Helminthic infections have been shown to inhibit allergy skin-prick tests and to modify the course of asthma. We evaluated Dermatophagoides pteronyssinus–specific immune responses in patients with asthma by measuring levels of T helper 2 (Th2) cytokines in peripheral blood mononuclear cell (PBMC) cultures. PBMCs from Schistosoma mansoni–infected patients with asthma living in an area of polyhelminthic endemicity produced lower levels of interleukin (IL)–5 and IL-4 in response to D. pteronyssinus antigen (Ag) 1 than did PBMCs from helminth-free patients with asthma. In contrast, D. pteronyssinus Ag 1–specific production of IL-10 was higher in helminth-infected patients than in helminth-free patients. The addition of recombinant human IL-10 to D. pteronyssinus Ag 1–stimulated cultures of PBMCs from helminth-free patients led to down-modulation of production of IL-5. After helminth-infected patients with asthma received antihelminthic treatment, there was down-modulation of D. pteronyssinus Ag 1–specific production of IL-10 in vitro. S. mansoni–infected patients with asthma produce lower levels of Th2 cytokines than do helminth-free patients with asthma, and this modulation is likely done by IL-10.

In spite of the high prevalence of helminthic infections in tropical regions, only a small percentage of individuals infected with these parasites actually develop disease. In Schistosoma mansoni infection, symptoms are usually mild, although 5% of infected individuals go on to develop the hepatosplenic form of disease. Because treatment is available to most infected individuals, the severe forms of schistosomiasis are becoming very rare, even though developing countries still have a high prevalence of infection [1–3].

Helminthic infections nearly always induce marked type 2 immune responses. The influence of parasitic infections on the development of atopy, which also induces a type 2 immune response, has become a topic of great interest. Studies performed by Lynch et al. have demonstrated that individuals living in an area where Ascaris lumbricoides is endemic have decreased reactivity to immediate-hypersensitivity skin-prick tests (SPTs) [4] and that treatment with antihelminthic drugs results in increased frequency of positive SPT results and production of aeroallergen-specific IgE [5]. In addition, our group has demonstrated that, among individuals who are chronically infected with S. mansoni, there is an inverse association between parasite load and positive SPT results for mite antigens [6]. There is a consensus that helminthic infections decrease skin reactivity to aeroallergens [4, 7–9], and studies in this field have shown lower prevalence and milder forms of asthma. The present study was designed to evaluate the T helper 2 (Th2) response to an aeroallergen in helminth-infected patients with asthma.
asthma [8, 10] in individuals living in areas where the parasite is endemic. However, the mechanisms by which helminthic infections interfere with the immediate-hypersensitivity reactions and allergy are unknown.

Hypotheses to explain the inhibition of the response to aeroallergens in individuals living in areas where helminths are endemic include the following: (1) competition between helminth-induced polyclonal IgE and aeroallergen-specific IgE for the high-affinity receptors present on mast cells; (2) the inhibition of aeroallergen-specific IgE synthesis by high levels of polyclonal IgE; and (3) high levels of regulatory cytokines produced during helminthic infections, such as interleukin (IL)–10, that suppress the immune response to unrelated antigens. In a previous study [6], we found no significant difference in total and Dermatophagoides pteronyssinus antigen (Ag) 1–specific production of IgE between S. mansoni–infected patients with low and those with high parasite loads, weakening the first 2 hypotheses. In the present study, we compared the cytokine profile produced by peripheral blood mononuclear cells (PBMCs) of patients with asthma in response to stimulation with D. pteronyssinus Ag 1 in vitro with that observed in S. mansoni–infected patients with asthma before and after antihelminthic treatment. In addition, the ability of IL-10 to down-modulate the type 2 immune response was evaluated.

PATIENTS AND METHODS

**Area of endemicity and selection of patients and control subjects.** Conde is a village located 150 km north of Salvador, the capital of Bahia, Brazil. It includes 5 communities, totaling ~1000 inhabitants. Because the sanitary conditions of this village are poor and because the principal occupation is fishing, the inhabitants are at great risk of helminthic infections; they had not been medically treated before the present study. Individuals living in 2 of 5 communities of Conde (n = 216) were screened for asthma and rhinitis through a directed questionnaire on the basis of the International Study of Asthma and Allergies in Childhood (ISAAC) [11]. In addition, questions concerning concomitant illnesses, socioeconomic status, and living conditions were added to the questionnaire. Individuals were selected if their responses to the ISAAC questionnaire corresponded with a personal history of asthma during the last 12 months, if they were currently infected with S. mansoni, and if they were 6–40 years old. Children <6 years old were excluded from this study because of the difficulty in performing the pulmonary function test, whereas subjects >40 years old were excluded because of their increased rates of chronic obstructive pulmonary disease. Subjects within the 6–40-year-old age group were excluded if they were currently smokers or using antihistamines, resulting in a total of 33 individuals included in the study. At enrollment, patients underwent clinical evaluations performed by 2 physicians, and were submitted to 3 stool sample examinations to identify intestinal parasites, SPT for aeroallergens, and in vitro analysis of the immune response. After the first evaluation, patients were treated for geohelminthic infections with albendazole (400 mg) and for S. mansoni infections with oxamniquine (25–30 mg/kg of body weight). Three months after antihelminthic treatment, patients with asthma were reevaluated. Patients with asthma who were 6–40 years old, who were being treated at the Allergy Service of the Professor Edgard Santos Hospital (Federal University of Bahia, Salvador, Bahia, Brazil), who were not infected with helminths, and who were of the same socioeconomic status as the patients from Conde were invited to be a control group for the present study; 10 individuals agreed to participate. Human-experimentation guidelines of the US Department of Health and Human Service were followed in the conduct of this study, and the Ethical Committee of Professor Edgard Santos Hospital approved the present study. Informed consent was obtained from all patients or their legal guardians.

**Fecal examinations for parasites.** Three stool samples from each individual were examined by use of the Hoffman sedimentation method, to identify helminths and enteric protozoa, and by use of the Kato-Katz method, to estimate parasitic load in patients with helminthic infection [12].

**SPTs and production of D. pteronyssinus Ag 1–specific IgE.** SPTs were performed by use of D. pteronyssinus, D. farinae, Blomia tropicalis, Periplaneta americana, and Blattella germanica glycerinated allergen extracts (International Pharmaceutical Immunology do Brasil LTDA [IPI-ASAC]). Histamine (1:1000) and glycerinated saline were used as positive and negative controls, respectively. Skin reactions were read 20 min after application and were considered to be positive if the mean diameter of the wheals was >3 mm. SPTs were considered to be positive if at least 1 of the 5 tested allergens induced a positive skin reaction. Levels of D. pteronyssinus Ag 1–specific IgE were determined by use of the enzyme-linked fluorescent assay in the CAP System (Pharmacia) for 33 patients with asthma living in the area of endemicity. The results were expressed in kilo-international units per liter.

**Cell culture and cytokine measurement.** PBMCs were obtained by use of the Ficoll-Hypaque gradient and adjusted to a concentration of 3 × 10⁶ cells/mL in RPMI 1640 medium containing 10% normal human serum (AB positive and heat inactivated), 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM/L l-glutamine, and 30 mM/L HEPES (all from Life Technologies GIBCO BRL). Cells were cultured in vitro with media and 25 µg/mL D. pteronyssinus Ag 1 (IPI-ASAC) in the presence or absence of recombinant human IL-10 (rhIL-10; concentrations, 10 and 50 ng/mL) or with the mitogen phytohemagglutinin (PHA; final dilution, 1:100). The cultures were incubated for 72 h at 37°C in 5% CO₂. After incubation, the supernatants were collected and maintained at −20°C for later
measurement of cytokines. Levels of interferon (IFN)–γ, IL-5, IL-10, and IL-13 in culture supernatants were determined by use of an ELISA sandwich technique, using commercially available kits (R&D Systems), and the results were expressed as picograms per milliliter, on the basis of a standard curve.

**Reverse transcription (RT) polymerase chain reaction (PCR) for IL-4.** RT-PCR was used to measure IL-4. In brief, after collection of supernatants at 72 h, cells were lysed in RNAzol, and RNA was prepared according to the manufacturer’s directions (Invitrogen). cDNA was generated from 1 μg of RNA by use of a first-strand cDNA synthesis kit (Invitrogen). IL-4–specific PCR was performed by use of cytokine-specific oligonucleotide primers (35 cycles of 1 min at 94°C, 2 min at 55°C, and 3 min at 72°C). PCR products were electrophoretically separated on 1.5% agarose gels (0.5% Tris-borate EDTA buffer) at 120 V. Ethidium bromide–stained gels were digitally recorded (UVP Labworks Laboratory Imaging and Analysis System; UVP).

**Statistical analyses.** Statistical analyses were performed by use of the software Statistical Package for Social Science (version 9.0 for Windows; SPSS). The frequency of positive SPT results and the presence of intestinal parasites were expressed as percentages. Fisher’s exact test was used to compare proportions. The difference in mean age was assessed by the Student’s test for independent samples. The Mann-Whitney U tests was used to compare the levels of cytokines between groups, and the Wilcoxon matched-pairs signed rank test was used to compare the levels of IL-5 in PBMC cultures, with or without rhIL-10, and the levels of IL-10 before and after antihelminthic treatment. Statistical significance was established at the 95% confidence interval.

**RESULTS**

**Prevalence of intestinal parasites and atopy.** We screened 216 people for asthma, rhinitis, and intestinal parasites in 2 communities of Conde. The prevalence of S. mansoni infection in these communities was 90.2%. The prevalences of other helminthic infections, including *A. lumbricoides* and *Trichuris trichiura*, were 79.7% and 77.2%, respectively. Infections with intestinal protozoa, such as *Giardia lamblia*, *Entamoeba coli*, and *Entamoeba histolytica*, were found in ~50% of the population. The degree of *A. lumbricoides* infection was quite variable in this population, and 30% of the individuals had >1000 eggs/g of stool, whereas 40% of studied individuals were heavily infected with *S. mansoni* (>400 eggs/g of stool).

Table 1 shows the characteristics of patients with asthma included in the study. There was no significant difference in age or sex between *S. mansoni*–infected and –uninfected patients. Positive SPT results for aeroallergens (IPI-ASAC) were observed in 12% of 33 patients with asthma living in the area of endemicity. Positive test results for *D. pteronyssinus* Ag 1 and *B. tropicalis* were observed in 6.2% and 9.3% of the patients, respectively. Positive SPT results for *B. germanica* were observed in only 1 patient, whereas no patients had positive SPT results for *P. americana*. Positive SPT results for *D. pteronyssinus* Ag 1 were observed in all helminth-free patients with asthma. *D. pteronyssinus* Ag 1–specific IgE antibodies above the reference values (0.035 kIU/L) were detected in 21 (65.6%) of 32 helminth–infected patients with asthma.

The prevalences of asthma and rhinitis in the area of endemicity, as assessed by the ISAAC questionnaire, were 14.4% and 28%, respectively. All patients with asthma were considered to have mild cases, according to results of the pulmonary function tests, results of a questionnaire performed to evaluate severity of asthma (which considers the number and severity of asthmatic episodes, use of antiasthmatic drugs and corticosteroids, and need of emergency care or hospitalization), and results of physical examinations performed by 2 physicians to check for abnormal findings, such as dyspnea and wheezing [10].

**Table 1. Characteristics of patients with asthma infected or not with *Schistosoma mansoni*.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Infected (n = 33)</th>
<th>Uninfected (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), years</td>
<td>10 (6–34)</td>
<td>15.5 (6–39)</td>
<td>.21</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>33.3</td>
<td>30</td>
<td>1.00</td>
</tr>
<tr>
<td>Skin test, % positive</td>
<td>12</td>
<td>100</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td><em>S. mansoni</em> burden, eggs/g of stool</td>
<td>100 ± 123.8</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td><em>A. lumbricoides</em> burden, eggs/g of stool</td>
<td>13,942 ± 22,130</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>Income, US$/year</td>
<td>&lt;1000</td>
<td>&lt;1000</td>
<td>...</td>
</tr>
<tr>
<td>FEV1, mean, L</td>
<td>91 ± 14.1</td>
<td>88.06 ± 18.91</td>
<td>...</td>
</tr>
<tr>
<td><em>D. pteronyssinus</em> Ag 1–specific IgE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive, no./total, no. (%)</td>
<td>21/32 (65.6)</td>
<td>ND</td>
<td>...</td>
</tr>
<tr>
<td>Mean ± SD, kIU/L</td>
<td>3.38 ± 6.58</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Median, kIU/L</td>
<td>0.58</td>
<td></td>
<td>...</td>
</tr>
</tbody>
</table>

**NOTE.** Ag, antigen; *A. lumbricoides*, *Ascaris lumbricoides*; *D. pteronyssinus*, *Dermatophagoides pteronyssinus*; FEV1, forced expiratory volume in 1 s; ND, not done.
Cytokine profile produced by PBMCs of patients and control subjects. Figure 1 shows production of Th2 cytokines by PBMCs stimulated in vitro with *D. pteronyssinus* Ag 1. Helminth-free patients with asthma who lived outside the area of endemity (n = 10) produced high levels of IL-5 (range, 46–2301 pg/mL; median, 341 pg/mL; figure 1A), compared with helminth-infected patients with asthma (range, 15.6–641 pg/mL; median, 15.6 pg/mL; P < .0001, Mann-Whitney U test). Of note, all but 1 of 33 *S. mansoni*–infected patients with asthma produced low levels of IL-5 (median, 15.6 pg/mL; range, 1.14 ± 0.20 pg/mL) compared with helminth-free patients with asthma (median, 341 pg/mL; range, 46–2301 pg/mL; P < .012, Mann-Whitney U test).

There was no significant difference between the levels of IL-13 in supernatants of *D. pteronyssinus* Ag 1–stimulated PBMCs from *S. mansoni*–infected patients with asthma (105.7 ± 42.5 pg/mL) and those from control patients with asthma (93.2 ± 39 pg/mL).

In contrast to IL-4 and IL-5, IL-10 was produced more readily by *D. pteronyssinus* Ag 1–stimulated PBMCs from the helminth-infected patients with asthma (range, 8.0–562 pg/mL; median, 98 pg/mL) and was not detected or was detected only at low levels (range, 8.0–97 pg/mL; median, 8.0 pg/mL) in the helminth-free patients with asthma (P = .0018; figure 2A). There was a positive association between *S. mansoni* egg count and *D. pteronyssinus* Ag 1–specific production of IL-10 (β = 0.3212; P = .012; figure 2B). IFN-γ was not detectable in the culture supernatants of *D. pteronyssinus* Ag 1–stimulated PBMCs from any of the study participants in either group of patients with asthma (data not shown). Levels of IL-5, IL-10, IL-13, and IFN-γ were below the detection limit in unstimulated cultures of PBMCs.
from patients with asthma, regardless of infection status, and, when cultures were stimulated with PHA, all cytokines were detected in high levels in the supernatants of PBMCs from both groups of patients with asthma (data not shown).

Role of IL-10 in modulating production of IL-5. Because low levels of IL-5 and high levels of allergen-induced IL-10 (an immunomodulator) were observed in helminth-infected patients with asthma, exogenous IL-10 was added to D. pteronyssinus Ag 1–stimulated PBMC cultures from helminth-free patients with asthma. The addition of rhIL-10 (at concentrations of 10 and 50 ng/mL) resulted in significant down-modulation of production of IL-5 in 6 of 7 subjects evaluated. The median level of IL-5 in cultures stimulated only with D. pteronyssinus Ag 1 was higher (range, 80–772 pg/mL; median, 497 pg/mL) than that in cultures stimulated with D. pteronyssinus Ag 1 in the presence of 50 ng/mL exogenous IL-10 (range, 32–351 pg/mL; median, 97 pg/mL; P < .05) (figure 3). The addition of anti–IL-10 antibody (10 and 50 ng/mL) to the D. pteronyssinus Ag 1–stimulated PBMC cultures from patients with asthma living in the area of endemicity did not change the level of production of IL-5 (data not shown).

As stated above, most helminth-infected patients with asthma had mild asthma, as determined by a questionnaire performed to evaluate the severity of asthma, by physical examinations performed by 2 physicians, and by the pulmonary function test (mean forced expiratory volume in 1 s, 91 ± 14.1 L). In a 1-year follow-up study, it was observed that, beginning 3 months after receipt of antihelminthic treatment, 9 (47%) of 19 patients evaluated before and after treatment had higher frequencies of asthma symptoms, abnormal findings (e.g., dyspnea and wheezing) by physical examination, and consumption of antiasthma drugs (e.g., β2 agonists and corticosteroids) after treatment than they did before treatment. In addition, it was observed that antihelminthic treatment resulted in down-modulation of D. pteronyssinus Ag 1–specific production of IL-10 (figure 4); the levels of IL-10 decreased from

82 pg/mL (range, 15.6–433 pg/mL) to 47 pg/mL (range, 15.6–120 pg/mL) after therapy (P < .05).

DISCUSSION

Studies demonstrating an inverse relationship between helminthic infection and a decreased SPT response to aeroallergens and decreasing prevalence and severity of asthma in helminth-infected patients [4, 6, 7, 9, 10] support the notion that helminthic infection down-modulates atopy. However, the mechanism involved in these phenomena has not been established. Our study has demonstrated that helminthic infections decrease the ability of patients with asthma to respond to a major allergen, D. pteronyssinus Ag 1, and that this down-modulation is mediated, at least in part, by IL-10. D. pteronyssinus Ag 1 was chosen as the antigen to test the immune response of helminth-infected patients with asthma to aeroallergens because it is the predominant mite species found in houses in Brazil [13–16] and because there is no significant difference between the population living in the areas of endemicity and from the areas of nonendemicity, with regard to the levels of exposure to this mite species [16].

In patients with asthma, as well as in helminth-infected individuals, the specific immune response is predominantly Th2. Here, we have shown that D. pteronyssinus Ag 1 induces high levels of IL-5 and high levels of IL-4 mRNA in patients with asthma but that the production of these cytokines is significantly decreased in helminth-infected patients with asthma. In contrast, production of IL-10 in response to stimulation with D. pteronyssinus Ag 1 was detected in cultures of PBMCs from patients with asthma living in the area of endemicity. Although IL-10 is found in high levels during chronic helminthic infections [17–19], low levels of this cytokine have been reported

![Figure 3.](image-url) Dermatophagoides pteronyssinus antigen 1 (Der p)–induced production of interleukin (IL)–5 by peripheral blood mononuclear cells (measured by ELISA) from patients with asthma, in the absence (range, 80–772 pg/mL; median, 497 pg/mL) or presence (range, 32–351 pg/mL; median, 97 pg/mL) of 50 ng/mL exogenous recombinant human IL-10 (P < .05, Wilcoxon matched-pairs signed rank test).
to be produced by PBMCs from patients with asthma, despite the predominant Th2 response [20]. Previously, we showed that *S. mansoni* infection induces production of IL-10 [17]; here, we have demonstrated that helminthic infection is likely to increase the production of IL-10 in response to *D. pteronyssinus* Ag 1.

IL-10 is able to inhibit both Th1 and Th2 immune responses [21] and seems to be protective against asthma, because it is detected in high levels after allergen immunotherapy [22]. Furthermore, it has been demonstrated that IL-10–secreting cells inhibit airway hyperreactivity and inflammation induced by Th2 cells and that neutralizing of IL-10 abolishes the effect [23]. Macaubas et al. [24] demonstrated a negative correlation between the sizes of skin wheals in response to mite antigen and allergen-specific production of IL-10. Recently, a low frequency of positive SPT results for aeroallergen in *S. haematobium–*infected individuals and high levels of *Schistosoma* antigen–specific production of IL-10 were demonstrated [25]. Moreover, Royer et al. showed that IL-10 prevents the immediate-hypersensitivity reaction through inhibition of mast-cell degranulation and release of histamine [26]. Because this is the basic mechanism of allergic inflammation and asthma, it is possible that IL-10 produced by chronically helminth-infected individuals is involved in the inhibition of the SPT reaction observed in atopic individuals. According to this premise, less-severe asthma would be expected in helminth-infected patients with asthma. In the present study, we have demonstrated down-modulation of production of IL-4 and IL-5 in helminth-infected patients with asthma. It is well known that IL-4 and IL-5 stimulate IgE differentiation and expression of high-affinity IgE receptors on mast cells and eosinophilia, all of which are involved in the pathogenesis of asthma [27]. In addition, IL-10 suppresses the synthesis of tumor necrosis factor–α and nitric oxide, which are mediators involved in the inflammatory response during asthma [26]. Therefore, IL-10 can act at different steps of allergic inflammation to down-modulate production of cytokines and other mediators, which might explain the low prevalence of positive SPT results and less-severe asthma observed in patients who are chronically infected with *S. mansoni* [10].

The observation that neutralization of IL-10 does not enhance the production of IL-5 in helminth-infected patients is not contrary to the role this cytokine plays in modulating the immune response to aeroallergens. Down-modulation of the immune response is so strong in helminthic infection that, in individuals heavily infected with *S. mansoni* who have been vaccinated with tetanus toxoid, there is no mRNA expression for IFN-γ, besides the fact that this cytokine was not detected in supernatants of PBMC cultures stimulated with tetanus toxoid antigen [28]. In such cases, the neutralization of IL-10 for a short period of time in cultures would not be sufficient to restore the ability of the cells to produce IL-5. Indeed, it was demonstrated that IL-10 induces long-term, antigen-specific energy in human CD4+ T cells [29, 30]. Moreover, it can not be ruled out that, in addition to production of IL-10, the down-modulation of immune responses is also being performed by regulatory T cells that also express IL-10, such as Th3 cells [31], CD4+CD25+ T cells [32], or T regulatory type 1 (CD4+ TR1) cells, which produce low levels of IL-2, no IL-4, and high levels of IL-10 [33]. IL-10 and transforming growth factor–β, cytokines related to the Th3 response, were associated with T cell proliferative hyporesponsiveness in individuals with generalized onchocerciasis [34].

Surprisingly, there was no difference in the levels of *D. pteronyssinus* Ag 1–induced IL-13 between the 2 groups. This could be due to the low sensitivity of the ELISA. In addition, it could not be ruled out that IL-13 is less sensitive to the modulatory activity of IL-10.

The results of the present study of patients with asthma living in an area of polyhelminthic endemicity suggest that IL-10 is the key cytokine that inhibits the inflammatory Th2 immune response. Data that support this argument include the following: (1) higher levels of allergen-specific production of IL-10 and lower levels of allergen-specific production of IL-4 and IL-5 in helminth-infected patients, compared with those observed in helminth-free patients with asthma; (2) decreasing *D. pteronyssinus* Ag 1–specific production of IL-10 by PBMCs from infected patients with asthma after antihelminthic treatment; and (3) appearance of more-severe symptoms of asthma after antihelminthic treatment. Furthermore, the addition of rIL-10 to the cultures of PBMCs from helminth-free patients with asthma decreased *D. pteronyssinus* Ag 1–specific production of IL-5. An understanding of the mechanisms by which atopic patients living in areas where helminths are endemic have a decreased response to the SPT and do not develop severe asthma could lead to new perspectives on the development of prevention and treatment of asthma.

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