regarded as master manipulators of the host immune response, being associated with chronic but generally asymptomatic infections. Although helminth infections induce strong Th2 responses, parasitic worms may survive in their mammalian hosts by switching off inflammatory immune responses and inducing a tolerant response to parasite antigens (38).

Atopy, characterized by raised immunoglobulin E (IgE) levels, is considered a major mediator of allergic diseases such as asthma, rhinoconjunctivitis, and eczema. The interaction of an environmental allergen with the innate immune system, uptake by antigen-presenting cells, and subsequent T-cell priming lead to the stimulation of Th2 cytokines, such as interleukin-4 (IL-4), IL-5, and IL-13. These cytokines stimulate IgE production and increased numbers of eosinophils and mast cells, which together may cause allergic inflammation in the respiratory tract (37).

The hygiene hypothesis has tried to explain the temporal trends of increased allergic disease prevalence over recent decades in industrialized countries by alterations in the host response to environmental allergens caused by decreased exposure to childhood infections through improvements in hygiene and greater access to antibiotics and vaccines (32). Such improved hygiene has been considered to alter the balance between type 1 (Th1) and type 2 (Th2) immune responses due to a failure of immune regulation resulting in allergy-mediating Th2 responses (22).

Exposure to pathogens and their products, and to helminths in particular, has been shown to protect against the development of autoimmune and allergic diseases in experimental animal models (7, 8, 10, 23, 25, 26, 28), and some evidence in support of this has been observed in human populations (3, 8, 10, 23, 29, 30, 31).

We previously demonstrated that children living in circumstances of poor hygiene without access to sanitation or clean water during the first 3 years of life have elevated spontaneous production of IL-10 up to 8 years later in life (13).

In the present study, we compared cytokine profiles from whole-blood cultures and antibody responses among children stratified by intestinal helminth infection status, determined at two separate time points in childhood.

MATERIALS AND METHODS

Study population and data collection. This study was conducted in the city of Salvador in northeastern Brazil, which has a population of 2.5 million. The prevalence of wheezing in this city in the past 12 months in school children aged 12 to 13 years was reported to be very high (27.1%) (29). The design of this study has been reported elsewhere (6, 13, 29). In short, the study population included 1,445 children recruited in infancy for a prospective study measuring the impact of a citywide sanitation program on childhood morbidity (5). Data were collected from children born between 1994 and 2001 who lived in sentinel neighborhoods in the city. Standardized questionnaires were administered to the children's guardians between 1997 and 2003 (baseline) to collect data on demographic and social variables as well as on the home environment. In 2000, stool samples were collected to detect intestinal helminth infection (33). The children were surveyed

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again in 2005 to collect data on the same variables and to obtain stool and blood samples. Of the 1,445 children included in the study, we assayed 1,006 for gamma interferon (IFN-γ), 1,356 for IL-10, 1,289 for IL-13, and 1,243 for IL-5. For the present analysis, we used data on 1,060 children who had information for at least one of the cytokines and for whom data were also available on levels of total IgE and anti-*Ascaris* IgE and IgG4 and on the covariates of interest obtained by questionnaire. Ethical approval for the study was obtained from the Brazilian National Ethical Committee, and written informed consent was obtained from the legal guardian of each child.

Parasitological analysis. Paired stool samples were collected from each child and analyzed for parasites at each of the two sampling times. Stools were analyzed using the gravitational sedimentation technique of Hoffman et al. to detect helminth eggs, protozoan cysts, and oocysts (17). Two slides were examined for each stool sample. Quantification of helminth eggs was performed using the Kato-Katz technique (18). All children with positive results were treated with appropriate antiparasitic drugs (6).

***Ascaris lumbricoides* antigens.** The *A. lumbricoides* extract was obtained by trituration of liquid nitrogen-frozen adult worms in phosphate-buffered saline (PBS), pH 7.4, by use of a blender (model 51BL30; Waring Commercial, Torrington, CT). The PBS-soluble fraction obtained by centrifugation was depleted of endotoxin by treatment with Triton X-114 (Sigma, St. Louis, MO), and protein content was determined by the Lowry method (20). Antigen was stored at −70°C until use.

Blood collection and whole-blood culture. We collected venous blood into heparinized tubes and cultured the blood at a dilution of 1:4 in RPMI (Gibco, Auckland, New Zealand) containing 10 mM glutamine (Sigma-Aldrich, St. Louis, MO) and 100 μg/ml gentamicin (Sigma-Aldrich, St. Louis, MO). The cells were cultured within 6 h of collection and were maintained in a humidified environment of 5% CO_2 at 37°C for 24 h for detection of IL-10 and for 5 days for the detection of IL-13, IL-5, and IFN-γ in the presence of *Ascaris lumbricoides* antigen (10 μg/ml), pokeweed mitogen (PWM; Sigma-Aldrich, St. Louis, MO) (2.5 μg/ml), or medium alone.

Cytokine production. We measured the production of IL-5, IL-13, IFN-γ, and IL-10 in whole-blood culture supernatants, using commercially available antibody pairs and recombinant cytokine standards (BD Pharmingen, San Diego, CA), by sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Cytokine concentrations were determined by interpolation of standard curves. The detection limits (low/high) for each cytokine were as follows: for IL-5, 15.63/500 pg/ml; for IL-13, 62.5/4,000 pg/ml; for IFN-γ, 18.5/300 pg/ml; and for IL-10, 31.25/500 pg/ml. Responders were defined as those children with cytokine concentrations above the lower detection limits.

IgE and IgG4 production. Determination of specific IgE serum concentrations was done for *A. lumbricoides* by use of the Immunocap assay (Phadia Diagnostics AB, Uppsala, Sweden). Children with an anti-*Ascaris* IgE level of ≥0.35 kilounits/liter were considered positive.

Anti-*A. lumbricoides* IgG4 was detected by indirect ELISA as follows. High-binding-level microassay plates (Costar, Cambridge, MA) were sensitized with 20 μg/ml of *A. lumbricoides* antigen diluted in carbonate-bicarbonate buffer, pH 9.6. Sera were diluted 1:50 in PBS containing 10% fetal calf serum (FCS) (Sigma Chemical Co., St. Louis, MO) and 0.1% Tween 20 (PBST). Plates were incubated with biotinylated anti-human IgG4 (Sigma Chemical Co., St. Louis, MO), followed by streptavidin-peroxidase (Pharmingen, San Jose, CA) and H_2O_2-OPD substrate (Merck & Co., Inc., White House Station, NJ), and were read using a 480-nm filter.

Total IgE was measured using high-binding-level microassay plates (Costar, Cambridge, MA) coated with 4 μg/ml of an anti-human IgE antibody (Pharmingen, San Diego, CA) overnight at 4°C. Plates were blocked with 0.15 M PBS, pH 7.2, containing 10% FCS and 0.05% Tween 20 (Sigma, St. Louis, MO) overnight at 4°C. Samples were diluted 1:10 in PBS containing 5% FCS and 0.05% Tween 20 and incubated overnight at 4°C. Plates were incubated with biotinylated anti-human IgE (Sigma Chemical Co., St. Louis, MO), followed by streptavidin-peroxidase (Pharmingen, San Jose, CA) and H_2O_2-OPD substrate (Merck & Co., Inc., White House Station, NJ), and were read using a 480-nm filter. A pool of parasite-infected patient sera was used as a positive control. Umbilical cord serum from a newborn of a nonatopic and nonparasitized mother was used as a negative control.

The assay cutoff for total IgE (0.2 μg/ml) was determined as the median plus the semi-interquartile deviation for negative controls (54 sera from children with 3 negative stool samples collected serially, specific IgE levels of <0.35, and <2% eosinophils in peripheral blood). The assay cutoff for IgG4 (optical density [OD] of 0.4) for *A. lumbricoides* was determined as the mean plus 3 standard deviations for negative controls (sera from children with 3 negative stool samples collected serially). The cutoff for *Ascaris*-specific IgE was 0.35 kilounits/liter, as recommended by the manufacturer. Antibody levels of anti-*Ascaris* IgG4 and total IgE were defined as positive or negative by using the above cutoffs.

Statistical analyses. The occurrence and chronicity of infections with *A. lumbricoides* and *T. trichiura* were defined as follows: (i) past infection, infection with either parasite detected in early life (i.e., survey conducted in 2000); (ii) current infection, infection with either parasite detected later in childhood (i.e., survey conducted in 2005); (iii) chronic infection, infection with either *A. lumbricoides* or *T. trichiura* in both 2000 and 2005; and (iv) coinfection, infection with both helminths in 2005. Associations between cytokine responsiveness (responders versus nonresponders) and intestinal helminth infection status for the different stimuli (spontaneous [medium control], mitogen, or *Ascaris*) were assessed using the Pearson chi-square test or trend test, as appropriate. We also compared cytokine concentrations by helminth infection status. The proportion of individuals with cytokine concentrations greater than or equal to a certain value was estimated and is presented graphically (Fig. 1A to D). Measurements above the detection limits of the assays were censored at the threshold value, and standard statistical methods for analyses of censored (or nondetectable) data were used, including the Kaplan-Meier estimator and log rank test (11, 15, 16, 18). The Kaplan-Meier method is the standard nonparametric method for computing summary statistics for censored data (34). A proportion was estimated for each observed concentration, and the results are summarized in plots in Fig. 1A to D. Comparisons of cytokine concentrations according to infection groups were performed using the log rank test. The rejection of the null hypothesis for the log rank test means that not all groups have the same distribution. In addition, geometric means and bootstrap standard errors were computed for cytokine concentrations from PWM stimulations for each infection group. These results are presented graphically in Fig. 1E and F. We estimated odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for production of anti-*Ascaris* IgE, total IgE, and anti-*Ascaris* IgG4 by helminth infection status, using logistic regression (1). All models were adjusted for age and sex. Estimated ORs for *A. lumbricoides* infection were adjusted for *T. trichiura* infection and vice versa. Statistical analyses were performed using SPSS v15, R v2.9, and STATA v.9 software.

RESULTS

Study population. The study population had an age range of 4 to 11 years, 41.1% of the population was 6 to 7 years of age, and the majority of participants were boys (54.3%). *Ascaris lumbricoides* and *Trichuris trichiura* were the most common helminth parasites in our population. The percentages of children with *A. lumbricoides* and *T. trichiura* infections in 2005 were 16.1% and 13.8%, respectively. In addition, 7% were chronically infected with *A. lumbricoides* and 6.3% were chronically infected with *T. trichiura* (infected in both surveys). A total of 7.0% of the children were coinfected with *A. lumbricoides* and *T. trichiura* in 2005.

Effects of *A. lumbricoides* and *T. trichiura* infections on whole-blood cytokine production. To measure the impact of helminth infections on cytokine production in whole-blood cultures, we measured Th1 (IFN-γ), Th2 (IL-5 and IL-13), and Treg (IL-10) cytokines. The percentage of children producing IL-10 spontaneously in whole-blood cultures was higher among children with current *A. lumbricoides* (16%; $P < 0.001$) and *T. trichiura* (15.1%; $P = 0.001$) infections in the 2005 survey, as well as those with coinfections (21.6%; $P < 0.001$), than among noninfected children in the respective infection groups (Table 1). Furthermore, the proportion of children producing IL-10 was higher among children with chronic *A. lumbricoides* (23.3%; $P < 0.001$) and *T. trichiura* (22.4%; $P < 0.001$) infections than among nonchronically infected children (i.e., those classified into the "never," "past," and "present" groups). Children with current *T. trichiura* infections were associated more frequently with production of IL-13 than uninfected children were ($P = 0.01$).

Current or chronic infections with *A. lumbricoides* ($P \leq 0.01$)

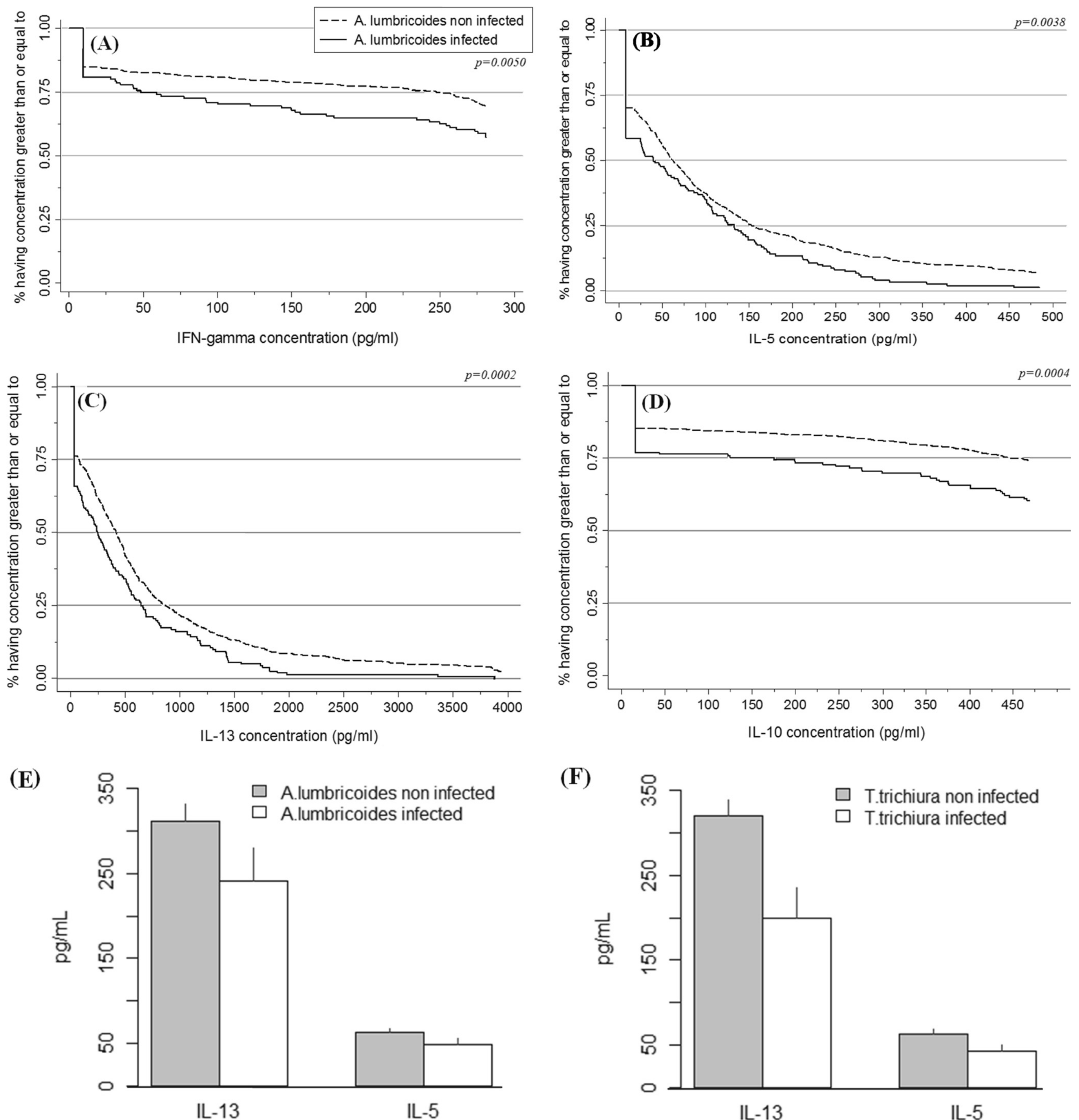

FIG. 1. Comparison of cytokine concentrations from PWM-stimulated whole-blood cultures by presence (continuous line) or absence (dotted line) of current infection with *Ascaris lumbricoides*. Cytokine levels were compared using nonparametric Kaplan-Meier estimator and log rank tests for IFN-γ (A), IL-5 (B), IL-10 (C), and IL-13 (D), and cytokine concentrations are presented according to the presence or absence of *Ascaris* (E) and *Trichuris* (F) infections.

and *T. trichiura* ($P < 0.05$) were associated with larger proportions of children producing IL-5 (Table 2) upon *A. lumbricoides* stimulation. A similar pattern was observed for coinfected children (27.3%; $P < 0.001$). *T. trichiura*-infected children were also associated with IL-13 responsiveness ($P < 0.05$). However, elevated frequencies of IL-10 responder children were observed only among those with chronic *T. trichiura* infections, not among the groups of children without chronic *T. trichiura* infections ($P = 0.02$).

Following stimulation with PWM, we observed a generalized downregulation of the majority of the cytokines among children with intestinal helminth infections (Table 3). For all in-

TABLE 1. Effect of helminth infection on spontaneous cytokine production in whole-blood cultures

Type of infection	No. of individuals (% with spontaneous cytokine production in whole-blood cultures)[a]			
	IFN-γ	IL-5	IL-10	IL-13
Overall	774 (11.0)	955 (6.1)	1,045 (8.2)	990 (34.3)
Current *Ascaris* infection				
Negative	643 (10.7)	806 (6.2)	**876 (6.7)**	829 (35.3)
Positive	131 (12.2)	149 (5.4)	**169 (16.0)***	161 (29.2)
Chronic *Ascaris* infection				
Never	539 (10.9)	675 (6.8)	**728 (6.3)**	687 (35.5)
Past	104 (9.6)	131 (3.1)	**148 (8.8)**	142 (34.5)
Present	74 (12.2)	87 (4.6)	**96 (10.4)**	91 (23.1)
Both	57 (12.3)	62 (6.5)	**73 (23.3)+**	70 (37.1)
Current *Trichuris* infection				
Negative	665 (10.5)	824 (5.9)	**899 (7.1)**	**853 (32.7)**
Positive	109 (13.8)	131 (6.9)	**146 (15.1)***	**137 (44.5)***
Chronic *Trichuris* infection				
Never	539 (10.6)	734 (6.3)	**809 (7.2)**	763 (32.9)
Past	104 (10.1)	90 (3.3)	**90 (6.7)**	90 (31.1)
Present	74 (11.3)	73 (5.5)	**79 (8.9)**	72 (54.2)
Both	57 (16.1)	58 (8.6)	**67 (22.4)+**	65 (33.8)
Coinfection with *Ascaris* and *Trichuris*				
Negative for both	593 (10.8)	741 (6.3)	**804 (6.6)**	762 (34.0)
Positive for only one	122 (9.0)	148 (3.4)	**167 (10.2)**	158 (34.2)
Positive for both	59 (16.9)	66 (9.1)	**74 (21.6)+**	70 (38.6)

[a] Differences between groups were assessed using the chi-square test or trend test, as appropriate. Numbers in bold show statistically significant results. *, $P \leq 0.05$ (χ^2); +, $P \leq 0.05$ (χ^2 trend test).

fection groupings, but especially chronic *T. trichiura* infections, we observed significant reductions in the proportions of cytokine responders among infected compared to noninfected children. The proportions of children with detectable IFN-γ, IL-5, IL-10, and IL-13 were lower among children with current *A. lumbricoides* infections than among noninfected children ($P < 0.01$; log rank test) (Fig. 1A to D). This is illustrated in Fig. 1 by the differences between the individual Kaplan-Meier graphs representing the proportions of children with each concentration of cytokine among those with and without *A. lumbricoides* infection. The proportions of children expressing each concentration of cytokine were lower for infected than noninfected children for all 4 cytokines measured. For example, 75% of the children infected by *A. lumbricoides* had IL-10 concentrations of ≥125 pg/ml, while 75% of the children not infected with *A. lumbricoides* had IL-10 concentrations of ≥425 pg/ml (Fig. 1C). We also observed that approximately 38% of noninfected children and 31% of infected children had IL-13 concentrations of ≥500 pg/ml (Fig. 1D). Similar effects were observed for children with chronic *A. lumbricoides* or *T. trichiura* infections and coinfections compared to the appropriate noninfected groups ($P < 0.05$ for all comparisons [see the supplemental material]). An alternative way of presenting the data, using geometric means and bootstrap-estimated standard errors, is shown in Fig. 1E (*A. lumbricoides* infection status) and Fig. 1F (*T. trichiura* infection status). Geometric mean cytokine concentrations for IL-5 and IL-13 were lower in infected than noninfected children for both *A. lumbricoides* and *T. trichiura* infections.

Effects of *Ascaris lumbricoides* and *Trichuris trichiura* infections on total IgE and *Ascaris*-specific IgE and IgG4. Among the mechanisms postulated to explain helminth-mediated modulation of allergy, the production of polyclonal IgE at levels that can saturate mast cells and of IgG4 blocking antibodies is commonly mentioned (10, 37). To address these mechanisms, we estimated the effects of *A. lumbricoides* and *T. trichiura* infections on the presence of total IgE and *Ascaris*-specific IgE and IgG4 (Fig. 2A to D). All infection groups (current and chronic infections and coinfections) were associated with increased proportions of children with these antibodies. Children with current *A. lumbricoides* infections had a greater chance of having IgE for *A. lumbricoides* (OR, 2.16; CI, 1.48 to 3.16), total IgE (OR, 1.36; CI, 0.93 to 1.99), or IgG4 for *A. lumbricoides* (OR, 2.63; CI, 1.75 to 3.95) than those without current *A. lumbricoides* infection (Fig. 2A). The same pattern of response was observed for coinfections (Fig. 2B) and chronic infections (Fig. 2C and D). The presence of coinfection increased the chance of producing antibodies compared to that for the group without current *A. lumbricoides* and *T. trichiura* infections (for anti-*Ascaris* IgE, OR = 3.78 and CI = 2.15 to 6.64; for anti-*Ascaris* IgG4, OR = 7.08 and CI = 4.28 to 11.71; and for total IgE, OR = 1.93 and CI = 1.12 to 3.31). Chronic infections were strongly associated with the presence of these antibodies. For example, the chance of producing anti-*Ascaris* IgE among children with *T. trichiura* infection in 2000 and 2005 was 3.20 times higher than that for individuals without *T. trichiura* infection in both periods (OR, 3.20; CI, 1.67 to 6.13) (Fig. 2C). In addition, the ratio of IgG4/total IgE was higher

TABLE 2. Effect of helminth infection on cytokine production in *Ascaris lumbricoides*-stimulated whole-blood cultures

Type of infection	No. of individuals (% with cytokine production in *A. lumbricoides*-stimulated whole-blood cultures)[a]			
	IFN-γ	IL-5	IL-10	IL-13
Overall	774 (2.5)	955 (12.1)	1,045 (4.1)	990 (20.5)
Current *Ascaris* infection				
Negative	643 (2.2)	**806 (10.7)**	876 (3.8)	829 (20.3)
Positive	131 (3.8)	**149 (20.1)***	169 (5.9)	161 (21.7)
Chronic *Ascaris* infection				
Never	539 (2.4)	**675 (11.0)**	728 (3.3)	687 (20.2)
Past	104 (1.0)	**131 (9.2)**	148 (6.1)	142 (20.4)
Present	74 (2.7)	**87 (16.1)**	96 (6.3)	91 (16.5)
Both	57 (5.3)	**62 (25.8)+**	73 (5.5)	70 (28.6)
Current *Trichuris* infection				
Negative	665 (2.3)	**824 (10.4)**	899 (3.7)	**853 (19.3)**
Positive	109 (3.7)	**131 (22.9)***	146 (6.8)	**137 (27.7)***
Chronic *Trichuris* infection				
Never	539 (2.3)	**734 (10.5)**	**809 (3.3)**	**763 (19.4)**
Past	104 (1.4)	**90 (10.0)**	**90 (6.7)**	**90 (18.9)**
Present	74 (3.8)	**73 (21.9)**	**79 (5.1)**	**72 (29.2)**
Both	57 (3.6)	**58 (24.1)+**	**67 (9.0)+**	**65 (26.2)+**
Coinfection with *Ascaris* and *Trichuris*				
Negative for both	593 (2.0)	**741 (10.0)**	804 (3.4)	762 (19.9)
Positive for only one	122 (4.1)	**148 (16.2)**	167 (7.2)	158 (18.4)
Positive for both	59 (3.4)	**66 (27.3)+**	74 (5.4)	70 (31.4)

[a] Differences between groups were assessed using the chi-square test or trend test, as appropriate. Numbers in bold show statistically significant results. *, $P \leq 0.05$ (χ^2); +, $P \leq 0.05$ (χ^2 trend test).

for children with chronic helminth infections or coinfections with *T. trichiura* and *A. lumbricoides* than for children with current infections. Ratios were lowest for noninfected children (Fig. 2E).

DISCUSSION

There is some evidence from experimental animal models and epidemiological studies of human populations indicating that the development of autoimmune and allergic pathologies could be modified by helminth infections (3, 7, 8, 10, 23, 28, 29, 31). Several mechanisms have been proposed to explain the anti-inflammatory effects of helminth infections, including the production of IL-10 and transforming growth factor beta (TGF-β) by regulatory T cells, production of IgG4 isotype blocking antibodies, and suppression of mast cells, basophils, and eosinophils (2, 10). Helminth infections induce strong Th2 responses, as described previously (10, 27). The findings of the present study support previous data and show the presence of Th2 immune polarization during intestinal helminth infection, in which the presence of anti-*Ascaris* IgE and the production of IL-5 upon stimulation of whole blood with parasite antigen were particularly prominent among children infected with both *A. lumbricoides* and *T. trichiura* (Table 1). Furthermore, we observed an upregulation of IL-10 by whole-blood cultures stimulated with *A. lumbricoides* antigens, especially during *T. trichiura* infections and coinfections. Survival of adult worms in the human host is thought to be facilitated by the induction of an immune regulatory network (23, 35).

We previously demonstrated that children whose household had no sewage system or potable tap water from early life were likely to produce more spontaneous IL-10 than those children living in households with access to a sewage system and clean drinking water, suggesting a possible homeostatic effect induced by these exposures (13) or factors associated with them. In this study, our data suggest that intestinal helminths may contribute to this immune regulation, as a large proportion of children with current infections with either or both *A. lumbricoides* and *T. trichiura* or with chronic infections showed evidence of spontaneous IL-10 production by unstimulated whole blood. Recently, Turner et al. (35) described that African children under conditions of hyperendemic exposure to the intestinal nematodes *A. lumbricoides* and *T. trichiura* constitutively secrete more immunoregulatory cytokines (IL-10 and TGF-β1) than do children living under conditions of mesoendemic exposure. The activation of this regulatory network induced by helminths has also been suggested to be responsible for preventing the elimination of the worms and for protection of the host from immunopathology that would otherwise result from excessive inflammation (21, 36). Looking at the effect of helminth infections on cytokine production induced by PWM, we did see a generalized suppression of cytokine production. This hyporesponsiveness occurred during current and chronic infections and during coinfections.

The hyporesponsiveness associated with helminths may extend to third-party antigens, such as vaccines, and has been suggested by the findings of several studies of humans (9, 25, 30, 35). Such suppression could also affect immunity to aeroallergens, as suggested by the observation of an inverse associ-

TABLE 3. Effect of helminth infection on cytokine production in PWM-stimulated whole-blood cultures

Type of infection	No. of individuals (% with cytokine production inPWM-stimulated whole-blood cultures)[a]			
	IFN-γ	IL-5	IL-10	IL-13
Overall	774 (84.2)	955 (68.3)	1,045 (83.9)	990 (74.4)
Current *Ascaris* infection				
Negative	643 (84.9)	**806 (70.1)**	**876 (85.3)**	**829 (76.1)**
Positive	131 (80.9)	**149 (58.4)***	**169 (76.9)***	**161 (65.8)***
Chronic *Ascaris* infection				
Never	539 (85.0)	**675 (71.4)**	**728 (86.7)**	**687 (77.1)**
Past	104 (84.6)	**131 (63.4)**	**148 (78.4)**	**142 (71.1)**
Present	74 (78.4)	**87 (60.9)**	**96 (80.2)**	**91 (67.0)**
Both	57 (84.2)	**62 (54.8)+**	**73 (72.6)+**	**70 (64.3)+**
Current *Trichuris* infection				
Negative	**665 (85.9)**	**824 (70.4)**	**899 (86.1)**	**853 (77.1)**
Positive	**109 (74.3)***	**131 (55.0)***	**146 (70.5)***	**137 (57.7)***
Chronic *Trichuris* infection				
Never	**539 (86.1)**	**734 (71.0)**	**809 (86.4)**	**763 (77.5)**
Past	**104 (84.1)**	**90 (65.6)**	**90 (83.3)**	**90 (74.4)**
Present	**74 (79.2)**	**73 (58.9)**	**79 (73.4)**	**72 (62.5)**
Both	**57 (69.6)+**	**58 (50.0)+**	**67 (67.2)+**	**65 (52.3)+**
Coinfection with *Ascaris* and *Trichuris*				
Negative for both	**593 (86.0)**	**741 (71.4)**	**804 (86.8)**	**762 (78.1)**
Positive for only one	**122 (79.5)**	**148 (58.8)**	**167 (74.9)**	**158 (62.7)**
Positive for both	**59 (76.3)+**	**66 (54.5)+**	**74 (73.0)+**	**70 (61.4)+**

[a] Differences between groups were assessed using the chi-square test or trend test, as appropriate. Numbers in bold show statistically significant results. *, $P \leq 0.05$ (χ^2); +, $P \leq 0.05$ (χ^2 trend test).

ation between the production of parasite antigen-induced IL-10 by peripheral blood lymphocytes and skin test reactivity to house dust mites among children from Gabon living in an area where *Schistosoma mansoni* infection is endemic (35).

The upregulation of IL-10 during helminth infection has been described previously (3). A study from Brazil comparing asthmatic subjects infected with *S. mansoni* to uninfected subjects (from a different population) showed that *Dermatophagoides pteronyssinus*-stimulated peripheral blood mononuclear cells from infected asthmatics produced fewer Th2 cytokines and more IL-10 than control cells did (3). However, other studies conducted in areas where *A. lumbricoides* was the predominant helminth did not provide evidence for either enhanced IL-10 responses to aeroallergens (27, 28) or an increase in the frequency of regulatory T-cell populations induced by aeroallergen stimulation of peripheral blood lymphocytes (8). Based on our findings, we hypothesize that exposure to helminths and their products produces a homeostatic effect (through spontaneous IL-10 production) by inducing the generalized suppression of cytokine production, mainly during chronic infections but also during coinfection, which seems to have an additive effect.

Nondetectable data were obtained for a large number of assays, particularly for unstimulated and *Ascaris* antigen-stimulated cultures. A high frequency of nondetectable data for unstimulated cultures is not surprising, and the low level of detection for *Ascaris*-stimulated cultures may be explained by the relatively low prevalence of intestinal helminth infections in this population—the 2005 survey showed the prevalences of *A. lumbricoides* and *T. trichiura* infections to be 16.1% and 13.8%, respectively. Nondetectable data occur not only in immunology but also in other areas, such as astronomy, pharmacology, environmental control, and ecology (16). In order to take into account such a data structure, several statistical methods have been discussed in the literature (12, 15, 19), from descriptive methods to regression models. The most common approach used is to replace nondetectable data by some fraction of the assay's detection limit. However, such a procedure may lead to spurious results when frequencies of nondetectable data are high (16). To avoid this possibility, in the present analysis we favored a statistical approach designed for the analysis of nondetectable data (15, 16).

Looking at the effect of helminth infections on IgE and IgG4 antibodies, we observed that both intestinal helminth infections, especially chronic infections, were more likely to be associated with the presence of total IgE and anti-*Ascaris* IgG4 and IgE and may reflect cross-reactivity between antigens of different helminth parasites. Anti-*Ascaris* IgG4 may contribute to the suppression of skin prick test (SPT) reactivity and allergic reactions, as proposed by others (10, 21, 27, 29, 37). Furthermore, there was some evidence that chronic infections and coinfections were more strongly associated with the presence of anti-*Ascaris* IgG4 and with greater ratios of anti-*Ascaris* IgG4 to IgE than current infections were. IL-10 is considered to promote the production of IgG4 over IgE (23), and a positive but nonsignificant association was observed between spontaneous IL-10 production and the presence of anti-*Ascaris* IgG4 (data not shown).

In summary, our data from a population-based prospective study among children living in an urban region of Brazil pro-

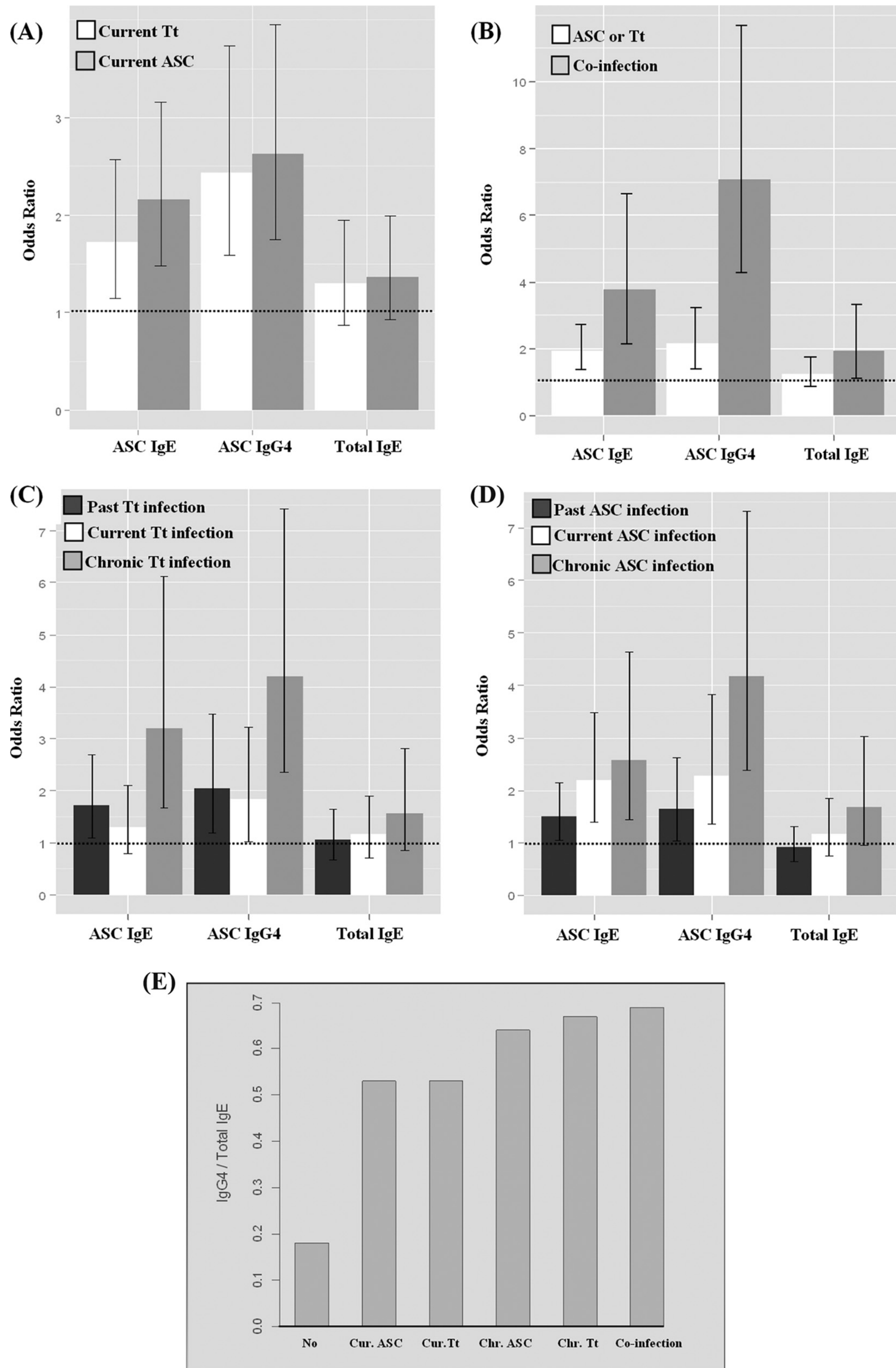

FIG. 2. Effects of helminth infection on the presence of antibodies, represented by ORs and 95% CIs. (A) Effects of current helminth infections on anti-*Ascaris* IgE and IgG4 and on total IgE. White bars represent *Trichuris* infection (Tt), and gray bars represent *Ascaris* infection (ASC). (B) Effects of helminth infections on anti-*Ascaris* IgE and IgG4 and on total IgE during single helminth infections (white bars) and coinfections (gray bars). (C and D) Effects of chronic *Trichuris* (C) and *Ascaris* (D) infections on anti-*Ascaris* IgE and IgG4 and on total IgE. Dark gray bars represent early-life infection, white bars represent current infections, and light gray bars represent infection in both periods analyzed. (E) Bars represent the ratio of IgG4/IgE as percentages among responders for IgG4 and total IgE. No, no infection; Cur. ASC, current *Ascaris* infection; Cur. Tt, current *Trichuris* infection; Chr. ASC, chronic *Ascaris* infection; Chr. Tt, chronic *Trichuris* infection; co-infection, current infection with both parasites.

vide evidence that chronic infections with intestinal helminths are associated with a generalized suppression of mitogen-induced cytokine production by peripheral blood leukocytes but with enhanced production of spontaneous IL-10. Such a regulated immune response phenotype induced by intestinal helminths may contribute to the development of an anti-inflammatory phenotype that may mediate the modulation of allergy that has been attributed to these infections.

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