Allergy and Parasites
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Editorial

Allergy and Parasites

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Received 12 December 2012; Accepted 12 December 2012

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It is estimated that at least one-fifth of the world’s population suffers from allergic diseases (atopic asthma and allergic rhinitis are the most common). Different factors contribute to the development of allergies; a predisposing genetic background is needed; however, environmental factors play an important role, among them is the exposure to infectious agents.

Allergy is increasing in the recent decades particularly in Western industrialized countries, and according to the so-called hygiene hypothesis this might be ascribable to the lower exposure to infectious agents, including parasitic helminths. The reasons for such a situation are several: small family size, stability of intestinal microflora, affluent urban homes, high use of antibiotics, good sanitation which means low oral-fecal pathogen burden, and finally low or absent helminth infections. On the other hand, the low prevalence of atopy in developing countries is generally associated with high exposure to parasites in these regions.

The hygiene hypothesis has been revisited pointing out, for example, the role of IL-10-producing Treg cells, which are induced during chronic infections and play an essential role in downregulating allergic responses.

To verify the above hypotheses, a number of publications have appeared, studying the effects of parasitic infections or of molecules derived from parasites on the well-established experimental models of allergy, along with epidemiological studies, which have tried to correlate these conditions.

This special issue on allergy and parasites is composed of papers, which address this topic.

A review by N. Rujeni et al. focuses on the relationship between helminth parasites and allergic reactivity, taking into account the new concept to explain why Th2 immune responses have evolved. The Th2 response has been associated with host resistance to helminth infections and generally considered a harmfully allergic response. The review discusses these concepts and also the mechanisms underlying the interface between allergy and helminth infections and the evolutionary processes of the immune response associated with these conditions.

In the paper by D. Ball et al., the influence of ES-62, a glycoprotein produced by Acanthocheilonema viteae, on the activity of mast cells of different sources (peritoneal, connective tissue, and bone marrow derived) was assayed, comparing surface phenotype, degranulation responses to LPS and FcεR1 cross-linking, and cytokine production in response to those stimuli. The authors show the activation pathways in mouse mast cells that may be altered by ES-62. These results will advance our understanding of the immunomodulatory activity directed against the allergy effector cells by this parasite-derived glycoprotein.

C. Aranzamendi et al. observe that the association between allergy and helminth infections is not always the same. These authors review the literature on some of the cell types that play an essential role in helminth-induced immunoregulation and the consequences for inflammatory diseases. In addition, epidemiological as well as experimental studies indicating the contrasting effects of Toxocara and Trichinella infection on allergic asthma are discussed.
According to the authors, this diversity might depend on the helminth species, whether it is a chronic or acute infection, the different role played by the host in the various infections (natural or occasional), as well as on the different life cycles in which the parasite may affect the lung (in toxocarsis) or not (in trichinellosis), and on parasite burden.

Several epidemiological studies have consistently shown an inverse association between clinical asthma and *Schistosoma* infections. M. C. F. Almeida et al. carried out an elegant intervention study using the appropriate method to assess whether helminth infections are associated with asthma symptoms. The method used was a prospective, double-blinded, placebo-controlled study evaluating the influence of antihelminthic treatment on asthma severity, carried out in an endemic area for schistosomiasis. Findings on worsening of asthma severity after repeated antihelminthic treatments support the hypothesis on the protective effect of helminth infections in inflammatory diseases. Additional studies, such as the one described by Almeida et al. which would include a larger number of participants and continue for a longer period of time, are necessary in order to elucidate further the association between different helminth infections and inflammatory diseases.

In the paper by L. S. Cardoso and colleagues, risk factors for asthma were investigated in an area of helminth transmission in Bahia, Brazil, by the use of questionnaires. The effect of *Schistosoma mansoni* and also various gastrointestinal nematodes was investigated. Although the authors could not conclude that schistosomes offered a protective effect, the results indicated that *Ascaris lumbricoides* infection was negatively associated with asthma. This paper may thus be consistent with the idea referred to earlier that parasitic worm species vary in their immunomodulatory effects, but it should also be noted (as outlined above) that other studies show schistosome infection to be associated with protection against allergy.

In conclusion, the editors hope to have stimulated, with this special issue, the interest on the relationship between allergy and parasitic infections; envisaging new research work is needed not only to clarify many aspects which remain uncertain, but also to open the way to possible new strategies to control allergic diseases.

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Mast Cell Subsets and Their Functional Modulation by the Acanthocheilonema viteae Product ES-62

Dimity H. Ball, Hwee Kee Tay, Kara S. Bell, Michelle L. Coates, Lamyaa Al-Riyami, Justyna Rzepecka, William Harnett, and Margaret M. Harnett

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Received 3 September 2012; Accepted 16 October 2012

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ES-62, an immunomodulator secreted by filarial nematodes, exhibits therapeutic potential in mouse models of allergic inflammation, at least in part by inducing the desensitisation of FcεRI-mediated mast cell responses. However, in addition to their pathogenic roles in allergic and autoimmune diseases, mast cells are important in fighting infection, wound healing, and resolving inflammation, reflecting that mast cells exhibit a phenotypic and functional plasticity. We have therefore characterised the differential functional responses to antigen (via FcεRI) and LPS and their modulation by ES-62 of the mature peritoneal-derived mast cells (PDMC; serosal) and those of the connective tissue-like mast cells (CTMC) and the mucosal-like mast cells derived from bone marrow progenitors (BMMC) as a first step to produce disease tissue-targeted therapeutics based on ES-62 action. All three mast cell populations were rendered hyporesponsive by ES-62 and whilst the mechanisms underlying such desensitisation have not been fully delineated, they reflect a downregulation of calcium and PKCα signalling. ES-62 also downregulated MyD88 and PKCδ in mucosal-type BMMC but not PDMC, the additional signals targeted in mucosal-type BMMC likely reflecting that these cells respond to antigen and LPS by degranulation and cytokine secretion whereas PDMC predominantly respond in a degranulation-based manner.

1. Introduction

Mast cells are increasingly recognised as playing an important pathogenic role in a variety of allergic and autoimmune diseases [1–4]. However, there is also developing evidence of their participation in tissue repair and resolution of inflammation [5–7], as well as for their exhibiting pathogenic and protective roles in cancer [8, 9]. Such contradictory evidence relating to mast cell function most likely reflects that mast cells, which are haematopoietic cells found in all vascularised organs [10–13], constitute a heterogeneous cell population [14] varying in morphology, function, and location with subpopulations being characterised by their differential protease, eicosanoid, and proteoglycan content [10, 13–17]. Such heterogeneity arises because bone marrow-derived mast cell progenitors [18, 19] arrive in tissue before they are fully matured [20] allowing the different cytokines, hormones, and reactive oxygen and/or nitrogen species produced by various microenvironments to essentially create “custom-made” site-specific mast cells [12–14].

Moreover, the functional response of mast cells depends on the stimuli received [12]; for example, following classical activation via the IgE receptor, FceRI, mast cells degranulate rapidly (within minutes) to exocytose prostaglandins and leukotrienes as well as preformed cytokines, tryptase, histamine, heparin, and platelet activating factor (PAF) whilst de novo synthesised cytokines exhibit a more delayed (hours) release [8, 21]. However, mast cells can also be activated independently of FceRI and this can be initiated by cytokines or other proinflammatory mediators [21] reflecting a direct interaction with triggering factors such as LPS, parasite molecules, or allergic stimuli in the skin or the mucosa. Hence, mast cells are frequently the first cell type to respond during inflammation [6], as evidenced by the important roles
played by mast cells in bacterial and parasitic infections [22–25]. However, mast cells are also able to influence disease progress subsequently; both directly via the release of proinflammatory mediators, and indirectly via their effects on other immune cells, including dendritic cells (DC), T and B cells, and macrophages [6].

Isolation of in vivo differentiated mast cell subsets is difficult due to their limited numbers in tissue [5, 26] and this has led to the development of in vitro culture protocols to generate human and mouse mast cells, as defined by their expression of CD117, FceRI and the IL-1 receptor family member, ST2 (suppression of tumorigenicity 2), that can be subclassified as distinct phenotypes due to their differential granular phenotypic and functional responses [14]. Thus, although mast cells make up <5% of the peritoneal cell population, they can be expanded by in vitro culture with SCF to create large numbers of homogenous peritoneal-derived mast cells (PDMC) that are serosal-type mast cells and retain most of the morphological, phenotypic, and functional features of mature mast cells [5, 14]. Such PDMC exhibit differential functional responses (generating less lipid mediators, chemokines, and cytokines but displaying stronger degranulation responses) [5, 14] to bone marrow-derived mast cells (BMMC), generated by culture in the presence of IL-3 and SCF [27–29] and often used to represent mucosal-type mast cells (mucosal-type BMMC) [10, 14]. Phenotypically, these BMMC, more closely resemble immature cells and have no identifiable physiological equivalent in tissues [5, 12]. Nevertheless, such BMMC can repopulate both the mucosal and serosal mast cell compartments when adoptively transferred to mast cell-deficient mice, consistent with the proposal that these cells represent precursor cells that require additional site-specific signals to develop into mature tissue mast cells [12, 14]. By contrast, coculture of BMMC with fibroblasts [30, 31] generates connective tissue mast cells (CTMC) which have been used to represent serosal mast cells [10, 14]; more recently it has been shown that these can be differentiated from bone marrow precursors using SCF and IL-4 [14, 32] and CTMC have been implicated as being involved in both autoimmunity [33] and contact hypersensitivity [34].

The phosphorylcholine (PC)-containing excretory-secretory filarial nematode product, ES-62 exhibits broad anti-inflammatory properties including the desensitisation of FceRI-mediated mast cell responses and displays therapeutic potential in associated mucosal allergic inflammatory disorders such as asthma [35, 36]. As ES-62 is also protective in autoimmune and allergic connective tissue inflammatory pathologies such as arthritis and contact hypersensitivity, respectively [35, 36], we have therefore investigated its effects on mature PDMC and also on both CTMC and mucosal-type BMMC function in order to better understand mast cell biology as a first step to producing disease tissue-targeted therapeutics based on ES-62 action.

2. Materials and Methods

2.1. Mice and Reagents. BALB/c and C57BL/6 mice were purchased from Harlan Olac and maintained at the Universities of Glasgow and Strathclyde. All procedures were conducted in accordance with Home Office, U.K. animal guidelines and with the approval of the local ethical committees. Unless otherwise stated, all reagents were obtained from Sigma.

2.2. Peritoneal Derived Mast Cells (PDMC). PDMC were expanded as described previously [5]. Briefly, cells were harvested following washing of the peritoneal cavity of 6–8 week-old mice with 5 mL sterile, cold RPMI 1640 by centrifugation at 400 g for 5 min and then resuspended in fresh complete RPMI (RPMI with 10% FBS, 4 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mM sodium pyruvate, 100 μM nonessential amino acids, and 50 μM β-mercaptopetanol; Invitrogen Life Technologies) before being incubated at 5 × 10^5 cell/mL for 2 h at 37°C in tissue culture-treated petri dishes (Corning) to remove adherent cells. The suspension cells were cultured at 0.3 × 10^6/mL in complete RPMI supplemented with 10 ng/mL recombinant SCF (Pepro Tech) or 4% conditioned medium from the SCF-secreting cell line KLS-C. KLS-C is a CHO (Chinese Hamster Ovary) cell line that produces SCF and was a kind gift from Dr Xiaoping Zhong, Duke University Medical Center. KLS-C cells were cultured at 37°C in Minimum Essential Medium alpha (MEMα) without nucleosides (Invitrogen Life Technologies) and with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and 2.5 μM methotrexate. SCF-enriched supernatant was filtered to remove cell debris and the concentration determined by ELISA and then adjusted with PBS to 550 ng/mL to be used at a final concentration of 22 ng/mL for PDMC.

2.3. Bone Marrow-Derived Mast Cells. Intact femurs and tibias were dissected from BALB/c or C57BL/6 mice and single cell suspensions were obtained by passing bone marrow cells through a 100 μM nylon monofilament gauze (Cadisch Precision Meshes) and lysis of red blood cells for 1 min at 22°C, followed by washing in PBS at 400 g. Mucosal-type BMMC were derived by culture of bone marrow progenitors [27–29] at 0.5 × 10^6/mL in RPMI with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mM sodium pyruvate, 10 mM HEPES, and 50 μM β-mercaptopetanol supplemented with conditioned medium from KLS-C (1%; SCF) and TOP3 (3%; IL-3) cell lines. TOP3 is a cell line that produces IL-3 and was a kind gift from Dr Massimo Gadina, NIH. TOP3 cells were cultured at 37°C in RPMI with 5% FBS, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mM sodium pyruvate, and 50 μM β-mercaptopetanol and 0.4 mg/mL G418. IL-3-enriched supernatant was filtered to remove cell debris and the concentration determined by ELISA, with the stock concentration adjusted with PBS to 1300 ng/mL to be used at a final concentration of 39 ng/mL. Alternatively, mucosal-type BMMC were derived using 10 ng/mL recombinant SCF and 10 ng/mL recombinant IL-3 (Pepro Tech).

CTMC were derived by culture of bone marrow progenitors [14, 32] at 0.8 × 10^6/mL in RPMI with 10% FBS, 4 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mM sodium pyruvate, 100 μM nonessential amino acids
and 50 μM β-mercaptoethanol supplemented with 1% KLS-C conditioned medium (or 10 ng/mL recombinant SCF) and 1 ng/mL recombinant murine IL-4 (Pepro Tech).

PDMC, mucosal-type BMMC, and CTMC were cultured at 37°C/5% CO₂ in tissue culture-treated flasks (Greiner Bio-one) for at least 28 days, with adherent cells being discarded. A purity of >95% mast cells was routinely obtained as evidenced by the surface expression of CD117, FcεRI and ST2 and viability was determined by Trypan Blue staining.

2.4. Mast Cell Stimulation. Unless otherwise stated, mast cells were sensitised with 0.5 μg/mL murine anti-DNP IgE for 18 h prior to stimulation. In experiments investigating immunomodulation by ES-62, mast cells were incubated with ES-62 (2 μg/mL) simultaneously with IgE during the sensitisation period. Cells were then stimulated (1 × 10⁶ cells/mL except where indicated) by addition of medium, 0.5 μg/mL DNP-HSA to cross-link FcεRI, 0.5 μg/mL LPS (Salmonella minnesota) or PMA (phorbol myristate acetate; 1 μM) plus ionomycin (1 μM). Reactions were terminated after the desired culture period by centrifugation at 400 g and supernatants aspirated for determination of mediator release whilst the cell pellets were stored at −20°C until subjected to Western Blot analysis.

2.5. Preparation of Endotoxin-Free ES-62. ES-62 was purified to homogeneity from spent culture medium of adult Acanthocheilonema viteae using endotoxin-free reagents as described previously [37]. The purity and identity of each batch were confirmed by SDS-PAGE and the level of endotoxin in the ES-62 sample was determined using a Limulus Amebocyte Lysate (LAL) QCL-1000 kit (Lonza Biologics). ES-62 is used at a working concentration of ≤0.003 endotoxin units/mL [37].

2.6. Mast Cell Phenotyping. To identify mast cells by positive staining of heparin with Toluidine Blue, cells (0.01 × 10⁶) were cytofluorographed using a Shandon Cytoflex3 (Thermo Shandon) at 500 rpm for 5 min. Slides were air dried before staining with 0.5% Toluidine Blue in 0.5 M HCl for 10–15 min. Images were obtained using an Olympus BX41TF microscope.

Mast cells were also phenotyped by flow cytometric analysis of lineage markers. Briefly, cells were pre-incubated with 50 μL Fc receptor (FcR) blocking buffer (anti-CD16/32, clone 2.4G2, hybridoma supernatant, 10% mouse serum, and 0.1% sodium azide) for 20 min at 4°C prior to incubation with the appropriate fluorochrome-conjugated or biotinylated antibodies (2 μg/mL; suspended in 50 μL Fc Block; CD117, eBioscience; FcεRI, eBioscience; ST2, MD Bioproducts; and TLR4/MD2, eBioscience) for 30 min, 4°C. Following washing, for biotinylated primary antibodies, fluorochrome-conjugated streptavidin was added for a further 30 min at 4°C. After labelling, cells were washed twice with 3 mL FACS buffer (PBS containing 2% FBS and 2 mM EDTA) at 1500 rpm for 6 min, 4°C and then resuspended in FACS buffer. To enable exclusion of dead cells from the analyses, cells were either stained with Live/Dead Viability/Cytotoxicity Kit (Invitrogen) before commencement of staining or by the addition of 1 μL 7-AAD (7-Amino Actinomycin D; eBioscience) immediately prior to data acquisition. Cellular fluorescence data were acquired using a Becton Dickinson LSR II or FACSCalibur flow cytometer and analysed using FlowJo software (Tree Star Inc). Analysis was performed on a minimum of 10,000 events.

2.7. Calcium Mobilisation. Cells were loaded with the fluorescent calcium-sensing dye Fura-2/AM (5 μM; Invitrogen) in HBSS (145 mM NaCl, 5 mM KCl, 1 mM MgSO₄, 1 mM CaCl₂, and 10 mM HEPES) supplemented with 0.18% (w/v) D-glucose and 0.2% (w/v) BSA for 30 min at 37°C in the dark. For measurement of intracellular calcium mobilisation in the absence of extracellular calcium, calcium-free HBSS supplemented with 100 μM EGTA (ethylene glycol tetracetic acid) to chelate any remaining free calcium, was used. Cells (10⁶) were added to a stirred glass cuvette in a Hitachi F-7000 fluorescence spectrophotometer at 37°C and stimulated as indicated at t = 50 s and measurements acquired for a total of 180 s. Calcium levels were detected every 500 ms using excitation-emission ratios of 340/380 nm. Following each experiment R_max and R_min values were determined by the addition of 1% Triton-X100 and subsequent addition of 20 mM EGTA pH 7.4, respectively.

2.8. Cytokine and Prostaglandin D₂ (PGD₂) Release. ELISAs for IL-6, IL-13, MCP-1, and TNFα (limits of detection 4 pg/mL, 4 pg/mL, 15 pg/mL, and 8 pg/mL resp.; eBioscience) and PGD2 (Cayman Chemicals) were performed on triplicate samples according to the suppliers’ recommendations and developed using TMB substrate and absorbances were determined using a TECAN Sunrise Microplate Reader.

2.9. Mast Cell Degranulation. The level of degranulation was determined using a modified colorimetric assay to assess the release of β-hexosaminidase. Mast cells (0.2 × 10⁶) were suspended in 200 μL Tyrode’s buffer supplemented with 1% FCS and stimuli were added for 30 min at 37°C. Reactions were terminated by centrifugation (400 g) and 50 μL aliquots of supernatants assayed for release of β-hexosaminidase and normalised to total cellular β-hexosaminidase following cell lysis by the addition of 1% Triton-X 100 and by incubation with 1 mM p-nitrophenyl-N-acetyl-β-d-glucosamine (NAG) in 200 μL 0.05 M citrate buffer, pH 4.5. After incubation in the dark at 37°C for 1 h the reaction was quenched by the removal of 62.5 μL of the reaction mix into a clean well and the addition of 125 μL well 0.1 M sodium bicarbonate buffer and optical density determined by a TECAN Sunrise Microplate Reader at 405 nm.

2.10. Western Blotting. Mast cells (2 × 10⁶/mL) were stimulated as indicated and reactions terminated by the addition of ice-cold PBS and centrifugation at 400 g at 4°C for 5 min. Lysis was performed by the addition of 50 μL ice-cold, modified RIPA lysis buffer (50 mM Tris buffer, pH 7.4 containing 150 mM sodium chloride, 2% (v/v) NP40, 0.25% (w/v) sodium deoxycholate, 1 mM EGTA, 10 mM...
3. Results and Discussion

3.1. Phenotyping of PDMC, Mucosal-Type BMMC, and CTMC Mast Cell Subsets. Following expansion of PDMC and derivation of mucosal-type BMMC and CTMC for 4–6 weeks in vitro, these mast cell populations were phenotyped for mast cell lineage markers (CD117, FceRI, and ST2) and also for TLR4 (toll-like receptor-4) and proteoglycan (heparin) expression (Figure 1). This analysis revealed three mouse subsets to be of similar size, although the CTMC appeared rather less granular (Figure 1(a)), with each subset comprising a homogeneous population of CD117/FceRI+ mast cells (Figure 1(b)). Moreover, all 3 populations expressed TLR4 (Figure 1(c)) and ST2 (Figure 1(d)), with BMMC typically expressing the highest levels of TLR4 and CTMC showing most ST2 expression. Similarly, PDMC, mucosal-type BMMC, and CTMC all contained heparin-containing granules as indicated by toluidine blue staining (Figure 1(e)), although there was some heterogeneity in the CTMC, and to a lesser extent, the mucosal-type BMMC populations, perhaps reflecting their differences in granularity (Figure 1(a)). Mucosal-type mast cells from mice have generally been considered to express little or no heparin, but in agreement with our results, it has been reported that heparin expression can be upregulated in response to SCF in these cells [14]: this functional plasticity is consistent with the ability of mouse BMMC to repopulate both serosal and mucosal compartments of mast cells in vivo [14].

3.2. Differential Functional Responses of Mast Cell Subsets. It has previously been reported that serosal- and mucosal-type mast cells exhibit differential functional responses with PDMC displaying strong degranulation responses whilst BMMC preferentially produce chemokines and cytokines, and that these responses can be further “fine-tuned” selectively in response to inflammatory stimuli and microenvironment [14]. We therefore characterised the differential responses of PDMC, mucosal-type BMMC, and CTMC in response to Ag-mediated crosslinking of FceRI, LPS/TLR4 signalling and also the pharmacological stimulus PMA plus ionomycin (P/I) in terms of degranulation (β-hexosaminidase), eicosanoid (PGD2), chemokine (MCP-1), and cytokine (IL-6, IL-13 and TNFα) release (Table 1). These data confirmed that PDMC were the subtype that degranulated most strongly in response to FceRI and PMA plus ionomycin and demonstrated that all of these subtypes generated in vitro exhibited little or no degranulation in response to LPS. All of the mast cell populations constitutively secreted high levels of PGD2 (lowest in PDMC); however, whilst PDMC did not produce any more PGD2 in response to FceRI crosslinking, both mucosal-type BMMC and CTMC responded further to this stimulus and also to LPS. Further differential responses were observed in terms of chemokine release as whilst LPS stimulated release of MCP-1 in all subtypes, FceRI crosslinking induced little or no release of MCP-1 over the basal levels in PDMC, yet strongly stimulated release of this chemokine from mucosal-type BMMC and CTMC. Only PMA plus ionomycin were able to induce substantial secretion of IL-6 and IL-13 by PDMC, and none of the stimuli were routinely able to trigger TNFα release by these cells. Similarly, whilst FceRI- and LPS/TLR4-signalling induced little or no IL-13 or TNFα production by CTMC, LPS, but not FceRI crosslinking, triggered strong secretion of IL-6. By contrast, FceRI and LPS/TLR4 signalling both induced the production of all three cytokines (IL-6, IL-13 and TNFα) by mucosal-type BMMC. It has been reported that BMMC and freshly isolated PDMC derived from C57BL/6 mice exhibited higher levels of degranulation (β-hexosaminidase) and generated lower levels of cytokine and prostaglandin production than those derived from BALB/c mice [38] but we did not find this to be a significantly reproducible trend in this study (data are not shown).

3.3. ES-62 Inhibits Functional Responses in Both Serosal and Mucosal Mast Cell Subtypes. Given the differential functional responses of the mast cell subtypes, we next investigated whether the filarial nematode product showed selectivity in its desensitisation of mast cell responses, both in terms of the mast cell subtype targeted and also with respect to their differential responses to the individual proinflammatory stimuli. These studies showed that exposure to ES-62 significantly
Figure 1: Phenotyping of mast cell subsets. Exemplar plots of mast cell phenotyping by flow cytometric analysis are shown in (a)–(d). FSC and SSC parameters of PDMC, mucosal-type BMMC, and CTMC cultured in vitro for 28 days (a) and gating (relative to isotype controls; not shown) of the consequent CD117⁺ FcεRI⁺ cell population (>98%; (b)) prior to the analysis of their TLR4⁺ expression (c) are shown. In parallel experiments, ST2 expression of CD117⁺ FcεRI⁺ cells in the various populations was determined (d). Gray shaded plots (c-d) are isotype controls. In (e), exemplar images of toluidine blue staining of the mast cell populations (x10) are shown. The data are representative of at least 2 independent experiments.
Table 1: Differential functional responses of mast cell subsets.

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<th>Basal</th>
<th>FcεRI</th>
<th>LPS</th>
<th>P/I</th>
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<th>FcεRI</th>
<th>LPS</th>
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<th>FcεRI</th>
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<td>Degranulation (%)</td>
<td>4.48 ± 0.56</td>
<td>33.24 ± 4.07</td>
<td>2.87 ± 1.47</td>
<td>55.34 ± 4.24</td>
<td>7.46 ± 1.10</td>
<td>17.34 ± 2.19</td>
<td>2.87 ± 1.78</td>
<td>31.97 ± 3.78</td>
<td>3.95 ± 1.02</td>
<td>17.3 ± 2.90</td>
<td>2.173 ± 0.48</td>
<td>25.29 ± 4.62</td>
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<td>PGD2 (pg/mL)</td>
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<td>1907 ± 55</td>
<td>3257 ± 338</td>
<td>4711 ± 264</td>
<td>4509 ± 156</td>
<td>2888 ± 370</td>
<td>3229 ± 333</td>
<td>4764 ± 153</td>
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<td>IL-6 (pg/mL)</td>
<td>70 ± 27</td>
<td>67 ± 32</td>
<td>81.33 ± 7</td>
<td>408 ± 61</td>
<td>37.4 ± 14.7</td>
<td>560 ± 96</td>
<td>2557 ± 696</td>
<td>21976 ± 6589</td>
<td>87 ± 23</td>
<td>114 ± 19</td>
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<td>MCP-1 (pg/mL)</td>
<td>457 ± 73</td>
<td>387 ± 84</td>
<td>616 ± 109</td>
<td>102 ± 19</td>
<td>504 ± 212</td>
<td>1703 ± 393</td>
<td>862 ± 296</td>
<td>396 ± 58</td>
<td>1410 ± 226</td>
<td>511 ± 76</td>
<td></td>
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<tr>
<td>TNFα (pg/mL)</td>
<td>15.25 ± 2.48</td>
<td>15.57 ± 2.91</td>
<td>19.62 ± 3.38</td>
<td>32.33 ± 2.94</td>
<td>5.02 ± 2.12</td>
<td>506 ± 159</td>
<td>150 ± 38</td>
<td>399 ± 916</td>
<td>1.42 ± 0.19</td>
<td>1.41 ± 0.34</td>
<td>15.62 ± 1.61</td>
<td></td>
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</table>

Data are presented as the mean values ± SEM where n = number of independent experiments or mean values ± SD of triplicate samples in the case where data from a single experiment are presented.
Figure 2: Degranulation by mast cell subsets. Mucosal-type BMMC, CTMC and PDMC were sensitised with murine anti-DNP IgE (0.5 μg/mL) in the presence and absence of ES-62 (2 μg/mL) overnight. Cells were then stimulated with DNP (0.5 μg/mL) to induce FcεRI cross-linking (XL; (a)) or PMA plus ionomycin (both 1 μM; (b)) for 30 min at 37°C. Degranulation was determined as the % β-hexosaminidase release relative to the total enzyme activity of the cells and the data presented are from single experiments representative of at least 2 independent experiments. PDMC were sensitised with murine anti-DNP IgE (0.5 μg/mL) in the presence and absence of ES-62 (2 μg/mL) overnight and analysed for expression of FcεRI (c), CD117 (d), and ST2 (e). Grey shaded plots (c–e) are relevant isotype controls.

inhibited the degranulation of PDMC and the mucosal-type BMMC, but not CTMC, in response to FcεRI signalling (Figure 2(a)). Whilst the responses to LPS were typically too low (Table 1) to show significant effects of ES-62 (data not shown), degranulation in response to PMA plus ionomycin was also significantly inhibited in PDMC (Figure 2(b)) and likewise observed in CTMC (2/2 experiments) and the mucosal-type BMMC (3/5 experiments).

Although PDMC produced little or no chemokines/cytokines in response to either FcεRI crosslinking or LPS-stimulation (Table 1), generally, the very low levels of MCP-1, IL-6, and IL-13 observed were inhibited by ES-62 (data not shown). With respect to mucosal-type BMMC and CTMC, whilst ES-62 was only able to significantly inhibit MCP-1 production by FcεRI-stimulated mucosal-type BMMC (Figures 3(a) and 3(b)), it inhibited LPS-stimulated IL-6 production.
Figure 3: Chemokine and cytokine release by mast cell subsets. Mucosal-type BMMC (a), (c) & (e) and CTMC (b), (d) & (f) were sensitised with murine anti-DNP IgE (0.5 μg/mL) in the presence and absence of ES-62 (2 μg/mL) overnight. Cells were then stimulated with DNP (0.5 μg/mL) to induce FcεRI cross-linking (XL) or LPS (0.5 μg/mL) for 24 h at 37°C and MCP-1 (a) & (b), IL-6 (c) & (d), and IL-13 (e) & (f) release measured by ELISA. The data presented are single experiments representative of at least 2 independent experiments apart from the IL-13 release from CTMC, which could only be detected in a single experiment.

by both subtypes as well as that seen in mucosal-type BMMC in response to crosslinking of FcεRI (Figures 3(c) and 3(d)). By contrast, the IL-13 response to FcεRI crosslinking was suppressed by ES-62 in both subtypes (Figures 3(e) and 3(f)) whilst the FcεRI-mediated TNFα response only seen in mucosal-type BMMC was also inhibited by ES-62 (data not shown).

Collectively, therefore, we have shown that ES-62 can target both serosal- and connective-tissue phenotypes to render these cells hyporesponsive to proinflammatory stimuli. That ES-62 can modulate the responses of both mature and immature cells is consistent with our previous studies showing that the parasite product can target immature bone marrow progenitors of macrophages and dendritic cells to generate a more anti-inflammatory environment in vivo [37, 39]. The finding that ES-62 did not modulate expression of FcεRI, CD117, or ST2 on resting or sensitized mast cells (Figures 2(c)–2(e) and data not shown), however, suggested that it was not affecting their phenotypic status but rather targeting their functional plasticity. The observed differential targeting of particular responses may indicate selective actions in particular microenvironments and consequently, recruitment of other innate cells such as neutrophils to the site of inflammation as well as mast cell promotion of the polarisation of particular immune responses [4, 14], dependent on the site and type of inflammation (e.g., protective inflammation to fight infection versus aberrant autoimmune or allergic hyperinflammation).
3.4. ES-62 Targets Calcium and PKC Signalling in Mast Cells.

To address how ES-62 may be differentially targeting the functional responses of PDMC, the mucosal-type BMMC, and CTMC, we investigated the effects of the parasite product on calcium mobilisation and expression of PKCα, as we had previously shown modulation of these two key signals in degranulation and cytokine signalling [40–42] to be crucial to the desensitisation of FceRI-mediated human mast cell responses [35, 36]. Moreover, ES-62 exerts its effects via subversion of TLR4 signalling whilst the canonical TLR4 ligand LPS typically acts to enhance FceRI functional responses [43, 44], the latter accounting at least in part for the widely established finding that LPS exacerbates airway hyperresponsiveness [43]. LPS has been reported to do this by increasing FceRI-driven calcium mobilisation by upregulating the Orai1 and Stim1 subunits of the store-operated calcium (SOC) channel and hence stimulating calcium influx [43]. In addition, PKC signalling, including that of PKCα, has been shown to be important to LPS/TLR4 responses in a variety of innate cells [45].

As a first step, we investigated whether FceRI- and LPS/TLR-signalling induced calcium mobilisation in each of PDMC, mucosal-type BMMC and CTMC (Figure 4). Interestingly we found that not only as expected, IgE-sensitisation of mast cells was essential for FceRI-mediated calcium mobilisation, but also that it enhanced that seen in response to LPS (PDMC; Figures 4(a) and 4(b)). Moreover, it was clear that whilst the FceRI signal reflected a mix of mobilisation of intracellular calcium and calcium influx, as indicated by the observed transient spike in the absence of extracellular calcium (EGTA), the calcium response to LPS in all mast cell subtypes predominantly reflected calcium influx (Figures 4(c)–4(h)). Consistent with LPS inducing calcium influx, we also found as reported previously [43] that LPS enhanced FceRI-mediated calcium mobilisation (data not shown). By contrast, although preexposure to ES-62 did not modulate the baseline calcium levels, the parasite product suppressed the subsequent calcium mobilisation in response to both FceRI-crosslinking and LPS/TLR4 signalling in PDMC (Figures 5(a) and 5(b)).

In addition, ES-62 was found to downregulate PKCα expression in PDMC, mucosal-type BMMC and CTMC (Figures 5(c) and 5(d)), suggesting, that as with human mast cells [35], ES-62 was targeting this signal in PDMC, mucosal-type BMMC and CTMC to suppress degranulation and cytokine responses. PKCα has been shown to be degraded via both proteosomal and caveolae/lipid raft, lysosomal routes [46, 47] and our preliminary studies in human mast cells showed that the inhibitor of caveolae/lipid raft trafficking, nystatin could protect against such downregulation following
Figure 5: ES-62 modulates signalling in mast cell subsets. PDMC were sensitised with murine anti-DNP IgE (0.5 μg/mL) in the presence or absence of ES-62 (2 μg/mL) overnight. Following loading with Fura-2/AM such PDMC were stimulated at 50 s with DNP (0.5 μg/mL) to induce cross-linking (XL) of FcεR1 (a) or 0.5 μg/mL LPS (b) and intracellular calcium mobilisation and influx recorded in real time using excitation-emission ratios of 340/380 nm (a) & (b). Calcium levels were calculated from $R_{\text{max}}$ and $R_{\text{min}}$ values and data are presented as the mean calcium values of triplicate samples from a single experiment representative of at least 3 independent experiments. PDMC and mucosal-type BMMC (c) & (e) were cultured with ES-62 (2 μg/mL) for the indicated times and expression of PKCα (c), Cell Signalling Technology), MyD88 (e), Abcam) and PKCδ (e), Cell Signalling Technology) analysed by Western Blotting. In (d), following preincubation for 1 h with inhibitors of proteosomal degradation (10 μM Lactacystin, ENZO Life Sciences; LAC), caveolae/lipid raft trafficking (50 μg/mL Nystatin; NYS) and lysosomal degradation (E64d + pepstatin A both 10 μg/mL, ENZO Life Sciences), sensitised CTMC were cultured with ES-62 (2 μg/mL) for the indicated times and expression of PKCα analysed by Western Blotting. Actin was used as a loading control and ES-62-mediated downregulation of PKCα expression was observed in PDMC, Mucosal-type BMMC, and CTMC in at least 2 independent experiments.

exposure to ES-62 for 24 h [35]. Furthermore, our earlier studies [48] had shown that ES-62-mediated downregulation of PKCα expression in B cells could be prevented by treatment with the cysteine protease inhibitor leupeptin; findings also consistent with a lysosomal mechanism [49, 50] of degradation of this key signalling element. We have therefore further investigated the (differential) mechanisms involved in ES-62-driven degradation of PKCα in mast cell subtypes. Our data in CTMC are consistent with that of our previous study on human mast cells [35] as they showed that nystatin but not lactacystin protected against PKCα degradation and we have further confirmed the role of an endosomal route by showing that protection is also afforded by the combination of the lysosomal inhibitors, E64d plus pepstatin A (Figure 5(d)). However, our comprehensive analysis of mechanism in PDMC and the mucosal-type BMMC has revealed a more complicated scenario in which both nystatin and lactacystin can offer some protection at differential time points (data not shown). Interestingly, these findings are consistent with reports that both mechanisms can coexist in cells not only in a temporal and spatially distinct manner but can also be triggered to regulate PKCα expression in response to a single agonist [46]. Hence, the differential recruitment of one or more of these degradative pathways may provide a rationale for fine tuning the level of PKCα desensitisation required to downregulate hyperinflammatory responses; an attractive proposal gave that CTMC exhibit the least degranulation potential and are not as effective at producing cytokines as the mucosal-type BMMC. Overall, as we have also found that the inhibitors alone can modulate PKCα expression in PDMC and mucosal-type BMMC, these findings collectively indicate that regulation of this key signalling element in mast cells is tightly controlled by a complex and dynamic system involving both proteosomal and lysosomal routes of degradation.
Finally, whilst we have shown that ES-62 can suppress the cytokine responses of both PDMC and the mucosal-type BMMC, it is clear that the levels of cytokines produced by PDMC in response to FcεRI- and LPS/TLR4 signalling are very low compared to those secreted by mucosal-type BMMC. We have therefore addressed identifying which signals may be contributing to such higher levels of cytokine production by also determining the effects of ES-62 on PKCδ expression as this signalling element has not only been shown to be important for functional responses to FcεRI- and LPS/TLR4 signalling [40, 45, 51–53] but also to be a target for downregulation by the parasite product in human mast cells and B cells [35, 48]. In addition, we have also examined the effect of ES-62 on MyD88, a pivotal signal transducer of TLR4 [45, 51, 54] as we have shown it to be a target of ES-62 in countering Th17 pathology [37] and only the MyD88- and not the TRIF-dependent pathway of TLR4 signalling, appears to be active in BMMC [55]. Consistent with the hypothesis that additional signals such as MyD88 and PKCδ are required for the augmented cytokine responses observed in BMMC relative to PDMC, these studies show that whilst MyD88 expression in PDMC is unchanged by exposure to ES-62, culture with the parasite product results in downregulation of both MyD88 and PKCδ in mucosal-type BMMC (Figure 5(e)).

4. Conclusions

PDMC, mucosal-type BMMC, and CTMC mast cell populations display differential functional responses with mature serosal mast cells predominantly acting like cells that perform a specialised degranulation function. By contrast, BMMC, which have been reported to possess an immature mucosal-like phenotype that can further differentiate into mucosal or serosal mast cells display reduced degranulation and increased cytokine responses. Consistent with the idea that BMMC are plastic and can differentiate into either mucosal or serosal mast cells, CTMC display a comparable degranulation potential to that of mucosal-type BMMC and a cytokine profile intermediate of mucosal-type BMMC and mature serosal/connective tissue PDMC. All three mast cell populations can be rendered hyporesponsive by ES-62 but the selective nature of these effects suggests that ES-62 may be targeting functions of the individual subtypes that are
specific to the particular inflammatory microenvironment and phenotype.

The mechanisms underlying such desensitisation have not been fully delineated but our working model (Figure 6) is that reduced degranulation and low level cytokine secretion reflect desensitisation of FcepsilonRI- and LPS/TLR4-mediated calcium mobilisation and PKCs signalling whilst suppression of the high levels of cytokine production by mucosal-type BMMC in response to these signals requires downregulation of additional signals such as MyD88 and PKCδ. Such a rheostat effect allowing differential signal strength-dependent desensitisation of receptor signalling would allow ES-62 to provide an appropriate level of hyporesponsiveness that would prevent development of aberrant autoimmune and allergic inflammatory disorders whilst allowing appropriate levels of inflammation to generate protective immune responses to pathogenic infection.

Acknowledgments

The authors would like to thank the Wellcome Trust and the American Asthma Foundation for supporting this research and the following funding bodies for the awards of Ph D studentships to D. Ball (MRC and U of G); K. S. Bell (SULSA); and M. L. Coates (BBSRC). The authors have no conflict of interests.

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Review Article

Helminths: Immunoregulation and Inflammatory Diseases—Which Side Are *Trichinella* spp. and *Toxocara* spp. on?

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Received 30 September 2012; Accepted 1 December 2012

Academic Editor: Fabrizio Bruschi

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Macropathogens, such as multicellular helminths, are considered masters of immunoregulation due to their ability to escape host defense and establish chronic infections. Molecular crosstalk between the host and the parasite starts immediately after their encounter, which influences the course and development of both the innate and adaptive arms of the immune response. Helminths can modulate dendritic cells (DCs) function and induce immunosuppression which is mediated by a regulatory network that includes regulatory T (Treg) cells, regulatory B (Breg) cells, and alternatively activated macrophages (AAMs). In this way, helminths suppress and control both parasite-specific and unrelated immunopathology in the host such as Th1-mediated autoimmune and Th2-mediated allergic diseases. However, certain helminths favor the development or exacerbation of allergic responses. In this paper, the cell types that play an essential role in helminth-induced immunoregulation, the consequences for inflammatory diseases, and the contrasting effects of *Toxocara* and *Trichinella* infection on allergic manifestations are discussed.

1. Introduction

Immune responses induced by helminths are predominantly of the Th2 type involving cytokines such as interleukin-3 (IL-3), IL-4, IL-5, IL-9, IL-10, and IL-13. These cytokines mediate immune responses typically characterized by increased levels of circulating IgE antibodies, eosinophils, basophils, and mast cells [1]. During infection, the immune system is exposed to different helminth-derived molecules, including proteins, lipids, and glycoconjugates present either at the surface of the worms or in the excretory-secretory (ES) products [2]. Interaction of helminth-derived molecules with host cells can result in a shift of the immune response, from an inflammatory towards an anti-inflammatory type of response. Helminth-derived molecules can modify dendritic cells (DCs) function and downregulate adaptive immune responses, through the induction of a regulatory network that include regulatory T (Treg) cells, alternatively activated macrophages (AAMs), and regulatory B (Breg) cells. The induced immunosuppressive network, together with cytokines produced by diverse hematopoietic and non-hematopoietic cells as integral part of immunoregulatory pathways, appears to be essential for parasite survival and its effect can be extended to other inflammatory disorders such as allergies and autoimmune diseases [3, 4]. However, the association between helminth infections and allergy does not always have an unequivocal outcome. While certain helminth infections protect against allergic diseases (reviewed in [5]), other helminths can exacerbate this immunopathology (reviewed in [6]). Here, the role of DCs, Treg, and other regulatory cells in helminth-induced immunoregulation, the consequences for inflammatory diseases, and the contrasting effects of *Toxocara* and *Trichinella* infections on allergic manifestations are discussed.

2. Dendritic Cells

DCs are sentinels on alert for possible danger signals to immediately activate local innate immune responses and
subsequently, after antigen presentation, initiate the proper adaptive immune responses. Interaction with DCs determines the function and cytokine production of lymphocytes. DCs play therefore an essential role in shaping the immune response and controlling the course of infection [7]. These cells are located throughout the body forming a complex network that allows them to communicate with different populations of lymphocytes. Different DC subsets may have distinct locations, where they acquire antigens to be transported to the draining lymph nodes for T-cell priming [8]. DCs as well as other innate immune cells possess various families of pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), NOD-like receptors, RIG-like receptors, and the C-type lectin receptors (CLRs) that allow them to recognize a great variety of pathogen-associated molecular patterns (PAMPs). After pathogen recognition via various PRR, DCs produce molecules that induce polarization of different types of responsiveness such as Th1-, Th2-, Th17-, or Treg-related. The response of DCs to pathogens is mediated in large part via TLR, with input from other PRR resulting in changes in gene expression that leads to DCs maturation. Maturation of these cells refers to a transition from a resting state into a more dynamic state in which the cells present antigen in the context of MHC, express costimulatory molecules such as CD40, CD80, and CD86, and secrete a broad spectrum of cytokines and chemokines [9]. TLR-mediated responses are controlled mainly by the MyD88-dependent pathway, which is used by all TLR except TLR3- and by the TIR-domain-containing adapter-inducing interferon-β-(TRIF)-dependent pathway, which is used by TLR3 and TLR4 [10]. TLRs have been implicated in the recognition of helminth products by DC. For instance, Lacto-N-fucopentaose III (LNFPIII) produced by trematode Schistosoma mansoni, and ES-62, a phosphocholine-containing protein secreted by the nematode Acanthocheilonema vitaeae, can condition DCs to induce Th2 responses through TLR4 [11]. Likewise, monoacylated phosphatidyl serine lipids from schistosomes specifically instruct DCs to preferentially induce IL-10-producing Treg in a TLR2-dependent manner [12]. This was also demonstrated in TLR2-deficient mice that showed a reduced number of CD4+CD25+ Treg cells and immunopathology during schistosomiasis [13]. CLRs on DCs also play an important role in sensing helminth glycans. Studies using schistosomal antigens suggest that helminth glycans may be the conserved molecular pattern that instructs DCs via CLR to drive Th2-polarized responses [14]. Other recent studies demonstrate that host-like glycan antigens expressed by many helminth are recognized by DCs via lectin receptors [2]. Schabussova et al. reported on blood group-like glycans from T. canis that bind the lectin DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Nonintegrin) [15], which may enable the activation of signal transduction pathways involving Raf-1 and subsequent modulation of DC maturation resulting in skewing towards a Th2 responses [16]. Lewis X antigen, a host-like glycan expressed on the surface of schistosomes in all life stages which is also present in secreted products such as the soluble egg antigens (SEAs), also binds to DC-SIGN [17].

DC maturation is considered to be essential for DCs to induce T-cell responses. It has become clear that DCs responding to helminth products do not mature in the conventional way upon encountering parasitic antigens but acquire a semimatured status and are still capable of inducing T-cell polarization. Several studies support the findings that helminth products fail to directly activate DCs and other studies show that helminth products suppress DC maturation. For instance, SEA suppresses lipopolysaccharide (LPS-) induced activation of immature murine DCs, as indicated by decreased MHC class II and costimulatory molecules expression in addition to IL-12 production. This resulted in increased LPS-induced production of IL-10 by DC after incubation by SEA [18]. Pretreatment of DCs and macrophages with ES-62 also inhibits their ability to produce IL-12p70 in response to LPS [19]. In another study, a mixture of high molecular weight components from Ascaris suum was found to reduce the LPS-induced expression of MHCII, CD80, CD86, and CD40 molecules on mouse CD11c+ DCs and to hampered T-cell proliferative responses in vitro. This inhibitory effect was abolished in IL-10-deficient mice [20]. Fasciola hepatica tegumental antigen alone did not induce cytokine production or cell surface marker expression on murine DCs; however, it significantly suppressed cytokine production and cell surface marker expression in DCs matured with a range of TLR and non-TLR ligands [21]. In vitro studies on the impact of T. spiralis excretory/secretory products (TspES) on mice DCs revealed that these parasitic antigens suppress DC maturation induced by LPS derived from different bacteria [22]. In this study, different TLR agonists were used showing that the suppressive effect of TspES on DC maturation is restricted to TLR4. These helminth products were also shown to interfere with the expression of several genes related to the TLR-mediated signal transduction pathways. For rat bone-marrow-derived DC it has been shown that after incubation with TspES these DCs acquire a semimatured status which is reflected in moderate upregulation of CD86, significant upregulation of ICAM1 (Intercellular Adhesion Molecule 1), and no upregulation of MHC II, accompanied by impaired production of IL-12 p70 [23].

3. Regulatory T Cells

Treg cells control peripheral immune responses and are likely to play a central role in autoimmune, infectious, and allergic diseases. Three phenotypes of Treg have been described to date, categorized according to their origin, function, and expression of cell surface markers: natural Treg cells (CD4+CD25+Foxp3+) and inducible Treg cells that include the IL-10-producing Tr1 cells and the Foxp3+ T cells induced in the periphery [24]. In spite of the complexity of regulatory cell types, CD4+CD25+Foxp3+ Treg cells are the most prominent population of immunoregulatory cells known so far to be induced during helminth infections [4]. Early studies had already suggested regulatory T-cell activity during chronic helminth infections in humans. Doetze et al. reported that IL-10 and transforming
growth factor-β (TGF-β) production mediated the hyporesponsiveness observed in PBMC from individuals with generalized onchocercosis caused by the filarial nematode *Onchocerca volvulus* [25]. In a study with falciparum patients, lymphphedema was associated with a deficiency in the expression of Foxp3, GITR (glucocorticoid-induced tumour-necrosis-factor-receptor-related protein), TGF-β, and CTLA-4 (cytotoxic T-lymphocyte antigen 4), known to be expressed by Treg cells [26], while in children infected with intestinal nematodes (*Ascaris lumbricoides* and *Trichuris trichiura*) high levels of IL-10 and TGF-β in addition to generalized T-cell hyporesponsiveness were found [27, 28]. Likewise, *Schistosoma*-infected individuals in Kenya and Gabon had higher CD4+CD25+ and CD4+CD25+Foxp3+ T-cell levels compared with uninfected individuals [29]. One of the studies providing evidence on the suppressive effect of Treg cells from helminth-infected individual is the one reported by Wammes et al. [30]. In this study, carried out in Indonesia, Treg cells from geohelminth-infected individuals were more effective at suppressing proliferation and IFN-γ production by effector T cells in response to malaria antigens and BCG than Treg cells from healthy individuals. A filarial parasite of humans, *Brugia malayi*, was found to secrete TGH-2 (transforming growth factor homologue-2), a homologue of host TGF-β [31]. Since the recombinant TGH-2 can bind to the mammalian TGF-β receptor, it has been suggested that it can promote the generation of regulatory T cells, as it has been shown for mammalian TGF-β. In another study a significant increased expression of Foxp3 and regulatory effector molecules such as TGF-β, CTLA-4, PD-1 (programmed death 1) and ICOS (inducible costimulatory molecule) was found in filarial-infected compared to uninfected individuals in response to live infective-stage larvae or microfilariae of *Brugia malayi* [32].

Various studies on the role of Treg cells in helminth infections have used animal models. In mice, CD25+ Treg cells were shown to restrain the pathology to helminth eggs during schistosome infection [13] and to *Trichuris muris* in the gut [33]. Moreover, depletion of CD25+ Treg cells with combined antibodies to CD25 and GITR resulted in enhanced immunity to *larial* nematode *Litomosoides sigmodontis* in mice [34]. Generation of Treg cells with elevated expression of Foxp3 during helminth infection has also been demonstrated. For instance, infection of BALB/c mice with *Brugia pahangi* third-stage larvae (L3) resulted in expansion of a population of CD4+CD25+ T cells which was highly enriched in Foxp3 and IL-10 gene expression [35]. Induction of Treg cells was demonstrated to be necessary to establish a chronic *L. sigmodontis* infection since depletion of Treg cells in susceptible mice prior to infection enhanced parasitic killing and cleared the infection [36]. In chronic infection with the gastrointestinal helminth *Heligmosomoides polygyrus*, it was established that levels of Foxp3 expression within the CD4+ T-cell population of mice mesenteric lymph nodes were significantly increased and that purified CD4+CD25+ Treg cells possess suppressive activity *in vitro* [37, 38].

The effect of TspES on T-cell activation *in vitro* was investigated using splenocytes derived from ovalbumin- (OVA)-TCR transgenic D011.10 mice that were incubated with TspES-pulsed DC+OVA. Results indicate that the presence of TspES resulted in expansion of CD4+CD25+ T cells that express high levels of Foxp3+. These Treg cells were shown to have suppressive activity and to produce TGF-β. Together these results indicate that *T. spiralis* secretion products can induce expansion of functional Treg cells *in vitro* [22].

In a rat model, the infection with *T. spiralis* is accompanied with the increase proportion of Foxp3+ T reg cells [23]. *In vitro* studies showed that DCs stimulated with TspES caused strong Th2 polarization, accompanied by elevated production of the regulatory cytokines IL-10 and TGF-β [23]. However, unlike the mouse model described previously, conditioned rat DCs generated no increase in the proportion of CD4+CD25+Foxp3+ T cells. *In vivo* T-cell priming with TspES stimulated DCs resulted in mixed Th1/Th2 cytokine response, with the dominance of the Th2 type and elevated levels of regulatory cytokines. Significant increase in the proportion of CD4+CD25+Foxp3+ cells was found in spleen cells of recipients that received TspES stimulated DCs compared to the control value obtained from rats that received DCs cultivated in medium only.

### 4. Other Regulatory Cells

Helminth infections may also lead to expansion of immunoregulatory cells other than Treg cells, including alternatively activated macrophages (AAMs) and regulatory B cells. Signals encountered during migration by developing macrophages determine their function at sites of inflammation or infection. Among these signals, cytokines are responsible for the development of highly divergent macrophage phenotypes: classically activated and AAMs [39]. Prieto-Lafuente et al. reported that the homologues of the mammalian cytokine macrophage migration inhibitory factor (MIF) expressed by *Brugia malayi* synergized with IL-4 to induce the development of suppressive AAMs in *vitro* [40]. One pathway for this effect may be through the MIF-mediated induction of IL-4R expression on macrophages, amplifying in this way the potency of IL-4 itself. Thus, in a Th2 environment, MIF may prevent the classical activation of macrophages. The suppressive effect of AAMs on the immune response is most likely dependent on the expression of arginase-1 (Arg-1) as indicated by studies in which mice macrophages lacking Arg-1 failed to suppress Th2 responses (reviewed in [41]).

B cells possess a variety of immune functions, including production of antibodies, presentation of antigens, and production of cytokines. IL-10-producing regulatory B cells have great potential to regulate T-cell-mediated inflammatory responses [5] and to downmodulate experimental autoimmune encephalomyelitis, collagen-induced arthritis, and inflammatory bowel disease [42]. In addition, in mouse models of chronic parasitic inflammation, such as chronic schistosomiasis, IL-10-producing B cells were also reported to be associated with protection against anaphylaxis [43]. Moreover, *H. polygyrus*-infected mice induced regulatory B cells that can downmodulate both allergy and autoimmunity in an IL-10 independent manner [44].
5. Helminth Infections and Inflammatory Diseases

The helminth-induced immunosuppressive network may not only be beneficial for the host, but it can also have beneficial outcomes for the host, reducing allergic and autoimmune diseases [41, 45]. Epidemiological, cross-sectional studies support an inverse correlation between allergic diseases and helminth infection [46, 47] including infections by nematode species like *A. lumbricoides* and *Necator americanus* [48]. An increased skin reactivity to house dust mites was found after antihelminthic treatment against infection with *A. lumbricoides* and *T. trichiura* [49]. Studies performed in animal model system have confirmed that helminth infection can protect against allergic disease and in particular lung-associated inflammation. For instance, *S. mansoni*-infected BALB/c mice were protected against OVA-induced experimental allergic airway inflammation (EAAI) as indicated by reduction of eosinophils in BAL, Th2 cytokine production, OVA-specific IgE levels and reduction of the number of inflammatory cells in lungs. Here, induction of CD4+CD25+Foxp3+ regulatory T cells was independent of number of inflammatory cells in lungs. There, induction of *Litomosoides sigmodontis* suppressed all pathological features of the OVA-induced EAAI model [51]. Additionally, these authors observed significantly increased numbers of Treg cells in spleen and mediastinal lymph nodes in infected OVA-treated mice compared to OVA-controls animals. Suppression of EAAI during the course of *H. polygyrus* infection was shown to involve the induction of Treg cells [52]. Infection with the same parasite resulted also in the inhibition of allergic response to peanut extract [53].

Several epidemiological studies have investigated the protective effect of parasitic infections in different autoimmune diseases like multiple sclerosis and type 1 diabetes [54]. Studies indicate that persons infected with chronic parasitic worm infections have lower rates of inflammatory bowel disease (IBD) than persons without these infections [55]. Experiments carried out using animal models of human autoimmune diseases have shown that parasites can interfere with autoimmunity. *Schistosoma mansoni* infection has been shown to protect from type 1 diabetes [56] and reduces the severity of EAE [57] while infection with *H. polygyrus* suppresses the experimental colitis [58]. Infection with *L. sigmodontis* prevented diabetes in NOD mice. In this study, protection was associated with increased Th2 responses and Treg cell numbers [59].

The immunomodulatory effect of helminth-derived products has been extensively studied. Table 1 provides an overview of different helminthic antigens with immunoregulatory properties. Findings regarding the use of parasite antigens to suppress experimental inflammatory diseases are summarized in Table 2.

Although the majority of data suggest that infection with helminths is associated with a suppression of allergic and autoimmune responses, some examples provide the opposite view. Epidemiological studies indicate that infection with *Ascaris* spp, *Toxocara* spp, *Fasciola hepatica*, hookworms, or *Enterobius vermicularis* has no protective effects or even enhanced allergic responses (reviewed in [95]).

There are also experimental studies that show that infection with some helminths have a positive association with allergy. A study using a murine model has shown that *T. canis* infection results in exacerbation of EAE [96]. Other animal experiments provided evidence that parasites like *Nippostrongylus brasilienis* [97] and *B. malayi* [98] could also induce or exacerbate allergic responses. Exacerbation of anaphylaxis has been shown to occur during *T. spiralis* infection [99]. The links between infections and autoimmunity are complex and there is scarce evidence on the induction or exacerbation of autoimmune responses by helminths [100].

6. Contrasting Effect of *Toxocara* and *Trichinella* Infections on Inflammatory Diseases

*Toxocara canis* and *Toxocara cati* are roundworms of dogs and cats, respectively, that can also infect humans worldwide. After ingestion of the infectious *Toxocara* eggs, the larvae migrate to the intestine, liver, and lungs. While in dogs and cats under the age of 6 months, the larva migrate back to the intestine; in humans, migration continues to other organs where they can persist for many years [101]. *Toxocara* infection results in the induction of Th2 cells that make cytokines such as IL-4, IL-5, and IL-13, which induce responses to the parasite such as increased IgE levels and eosinophilia (reviewed in [6]). *Trichinella spiralis* is also a roundworm that infects different mammals including humans and mice. After ingestion of *Trichinella* infected meat, the larvae migrate to the intestine and matures to the adult stage, the parasites mate, and finally the newborn larvae (NBL) migrate to striated muscle cells where they become encysted. Infection with *T. spiralis* is characterized by the induction of a Th1 type of response at the beginning of the intestinal phase. When the NBL disseminate, a dominant Th2 type of response develops which is essential for parasite expulsion [102]. Ingestion of both *Toxocara* spp. and *Trichinella* spp. commonly results in chronic infections. Interestingly these helminths have a contrasting effect on inflammatory diseases, while infections with *Trichinella* spp. can suppress (reviewed in [103]) *Toxocara* spp. exacerbate inflammatory diseases [6]. Studies using animal models for human autoimmune and allergic diseases indicate that *Trichinella* infection ameliorates these immune disorders (Table 3). Khan et al. showed that *T. spiralis* infection reduces the severity of dinitrobenzenesulphonatic-acid- (DNBS-) induced colitis in C57BL/6 mice [88]. Motomura et al. demonstrated that in addition to the protection exerted by the actual infection, rectal submucosal administration of *T. spiralis* crude muscle larvae antigen can also protect [78]. *T. spiralis* infection also ameliorated autoimmune diabetes in NOD mice [89] and modulated severity of the disease in the experimental model of multiple sclerosis (MS), namely, experimental autoimmune encephalomyelitis (EAE) in Dark Agouti rats in a dosenon-dependent manner [90]. In this study severity of EAE as
### Table 1: Helminth-derived antigens with immunoregulatory properties.

<table>
<thead>
<tr>
<th>Helminth</th>
<th>Antigen</th>
<th>Immunoregulatory mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Schistosoma mansoni</em></td>
<td>LNFPIII (lacto-N-fucopentaoseIII)/SEA (soluble egg antigen)</td>
<td>Interact with TLR4 to produce Th2 polarizing DCs</td>
<td>[60]</td>
</tr>
<tr>
<td><em>Schistosoma mansoni</em></td>
<td>Schistosome lysophosphatidylserine</td>
<td>Interact with TLR2 to induce Treg polarizing DCs</td>
<td>[61]</td>
</tr>
<tr>
<td><em>Acanthocheilonema vitae</em></td>
<td>ES-62</td>
<td>Exert immunomodulatory effects on macrophages and DCs by a TLR4-dependent mechanism with consequent Th2 polarisation</td>
<td>[62–64]</td>
</tr>
<tr>
<td><em>Nippostrongylus brasiliensis</em></td>
<td>Excretory-secretory antigen (NES)</td>
<td>Potently induce Th2 type of response via DC</td>
<td>[65]</td>
</tr>
<tr>
<td><em>Brugia malayi</em> adult</td>
<td>Cystatins (cysteine protease inhibitors) CIP-2</td>
<td>Interfere with antigen processing in human cells and inhibits B cells</td>
<td>[66, 67]</td>
</tr>
<tr>
<td><em>Brugia malayi</em> microfilariae</td>
<td>Serpins (serine protease inhibitors) SPN-2</td>
<td>Block neutrophil protease and promote Th1 type of response</td>
<td>[68]</td>
</tr>
<tr>
<td><em>Brugia malayi</em> L3 larvae</td>
<td>ALT-1/2 proteins</td>
<td>Inhibit macrophage resistance and present good filarial vaccine candidate</td>
<td>[69]</td>
</tr>
<tr>
<td><em>Toxocara canis</em></td>
<td>TES32—C type lectin (CTL)</td>
<td>Inhibit TLR responses on DC and compete with host lectins for ligands, thereby blocking host immunity</td>
<td>[70]</td>
</tr>
<tr>
<td><em>Heligmosomoides polygyrus</em></td>
<td>Excretory-secretary antigen (HES)</td>
<td>Induce regulatory T cells through TGF-βR</td>
<td>[37]</td>
</tr>
<tr>
<td><em>Teladorsagia circumcincta</em></td>
<td>Excretory-secretary antigen</td>
<td>Induce generation of Foxp3+ regulatory T cells through TGF-β mimicking effect</td>
<td>[73]</td>
</tr>
<tr>
<td><em>Trichinella spiralis</em></td>
<td>Adult excretory-secretary antigen (AdES); newborn larva antigen (NBL); crude muscle larva antigen (MLCr)</td>
<td>All antigens from different life stages induce polarization towards mixed Th1/Th2 with predominance of Th2 response, via semimatured DC</td>
<td>[74]</td>
</tr>
<tr>
<td></td>
<td>Excretory secretory muscle larva antigen</td>
<td>Induce mixed Th1/Th2 response with the predominance of Th2 component and elicit regulatory arm of immune response</td>
<td>[75]</td>
</tr>
<tr>
<td></td>
<td>Excretory secretory muscle larva antigen</td>
<td>Interfere with LPS-induced DC maturation and induce expansion of Foxp3+ regulatory T cells</td>
<td>[22]</td>
</tr>
<tr>
<td><em>Fasciola hepatica</em></td>
<td>Thioredoxin peroxides</td>
<td>Alternatively activated macrophages</td>
<td>[76]</td>
</tr>
</tbody>
</table>

EAE: experimental autoimmune encephalomyelitis; DNBS: dinitrobenzene sulfonic acid; TNBS: trinitrobenzene sulfonic acid; DSS: dextran sodium sulfate; T1D: type 1 diabetes; EAAI: experimental allergic airway inflammation.

### Table 2: Suppression of experimental inflammatory diseases by parasite-derived antigens.

<table>
<thead>
<tr>
<th>Helminth</th>
<th>Antigen</th>
<th>Model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichinella spiralis</em></td>
<td>Soluble antigens of muscle larvae</td>
<td>EAE</td>
<td>[77]</td>
</tr>
<tr>
<td><em>Trichuris suis</em></td>
<td>Soluble antigens of adult worm</td>
<td>EAE</td>
<td>[77]</td>
</tr>
<tr>
<td><em>Ancylostoma ceylanicum</em></td>
<td>Soluble and excretory-secretory antigens of adult worm</td>
<td>DSS-induced colitis</td>
<td>[79]</td>
</tr>
<tr>
<td><em>Hymenolepis diminuta</em></td>
<td>Soluble antigens of adult worm</td>
<td>DSS-induced colitis</td>
<td>[80]</td>
</tr>
<tr>
<td><em>Heligmosomoides polygyrus</em></td>
<td>Excretory-secretary antigens (HES) of adult worm</td>
<td>EAAI</td>
<td>[73]</td>
</tr>
<tr>
<td><em>Ancylostoma caninum</em></td>
<td>Excretory-secretary antigens of adult worm</td>
<td>TNBS-induced colitis</td>
<td>[81]</td>
</tr>
<tr>
<td><em>Acanthocheilonema vitae</em></td>
<td>ES-62</td>
<td>Collagen-induced arthritis</td>
<td>[82]</td>
</tr>
<tr>
<td><em>Schistosoma mansoni</em></td>
<td>SEA and soluble adult worm antigen</td>
<td>T1D</td>
<td>[83]</td>
</tr>
<tr>
<td></td>
<td>Recombinant proteins (Sm22-6, Sm29) and soluble adult worm fraction (PIII)</td>
<td>EAAI</td>
<td>[84]</td>
</tr>
<tr>
<td><em>Schistosoma japonicum</em></td>
<td>SEA</td>
<td>EAAI</td>
<td>[85]</td>
</tr>
<tr>
<td><em>Nippostrongylus brasiliensis</em></td>
<td>Excretory-secretary antigens (NES) of adult worm</td>
<td>EAAI</td>
<td>[86]</td>
</tr>
<tr>
<td><em>Ascaris suum</em></td>
<td>Soluble antigens of adult worm</td>
<td>EAAI</td>
<td>[87]</td>
</tr>
</tbody>
</table>
judged by cumulative disease index, maximal clinical score, duration of illness, and the number of mononuclear cells infiltrating the spinal cord in *T. spiralis* infected animals were all reduced in comparison to the uninfected EAE-induced group. In a following study, these authors reported that alleviation of the disease in infected-EAE rats coincided with reduced IFN-γ and IL-17 production and increased IL-4, IL-10, and TGF-β production. They suggested that mechanisms underlying the observed beneficial effect include Th2 and regulatory responses provoked by the parasite. Transfer of T-cell-enriched spleen cells from *T. spiralis*-infected rats that contained a higher proportion of CD4+CD25+Foxp3+ regulatory T cells into rats in which EAE was induced caused amelioration of EAE, which indirectly points to the role of Treg in restraining inflammatory conditions [91]. Boles et al. have shown with another *Trichinella* species, namely, with *Trichinella pseudospiralis*, that infection results in suppression of MS in the rat [93]. These authors used this model to compare the anti-inflammatory effects of the intestinal and late migratory phases of *T. pseudospiralis* infection on development of myelin-basic-protein- (MBP-) induced MS-like debilitation. Findings from this study indicate that the late migratory phase of infection which occurred during the peak of MBP-induced debilitation significantly improved performance scores in mobility, coordination, and strength. Wu et al. also reported on amelioration of clinical severity and delayed onset of EAE after *T. pseudospiralis* infection. This effect was associated with suppression of Th17 and Th1 responses induced by infection [94]. *Trichinella pseudospiralis* is markedly different from *T. spiralis* in that it is smaller in size and that the muscle stage larvae are not surrounded by a capsule [104]. Whether the mechanisms involved in immunosuppression varies depending on *Trichinella* species remains to be investigated.

Infection with *T. spiralis* can also ameliorate EAAI [92]. In this study, the concentrations of IL-10 and TGF-β were significantly increased and the recruitment of Treg into draining lymph nodes was elevated as the result of *T. spiralis* infection. This protective effect has been recently shown to occur during acute as well as chronic phases of *Trichinella* infection [105]. Protection against EAAI to OVA was stronger during the chronic phase of infection and associated with increased numbers of splenic CD4+CD25+Foxp3+ Treg cells with suppressive activity. Adoptive transfer of CD4+ T cells from chronically infected mice with elevated numbers of Treg cells in the spleen induced partial protection against EAAI [105]. The possible mechanisms by which helminths or their products could inhibit allergic responses are depicted in Figure 1.

In contrast to the suppressive effect of *Trichinella* infections on allergic diseases experimental as well as epidemiological studies indicate that *Toxocara* infections are risk factors for allergies, including allergic asthma [6].

Studies using murine models for toxocariosis indicate that infection with *T. canis* leads to persistent pulmonary inflammation, eosinophilia, increase levels of circulating IgE, airway hyperreactivity, and production of Th-2 type cytokines. Pulmonary inflammation has been shown to develop as soon as 48 hours after infection, it occurs in a dose-dependent manner, and it can persist up to 2 or 3 months. Eosinophil counts also increase in the bronchoalveolar lavage (BAL) of *Toxocara*-infected mice [106, 107]. Relative quantification of cytokine expression in lungs of mice infected with different *T. canis* doses showed that while a proportional increased expression of the IL-4, IL-5, and IL-10 transcripts was observed, the expression of IFN-γ was not different from that of uninfected controls [107]. Results from this study indicate that infection of mice with *T. canis* results in a dominant Th2 type of immune response, independent of the inoculum size [108]. In addition, infection of BALB/c mice with 1,000 *T. canis* embryonated eggs results in hyperreactivity of the airways that persisted up to 30 days p.i. Evaluation of parasite burden revealed that few *T. canis* larvae were still present in the lungs of infected mice at 60 days p.i. which could explain the persistent pulmonary inflammation observed in these mice [107].

Common features between allergic asthma and toxocariosis are the induction of a Th2-mediated mediated immune response including the production of high levels of IgE, and eosinophilia. In addition, infection with *Toxocara* spp. shares common clinical features with allergic asthma such as inflammation of the airways accompanied with wheezing, coughs, mucus hypersecretion, and bronchial hyperreactivity. In order to study the effect of *Toxocara* infection on allergic manifestations two murine models were combined, namely, the murine model for toxocariosis described above and a murine model for allergic airway inflammation. For this study BALB/c mice were infected with 500 embryonated *T. canis* eggs and exposed to OVA sensitization followed by OVA-challenge. Results indicate that infection with *T. canis* in combination with OVA treatment led to exacerbation of pulmonary inflammation; eosinophilia; airway hyperresponsiveness; increase of OVA specific and total IgE; increased expression of IL-4 compared to mice that were only *T. canis* infected or OVA treated. The observed exacerbation of EAAI was independent of the timing of infection in relation to

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**Table 3: Experimental models of Th2-mediated inflammatory diseases successfully treated by *Trichinella* infection or administration of *Trichinella* antigens.**

<table>
<thead>
<tr>
<th><em>Trichinella</em> spp. or their products</th>
<th>Experimental disease model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. spiralis</em></td>
<td>Exp. colitis</td>
<td>[88]</td>
</tr>
<tr>
<td></td>
<td>T1D</td>
<td>[89]</td>
</tr>
<tr>
<td></td>
<td>EAE</td>
<td>[90]</td>
</tr>
<tr>
<td></td>
<td>EAE</td>
<td>[91]</td>
</tr>
<tr>
<td></td>
<td>EAAI</td>
<td>[92]</td>
</tr>
<tr>
<td></td>
<td>EAAI</td>
<td>[22]</td>
</tr>
<tr>
<td><em>T. pseudospiralis</em></td>
<td>EAE</td>
<td>[93]</td>
</tr>
<tr>
<td></td>
<td>Exp. colitis</td>
<td>[78]</td>
</tr>
<tr>
<td><em>T. spiralis</em> crude muscle larvae antigen</td>
<td>Exp. colitis</td>
<td>[77]</td>
</tr>
<tr>
<td></td>
<td>EAE</td>
<td>[94]</td>
</tr>
</tbody>
</table>

Exp. colitis: experimental colitis; T1D: type 1 diabetes; EAE: experimental autoimmune encephalomyelitis; EAAI: experimental allergic airway inflammation.
allergen exposure. In conclusion, infection with *T. canis* leads to exacerbation of EAAI [96].

Several factors may influence the differential effect of helminth infections on allergic diseases [6]. One of these factors is whether the host is definitive or accidental. The normal or definitive hosts for *T. canis* are dogs whereas humans are accidental hosts for this parasite. In an accidental host the parasite does not usually develop to the adult stage and in case of *Toxocara* spp. the continuous migration of the larvae through different organs including the lungs can cause more damage comparing to what happens in a definitive host where migration is transitory. *T. spiralis* can infect many different mammals including mice and humans in which the parasite completes its life cycle. The infected mammal is therefore a definitive host for this helminth. And although *T. spiralis* pass through the lung microvascular system on its way to the skeletal muscle, it is a rapid process in which the larvae are usually not trapped in the lungs [109]. It is likely that there are differences between parasites of humans such as *T. spiralis* that have evolved with their host and have developed strategies to survive without causing much damage compared to parasites such as *Toxocara* spp. for which humans are accidental host [6, 110].

In conclusion, helminths induce an anti-inflammatory response, which could ameliorate inflammatory diseases; however, this is not a universal property of all helminths and different factors such as the helminth species, and whether the host is definitive or accidental, the parasite load and acute versus chronic infections may all influence the overall effect of helminth infections on inflammatory diseases. Identification of the helminth molecules that induce immunosuppression and elucidation of the mechanisms involved is essential for the development of alternative strategies for prevention and/or treatment of inflammatory diseases.

References


Review Article

Human Schistosome Infection and Allergic Sensitisation

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Received 14 May 2012; Accepted 28 June 2012

Academic Editor: Maria Ilma Araujo

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Several field studies have reported an inverse relationship between the prevalence of helminth infections and that of allergic sensitisation/atopy. Recent studies show that immune responses induced by helminth parasites are, to an extent, comparable to allergic sensitisation. However, helminth products induce regulatory responses capable of inhibiting not only antiparasite immune responses, but also allergic sensitisation. The relative effects of this immunomodulation on the development of protective schistosome-specific responses in humans has yet to be demonstrated at population level, and the clinical significance of immunomodulation of allergic disease is still controversial. Nonetheless, similarities in immune responses against helminths and allergens pose interesting mechanistic and evolutionary questions. This paper examines the epidemiology, biology and immunology of allergic sensitisation/atopy, and schistosome infection in human populations.

1. Introduction

The major human helminth parasites belong to two phyla, the nematodes (or roundworms) which include intestinal soil transmitted helminths (STH) and filarial worms (which cause lymphatic filariasis and onchocerciasis), and the platyhelminths (or flatworms) which include the flukes (or trematodes, including schistosomes) and the tapeworms (or cestodes). Although common in most parts of the world sixty years ago [1], these parasites are currently mainly prevalent in sub-Saharan Africa, Asia, and South America [2–4], where they are responsible for considerable disabilities including blindness and elephantiasis (filarial worms). Furthermore, helminth infections are responsible for morbidities that include anaemia, stunted growth, poor cognitive development, and malnutrition [5–7], hence exert a negative socioeconomic impact in some of the poorest communities in the world.

Immune-mediated diseases including auto-immune diseases (such as type 1 diabetes, inflammatory bowel diseases, and rheumatoid arthritis) and allergic diseases (such as asthma, allergic rhinitis, and atopic eczema) are reported to be more prevalent in developed countries and in urban areas of developing countries [8, 9]. But studies from Africa are demonstrating that allergic diseases are common, if not acknowledged, clinical problems in this region [10]. Immune disorders have been responsible for increased mortality and morbidity worldwide [11–13] and they negatively impact on economic growth due to their elevated cost of their treatment [14, 15]. There is also mounting evidence that allergic disorders, especially allergic rhinitis, are associated with attention deficit disorder and hyperactivity in children [16, 17].

Increasing rates of childhood allergies have long been a puzzle to epidemiologists [18, 19]. Thus, studying cohorts of children born in 1946, 1958, and 1970, concluded that a “new environmental agent,” contained in breast milk and possibly infants’ food was responsible for the increase in eczema. Emmanuel, reviewing medical literature published from 1820 to 1900, suggested that the hay fever “epidemic” was associated with the rapid industrial growth of the 19th century since this disorder was rarely described prior that period [19]. It was Strachan who in 1989, observing that the rate of hay fever and eczema was consistently negatively associated with attention deficit disorder and hyperactivity in children [16, 17].
Table 1: Heterogeneity in studies investigating the effect of helminth infection on atopy.

<table>
<thead>
<tr>
<th>Parasite spp, References</th>
<th>Atopy outcome</th>
<th>Association</th>
<th>Population age</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[37]¹</td>
<td>Wheeze, SPT</td>
<td>Negative</td>
<td>1–4 years</td>
</tr>
<tr>
<td>[38]²</td>
<td>IgE, PK</td>
<td>Negative</td>
<td>5–15 years</td>
</tr>
<tr>
<td>[39]¹</td>
<td>SPT, airway responsiveness</td>
<td>Positive</td>
<td>8–18 years</td>
</tr>
<tr>
<td>[40]²</td>
<td>Allergen-induced Th2 cytokines</td>
<td>None</td>
<td>7–13 years</td>
</tr>
<tr>
<td>[41]²</td>
<td>SPT, wheeze</td>
<td>None</td>
<td>9 years mean age</td>
</tr>
<tr>
<td>[42]²</td>
<td>SPT</td>
<td>Negative</td>
<td>6–17 years</td>
</tr>
<tr>
<td></td>
<td>Wheeze, eczema, EIB</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichuris trichiura</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[38]²</td>
<td>IgE, PK</td>
<td>Negative</td>
<td>5–15 years</td>
</tr>
<tr>
<td>[37]¹</td>
<td>Wheeze, SPT</td>
<td>None</td>
<td>1–4 years</td>
</tr>
<tr>
<td>[43]²</td>
<td>SPT</td>
<td>Negative</td>
<td>2–8 years</td>
</tr>
</tbody>
</table>

*Hookworm*  

|               |               |             |                |
| [37]¹        | Wheeze, SPT   | None        | 1–4 years      |
| [42]²        | SPT           | Negative    | 6–17 years     |
|             | wheeze, eczema, EIB | None |                |

*Schistosoma mansoni*  

| [44]¹ | SPT, IgE | Negative | 18 ± 9.7 years |
| [45]² | SPT, asthma symptoms | Negative | 15 years mean age |

*Schistosoma haematobium*  

| [46]¹ | SPT | Negative | 5–14 years |

Cross-sectional ¹ and treatment followup ² studies are reported here. ²Longitudinal approach but treatment intervention was not the primary objective of the study. SPT: skin prick test; PK: Prausnitz-Kustner passive transfer test, EIB: exercise-induced bronchoconstriction.

the “hygiene hypothesis,” was subsequently supported by some epidemiological studies [20, 21] but contradicted by others [22, 23] (see summary in Table 1). In a retrospective case control study on Italian military cadets, Matricardi and colleagues were able to show that cumulative exposures to foodborne and oral-faecal infections, but not infections transmitted via other routes, were associated with a reduced risk of being atopic [24]. They suggested that the mode of transmission of the pathogen was a determining factor in subsequent protection (or lack of protection) against atopy and asthma, hence explaining inconsistencies in previous studies.

2. Global Burden of Schistosomiasis and Atopy

2.1. *Schistosomiasis*. Schistosomiasis accounts for up to 70 million DALYs annually [6], with an estimated 15,000 deaths [4], and children carry the heaviest burden of infection [47]. With these figures, schistosomiasis is classified second only to malaria in terms of human morbidity and mortality due to parasitic diseases [48]. Schistosomiasis is caused by infection with blood-dwelling trematodes of the genus *Schistosoma*, of which *S. haematobium*, *S. mansoni*, and *S. japonicum* are the main human schistosomes [49]. It is typically prevalent in rural areas where natural streams, ponds, rivers, and lakes harbouring the infected intermediate host snails, are the main sources of water for domestic or occupational purposes such as washing and fishing. School children usually become infected during swimming or collecting water, while younger children and infants become infected when accompanying adults (washing clothes or collecting water) or by being bathed in these water sources [50].

2.2. *Atopy*. Rising rates of atopic diseases have been reported in developed countries since the end of World War II [18] and currently constitute a major public health issue [51]. Demographic data in the US have shown an average increase in childhood asthma prevalence of 4.3% per year from 1980 to 1996, with associated deaths and hospitalisation increasing by 3.4% and 1.4%, respectively [52]. In the United Kingdom, according to the British Allergy Foundation, 1 in 3 people suffer from allergy at some time in their lives. This report indicates that 58% of allergic sensitisations are triggered by house dust mites (HDM), a known risk factor for developing asthma and allergic rhinitis [53, 54]. Increasing prevalence of asthma in adults over a period of 10 years and doubling in school children over 20 years have been reported in Australia [55]. A recent study involving 12 European countries and 19 centres reported incidences of asthma between 5 and 17% (average 8%), while allergic rhinitis varies between 23 and 44%, with an average of 30% [56].

In less affluent countries, comparable rates of atopic diseases are generally reported in urban and suburban areas. Thus, a prevalence of asthma of 9% was reported in urban
areas of Rwanda [57] while the International Study of Asthma and Allergies in Childhood (ISAAC) reported an overall prevalence of 10.9% across 22 centres in Africa [58]. Reported incidences of allergic rhinitis range from 14% to 54% in urban and suburban areas across African countries (reviewed by [59]). Importantly, according to the ISAAC phase three, although the prevalence was generally lower, there were more severe symptoms of rhinoconjunctivitis reported in urban centres of developing countries compared to those reported in developed countries [8]. However, studies from Africa suggest that allergic conditions may be underdiagnosed in Africa due to “inappropriate” diagnostic tests and these studies call for component-resolved allergy testing in Africa [60]. Indeed a recent study in Zimbabwe showed that schistosome-infection resulted in impaired diagnosis of cat allergy [61].

3. Atopy and Schistosome Life-Cycle Stages

In schistosome infection, the human immune system is exposed to schistosome larvae (cercariae and schistosomula), adult worm, and egg antigens. Animal studies as well as in vitro studies have demonstrated immunological changes and regulatory mechanisms associated with these different life-cycle stages. The surface of cercariae (enriched in carbohydrates [62]) and the newly transformed schistosomula activate the complement cascade [63, 64] and eliciting proinflammatory responses [65, 66]. An excessive immunological reaction to skin stage cercarial antigens results in cercarial dermatitis or swimmer’s itch [67], an allergic condition also occurring in contact with nonhuman schistosomes that is prevalent in developed countries [68–70]. This inflammatory reaction is rarely reported in populations in which schistosomiasis is endemic, possibly due to regulatory responses resulting from multiple exposures as has been demonstrated in mice [71]. Such regulatory responses may be induced by skin-stage schistosomula-derived molecules such as prostaglandin E₂ (PGE₂) which upregulates IL-10 production during skin penetration by the parasite [72]. The PGE₂ is also secreted by the lung-stage schistosomula during migration through the capillary beds of the lungs, and this is thought to diminish eosinophil infiltrates around the parasites (and thus inflammation [73]). In addition, these parasites are capable of inhibiting the expression of endothelial adhesion molecules such as E-selectin and VCAM-1, limiting leucocyte recruitment in the lungs [74]. These anti-inflammatory mechanisms in the lungs have been suggested as potential explanations for reduced severity of asthma symptoms in schistosome-infected asthmatic patients [45], although there are no mechanistic studies from human populations to support this.

Schistosome eggs are major Th2 triggers as demonstrated in murine studies [75, 76], and they induce formation of fibrotic lesions or granulomas [77–79]. Indeed, an S. mansoni egg-secreted glycoprotein, omega-1, has recently been identified that conditions dendritic cells for Th2 polarisation [25]. However, egg secretions are capable of inhibiting the specific binding of chemokines such as CXCCL8 (IL-8) and CCL3 (MIP-1α), therefore blocking chemokine-elicited migration of neutrophils and macrophages respectively during granuloma formation [80].

Schistosome adult worm antigens also induce Th2 responses and IgE in mice [81], baboons [82], and humans [83]. However, this parasite life stage elicits high levels of modulatory responses capable of inhibiting antiparasite [84] as well as allergic reactions [85]. The latter study demonstrated that worm infection induces IL-10, producing B cells that could protect mice against anaphylaxis. They later demonstrated that egg-laying worms exacerbate while single sex worms (precluding egg production) inhibit airway hyperresponsiveness [86].

Together, these studies show that the different parasite life-cycle stages are associated with different mechanisms of regulation and inflammation. Although concurrent exposure to all or most antigens is likely in endemic areas, and despite the fact that they may induce cross-reactive immune responses [87], the different parasite life-cycle stages may differentially affect atopic responses.

4. Epidemiology

4.1. Schistosomiasis. The epidemiological patterns of schistosomiasis differ from those of atopy, mainly because of their aetiology. Indeed, while schistosome infection is acquired as a result of exposure to parasites, atopy is a genetic predisposition (although the clinical manifestations are influenced by environmental factors). In schistosome endemic areas, infection levels follow a convex shape with host age, where infection intensity rises to peak in childhood-adolescence and decline in adulthood [88]. This peak was initially interpreted as arising from different water contact levels between age groups [89]. However, longitudinal studies showed that with the same exposure rate, “resistant” individuals were older than “susceptible” individuals [90–92], suggesting an age-dependent acquired resistance to reinfection. In addition, in communities of different parasite transmission, infection intensity peaks at a younger age in areas of high transmission compared to low-transmission areas [88, 93–95], a phenomenon referred to as a “peak shift.” This phenomenon has been interpreted as reflecting different rates of development of acquired resistance to infection during schistosome infection as has been reported for Plasmodium infections (which cause malaria) [96, 97]. This interpretation is supported by age-related changes in immune responses, with the peak of antibodies and cytokine associated with protection coinciding with the decline in infection levels have been reported in S. haematobium endemic areas [83, 98, 99]. More recently, Black and colleagues observed that the rate of acquisition of antischistosome protective responses by adults occupationally exposed to S. mansoni, following treatment, is dependent on their history of exposure, being faster in those with a longer history [100]. This study, consistent with earlier studies [101, 102], demonstrated that resistance to schistosome infection/reinfection is acquired independent of age related physiological changes [103].

4.2. Atopic Diseases. Atopy is the genetic predisposition to become excessively sensitised and produce high levels of
IgE [104]. However, atopic diseases result from a genetic predisposition in combination with environmental stimuli such as allergens, smoke, diet, and/or infectious agents [105, 106]. The epidemiology of atopic diseases is complex as some diseases may become more prominent with age while others diminish or disappear [107]. The earliest phases of atopic diseases usually manifest during the first five years of life and the severity (and prevalence) of clinical symptoms seem to increase in late childhood/adolescence and plateau throughout adulthood [108–110] or decline for some conditions [111, 112].

It has been suggested that a natural history of allergy manifestations in atopic individuals involves progression from atopic eczema (below one year of age) to asthma or allergic rhinitis (late childhood/adolescence), a phenomenon referred to as the “atopic/allergic march” [112]. However, this is not always consistent as some children may develop atopic dermatitis long after the onset of asthma [113], while some atopic individuals may only develop one of these conditions throughout life. Longitudinal studies indicate that atopy in infancy predicts the occurrence and severity of asthma [114] and bronchial hyperresponsiveness [115] in later life. Total and allergen-specific IgE levels also seem to increase throughout childhood in allergic individuals [116]. However, a number of events occurring in the first few years of life and in utero are likely to influence the onset and persistence of disease. Thus, Kliinert and colleagues have shown that respiratory infections during the first year of life and parenting difficulties (e.g., postnatal maternal depression) were independent predictors of the onset of asthma during early (3 years) and late (6–8 years) childhood in children at risk [114, 117].

Microbial exposures and diet of pregnant mothers may also alter early gene expression in neonates, influencing the onset of allergy in childhood (see [106, 118]). Consistent with this hypothesis is the finding that maternal exposure to farm milk and farm animals during pregnancy was associated with demethylation within the FOXP3 (Treg transcription factor) locus in cord blood and subsequent elevated levels of regulatory T cells (Tregs) (and their suppressive activity) in offspring [119]. Murine studies have also shown that, when exposed to a methyl rich diet during pregnancy (as may be the case for folate supplementation in humans), foetal DNA may undergo changes in methylation that results in decreased gene transcription activity, leading to subsequent enhanced development and severity of allergic diseases [120]. The study also showed that this diet-associated allergic phenotype was transgenerationally inheritable (persistence of high levels of IgE and eosinophilia into the F2 generation).

5. Effector Responses in Atopy and Schistosoma Infection

5.1. Immunoglobulin E. Identified in the 1960s as a “carrier of reaginic activity” [121, 122], IgE is well known as a central player in atopic diseases and anaphylactic reactions. This antibody is part of a protein network involving its 3 receptors, namely, the FceRI, the CD23 (or FcεRII) and galectin-3 [32], all of which can be found in soluble forms [123, 124]. The FceRI (also termed high-affinity receptor) is mainly expressed on mast cells and basophils but also on epidermal Langerhans cells [125] and eosinophils [126, 127]. Cross-linking of these high-affinity receptors by IgE induces activation of mast cells and basophils and their degranulation. The galectin-3 receptor is expressed on neutrophils and on trophoblast cells in placentas [128], where it is thought to facilitate IgE transport [129]. The CD23 receptor facilitates the transport of IgE-antigen complexes but is also involved in the regulation of IgE synthesis [32]. Highly conserved in mammalian lineages [130], IgE is thought to have evolved as a first line of defence against helminth parasites.

IgE antibodies are naturally strongly regulated and have the lowest concentrations of all antibodies in serum of healthy nonatopic individuals [32]. Mechanisms of regulation of IgE include its short half-life in serum (12 h for murine monoclonal antibodies [131]), the poor processing of mRNA for the membrane ε heavy chain [132], and the negative feedback regulation by the CD23 [133]. The latter has been a subject of investigations in terms of therapeutic application in atopic diseases but also in autoimmune diseases [134] and chronic lymphocytic leukaemia [135].

5.2. CD23. CD23 is the low-affinity receptor for IgE and differs from the high-affinity FceRI receptor in structure and function. Thus, while cross-linking of the latter results in degranulation of mast cells and release of mediators, engagement of membrane-bound CD23 suppresses the production of IgE by B lymphocytes [33]. CD23 has long been proposed as a natural regulator for IgE synthesis [133] although elevated levels of CD23+ B cells have been reported in atopic patients [136]. As initially suggested by Aubry and colleagues [137], CD23 not only binds IgE but also CD21, a cell-surface protein expressed on T-cell, B-cell, and follicular dendritic cells, classically identified as a receptor for complement proteins [138] or Epstein-Barr virus [137]. The interaction between CD23, IgE, and CD21 may lead to either negative or positive regulation of IgE synthesis (reviewed in [34–36]). The binding of IgE stabilises membrane-bound CD23 and inhibits IgE synthesis from activated B cells, while in the absence of IgE binding, CD23 is cleaved by ADAM10 (a disintegrin and metalloprotease protein 10), and this destabilisation enhances IgE synthesis [32]. Soluble CD23 (sCD23) fragments resulting from the cleavage can bind to IgE with different affinities and outcomes for IgE synthesis depending on their oligomerization state. Trimers bind IgE with high affinity and enhance IgE synthesis by their ability to also bind the CD21 receptor while monomers bind with low affinity but do not bind CD21 and hence inhibit IgE synthesis [36, 139].

5.3. Immunoglobulin 4. Serum IgG4 antibodies, the least abundant among human IgG subclasses, have long been associated with IgE-mediated diseases [140–142]. However, rather than the cause of disease, these antibodies seem to be involved in the regulation of IgE-induced anaphylactic reactions [143]. IgG4 may interfere with antigen recognition by IgE due to their similar antigenic specificity [144], although different epitope-binding [145]. In a process that
involves exchange of fab molecules, IgG4 are structurally hetero-bivalent (each heavy chain and light chain recognising a different epitope within a single IgG4 molecule) and often function as monovalent [141, 146], to bring about anti-inflammatory effects [147]. The interaction between IgG4 and a given antigen results in small and non-pathological immune complexes (since these antibodies cannot cross-link antigens) [146]. Furthermore, in contrast to other IgG subclasses, IgG4 cannot fix complement but inhibits complement activation by IgG1 [148]. IgG4 antibodies, in allergy or helminth infection, are secreted in response to high antigen loads [141, 149, 150] but levels of the antibodies are differentially regulated by the same cytokines [151] as those regulating IgE, suggesting an important homeostatic mechanism for controlling IgE-mediated responses.

6. Control of Effector Responses in Atopy and Schistosome Infection

In addition to the cross-regulation between Th1 and Th2 [152] (and potentially other T cell subsets), there is growing evidence that Th2 cells interact with a complex network of other T cell subsets as well as B cells and antibodies, naturally or during disease (atopic or infectious). Thus, it has emerged that Tregs play an important role in the tolerance of ubiquitous antigens and that alterations in Treg function [153, 154] and/or the fine balance between Tregs and Th2 cells [155, 156] determines the clinical manifestation of atopy. Indeed, in healthy (nonatopic) individuals T cell polarization occurs in contact with environmental allergens but higher levels of Tregs dampen the effect of Th2 cells, leading to peripheral tolerance [156]. Tregs modulate the activity of Th2 (and Th1) cells via several mechanisms including the secretion of anti-inflammatory cytokines such as IL-10 and TGF-β [155, 157]. As the description and role of other recently identified T-helper cells is clarified (e.g., Th17 cells shown to be important in nonatopic asthma) regulation of Th2 mediated responses will also become clearer [158]. The role of cells such as the T-helper cells recently shown to produce both IL-17 and Th2 cytokines (IL-4, IL-5, IL-9, and IL-13) [159] in pathogenesis is currently under intense investigation. Our own group has recently described a role for Th17 responses in human schistosome-acquired immunity (submitted).

IL-10 producing B cells (Bregs) are also involved in the recruitment of Tregs, hence contributing to the regulation of Th2 responses as demonstrated in murine models of helminth infection (see [31]). IL-10 can inhibit effector functions of mast cells and eosinophils, and regulate the growth of several cells including B cells, NK cells, mast cells, and dendritic cells. Furthermore, IL-10 modulates IgE: IgG4 ratios [154] possibly by indirectly inducing the antibody switch to IgG4 in the B-cell progeny while preventing IgE production [160].

IgG4 may control IgE-mediated histamine release as has been demonstrated in filarial infection [143]. Furthermore, it has recently been shown that the binding patterns of IgG4 antibodies correspond to natural recovery from childhood IgE-mediated milk allergy [161], suggesting their potential protective role in atopic diseases, although this is still controversial [141]. In addition, early observations that IgG4 antibodies were highly elevated in sera of patients receiving allergen immunotherapy [162] have prompted the use of IgE: IgG4 ratio as a marker for successful immunotherapy [142, 163, 164]. In helminth infections, high IgG4: IgE ratio has been associated with reduced pathology while favouring a heavy worm load [150, 165, 166]. Interestingly, IgG4 may be one of the “regulatory antibodies” resulting from IgG sialilation involved in the control of immune disorders [167, 168].

7. Immune Responses in Atopy

The human immune system must distinguish between a dangerous pathogen and ubiquitous environmental allergens and has evolved to mount appropriate defensive responses to the first while tolerating (or ignoring) the latter. However, a certain proportion of individuals fail to tolerate environmental allergens and develop allergic diseases such as asthma, atopic dermatitis and allergic rhinitis. These result from excessive sensitisation to ordinary exposures to allergens [104]. IgE antibodies are critical effector molecules in the pathogenesis of these diseases [169]. Mast cells and basophils are coated with specific IgE antibodies and this results in immediate hypersensitivity (release of mediators) and/or late-phase inflammatory reaction (cytokine secretion and recruitment of leucocytes).

In atopic individuals, allergen products (e.g., cysteine proteases) activate epithelial cells, which produce thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 which in turn initiate Th2 polarisation with increased production of IL-4, IL-5, IL-9, and IL-13 cytokines [170, 171]. Th2 cytokines are involved in the class-switching to IgE as well as the development and recruitment of basophils, mast cells, and eosinophils (see Figure 1(c)). IgE binds to the high-affinity FceRI receptor on mast cells, basophils, and eosinophils which (upon exposure to allergens) results in their activation and degranulation (via cross-linking of allergens), with the release of preformed mediators such as histamine, cysteinyl leukotrienes, and prostaglandin D2 [154, 172].

A complex interplay between innate and adaptive immune responses underlies the heterogeneous characteristics of atopic diseases. Thus, recruitment of eosinophils into the lungs of asthmatics may be promoted not just by Th2 (IL-5) alone but in conjunction with natural killer T cells (NKT) as well as CD8+ T cells (see [173]). In addition, IL-17-producing T cells (Th17 [174]) may be involved in the severity of asthma [175]. These promote the recruitment and activation of neutrophils and lead to corticosteroids—resistant asthma [175]. IL-9-producing T-cell subset (Th9), which probably derive from Th2 cells under the influence of TGFβ1 [176], may also be involved in the production of IgE and mast cell recruitment in the lungs [173]. In allergic rhinitis, mast cells accumulate in the epithelium of the nasal mucosa where they secrete inflammatory cytokines (IL-6, IL-8, and TNFα) in addition to Th2 cytokines [177].
**8. Immune Responses during Schistosome Infection**

Acquired immunity to schistosome infection was first proposed by Fisher in the 1930s when analysing data from animal studies as well as those from hospital-diagnosed *S. haematobium* infected people [88]. Subsequently, the susceptibility of schistosome larvae to immune attack was demonstrated by in vitro studies showing that sera from *S. mansoni* infected individuals could damage schistosomula in the presence of normal human peripheral blood leucocytes [178]. This “antibody-dependent” killing was subsequently shown to be eosinophil mediated [178, 179], and studies on monoclonal antibodies led to the identification of IgE antibodies with the highest cytotoxicity for the schistosomula [180–182]. Field studies were conducted to identify antibody responses predictive of resistance to reinfection following chemotherapy. Hagan et al. [83] demonstrated in a multivariate logistic regression that reinfection with *S. haematobium* was less likely in individuals producing high IgG4 levels against the worm antigens and more likely in those producing high levels of IgG against the worm or egg antigens. The role of IgE in resistance was also demonstrated by Rihet et al. [183], who identified specific antigens (120–165 KDa and 85 KDa) to which IgE reacted (on immunoblots) and showed that these antibodies, in contrast to IgM and IgG, were significantly higher in the sera of the most resistant individuals. This study showed that some of the immunogenic antigens were readily accessible to IgE on living *S. mansoni* larvae as they were located on the outer membrane. However, Dunne and colleagues, working on *S. mansoni* as well, showed that IgE (produced following treatment) against adult worm antigens, particularly a 22 kDa tegumental antigen (Sm22), but not against any other life-cycle stages, were associated with resistance to reinfection following treatment [184]. Both antiadult worm and anti-schistosomula tegument IgE antibodies were associated with resistance to *S. mansoni* reinfection in another study in Brazil [185] while antiegg IgE antibodies also could confer protection against *S. japonicum* reinfection [186].

Collectively, these studies and several others [187–190] have led to the conclusion that resistance to schistosome infection/reinfection is dependent on IgE antibodies. However, data on other antibody isotypes have been reported which correlated with resistance to infection/reinfection. For example, IgG3 against the recombinant antigen Sh13 has
been associated with resistance to *S. haematobium* infection [191], while antiworm and cercarial IgM were significantly higher in individuals more resistant to reinfection with *S. mansoni* [185]. Furthermore, a decline in IgA together with an increase in IgG1 were associated with resistance acquired with host age as well as following treatment in *S. haematobium* endemic area [192]. IgA against Sm28GST antigen has also been associated with reduced *S. mansoni* fecundity and increased host resistance to reinfection [193]. More recently, antiworm IgE antibodies, as well as eosinophilia and the low affinity receptor for IgE (the CD23) have been shown to correlate with resistance in individuals undergoing multiple rounds of treatment [100, 194], again suggesting that IgE may be directly involved in parasite killing via antibody-dependent cellular cytotoxicity (ADCC) in vivo. However, since schistosomula are more susceptible to ADCC, it is possible that adult-worm-specific antibody responses may rather target the incoming larvae, a process termed “concomitant immunity” (as predicted by Fisher [88]) and well demonstrated in rhesus monkeys [195].

As initially demonstrated by in vitro studies [196–198], ADCC is dependent on Th2 cytokines, and these have been involved in resistance to schistosome infection. Thus, higher ratios of IL-4/IFN-γ and IL-5/IFN-γ were produced by specific T-cell clones from *S. mansoni* resistant than susceptible individuals [199] and IL-5 correlated with lower levels of *S. haematobium* infection [99] and *S. mansoni* reinfection after treatment [200]. Furthermore, IL-4, IL-5, and IL-10 levels were associated with resistance posttreatment while IFN-γ was associated with susceptibility [201]. However, significantly higher levels of IFN-γ against adult worm and cercariae antigens by PBMCs from resistant individuals compared to those from susceptible individuals [202], suggesting that acquired resistance to human schistosomiasis cannot be exclusively classified into a single T helper cell subset.

Cellular immune responses, although involved in resistance, mediate most of schistosome-related pathology [203, 204], which can be divided into acute and chronic diseases based on disease progression. Acute schistosomiasis is a debilitating febrile disease which often occurs in individuals with no experience of infection. It is characterized by high percentage of eosinophilia, which may be reversed by chemotherapy [205], nausea, urticaria, dry cough, and fever [206, 207]. Anatomically, this stage is accompanied by a dissemination of large and destructive granulomas around the eggs [67, 208]. Chronic schistosomiasis is often referred to as a Th2 disease and accounts for most human immunopathologies in endemic areas [77, 204, 208–210]. As infection becomes chronic, schistosome eggs lodge in the liver, gut (*S. mansoni*), or bladder (*S. haematobium*), and the granulomatous response translates into extensive tissue damage and excessive extracellular matrix protein (ECMP) deposition, leading to fibrosis [210].

9. Immunological Interaction between Helminth Antigens and Allergens

9.1. Helminth Infection and the “Mast Cell Saturation” Hypothesis. The earliest protective mechanism of helminth infection suggested was “mast cell saturation,” whereby helminths induce high levels of nonspecific IgE that saturate Fc receptors on mast cells, thus inhibiting hypersensitivity reactions [211, 212]. Further supportive evidence for the Fc saturation hypothesis came from a study showing that histamine release of human mast cells from lung fragments could be blocked by preexposure of these fragments to high total IgE [213]. However, more recent studies on basophils have shown that high levels of polyclonal IgE and polyclonal/specific IgE ratios from filarial- and hookworm-infected patients do not prevent antigen-induced histamine release [214, 215]. Nevertheless, Mitre and colleagues were able to show that extremely high ratios of polyclonal/specific IgE, enhanced with polyclonal myeloma IgE in vitro, could prevent histamine release [214]. Although basophils and mast cells may be differentially regulated [216], these experiments suggested that the FcεRI receptor saturation may not be the primary mechanism by which helminths “protect” against allergy.

9.2. Helminth Infection and Cross-Reactive IgE Responses. Another hypothesis suggested was that helminth parasites induce a “clinically irrelevant” allergen-specific IgE response, which would be cross-reactive between helminths and allergens [217]. Cross-reactive anti-tropomyosin IgE antibodies between helminths and allergens have recently been demonstrated, where monkeys infected with *Loa loa* (filarial parasites) mounted an IgE cross-reacting between filarial tropomyosin and Derp 1 allergen but not with timothy grass [218]. Furthermore, cross-reactivity between ascariis and mites has been reported [219]. However, field studies report mixed results on the effects of helminth infections on allergen-specific IgE in endemic areas [220–222]. Our recent study has demonstrated that the levels of anti-Derp1 IgE antibodies inversely correlate with *S. haematobium* infection intensity in a high schistosome infection area in Zimbabwe [223].

9.3. Helminth-Induced Immunomodulation. Technological and scientific advances such as genomic sequences and proteomic approaches have generated molecular and evolutionary information on the relationship between helminth parasites and allergic reactivity. Helminth infections are generally characterised by a Th2-polarised immune response [25, 84, 224], which is often associated with host resistance to infection/reinfection [30]. However, this Th2 response is also associated with pathology [204], consistent with the role for Th2 in allergic diseases [171]. Nevertheless, helminth parasites are capable of modulating this response to prolong their survival and minimize severe pathology in their host [76, 84, 225]. This immunomodulation is thought to affect unrelated antigens such as allergens, hence dampening the clinical manifestation of allergy. Indeed, experimental studies have demonstrated helminth-induced suppression of allergic responses via multiple pathways (Figure 1). However, these observations and mechanisms remain to be rigorously tested in humans. Furthermore, biological and evolutionary differences in the mouse experimental host and the natural human host must be taken into account when extrapolating
mechanistic and phenomenological results from the mouse to the human, for example, differences in the IgE receptors [226].

Human studies investigating the regulatory mechanisms underlying the protective effect of helminth infections on atopy have primarily focused on IL-10. Thus, parasite-induced IL-10 production and skin prick reactivity were negatively associated in Ascaris lumbricoides [42] and Schistosoma haematobium [46] infected populations. Furthermore, allergen-induced IL-10 was associated with reduced Th2 responses (IL-4 and IL-5) in asthmatic schistosome infected patients [227]. More recently, it has been shown that the frequency of PBMCs expressing cytotoxic-T-lymphocyte antigen 4 (CTLA-4) and monocytes expressing IL-10 from asthmatic patients infected with S. mansoni was significantly higher compared to their asthmatic uninfected counterparts [228]. However, a study on an Ecuadorian population showed no association between skin prick reactivity with either IL-10 or IL-10-producing T cells induced by Ascaris lumbricoides [40].

The TGFβ is another cytokine involved in the modulation of immune responses, and is secreted by antigen-presenting cells (APCs) or regulatory T cells [229]. However, there is a paucity of human studies on this cytokine in the context of atopy and helminth infections. Interestingly, we have observed a negative association between atopy and the levels of soluble CD23 in S. haematobium infected populations (Rujeni et al., manuscript in preparation). The CD23 is the low affinity receptor for IgE and is involved in the regulation of these antibodies [35]. As illustrated in Figure 2, the soluble CD23 can either upregulate or downregulate IgE synthesis depending on their size and oligomerization state.

Of note, expression of this receptor has been associated with resistance to schistosome [194] and Ascaris [230] infections in humans, and suppressed airway allergy in helminth-infected mice [231].

As illustrated in Figure 1, immunomodulation during chronic helminth infection is driven by regulatory T and B cells (Tregs and Bregs, resp.), which secrete the above mentioned anti-inflammatory cytokines. Treg cells are either recruited by Bregs or induced and expanded by helminth-derived products [28]. Both T and B regulatory cells can suppress Th2 cells thereby regulating atopy and helminth-induced pathology [31, 232]. Indeed, a study in our lab has shown that Treg proportions correlate with the levels of schistosome infection in young children actively acquiring infection [233].

Helminth molecules have been identified from excretory-secretory (ES) products that are associated with immunomodulation during helminth infection. Thus, the ES-62 is a phosphorylcholine-containing glycoprotein secreted by Acanthocheilonema viteae, a rodent filarial nematode [234]. This protein presents anti-inflammatory properties and has been successfully tested in mouse models of allergy and autoimmune diseases [235, 236], and it is currently being exploited as a potential therapeutic agent for inflammatory diseases in humans [237]. The anti-inflammatory properties of this molecule include modulation of B-cell proliferation and cytokine production as well as hyporesponsiveness and desensitization of mast-cell degranulation [237–240]. The interleukin-4-inducing principle from S. mansoni egg IPSE/alpha-1, identified as one of the most abundant proteins secreted by S. mansoni eggs [241], has also been associated with immunomodulation, possibly by inducing
granulomatous responses [242]. Furthermore, IPSE/alpha-1 has been shown to induce antigen-independent IL-4 production by murine basophils in vivo [243]. The venom allergen-like (VAL) proteins are another group of helminth ES products involved in immunomodulation. Thus, Hewitson et al. have demonstrated that antibodies to these VAL proteins involved in immunomodulation. Thus, Hewitson et al. have demonstrated that antibodies to these VAL proteins are involved in immunomodulation. Furthermore, IPSE/alpha-1 and helminth parasites. Instead, this paper suggests that the relationship between allergic and antiparasite Th2 responses arises from a common response to different classes of environmental challenges which include helminth parasites, venoms and haematophagous fluids, and environmental irritants such as carcinogens and noxious xenobiotics, so that this diverse group of stimuli activates responses collectively known as “allergic host defences” [246]. Within this paradigm, these environmental challenges are characterised only by the type of response they elicit with multiple pathways leading to the activation of Th2 responses with the result of protecting against environmental challenges by either reduced exposure to, or elimination of the “irritant.” In this scenario, allergic reactivity is believed to have evolved as an important and essential mechanism against harm rather than a harmful overreaction of a misdirected immune system [247]. Studies in cancer patients also show a negative association between cancer and atopy which has led to the suggestion that allergy protects against some types of cancer [248, 249]. This suggests that the Th2 responses protecting against allergens, carcinogens, and helminths are complex. This presents a challenge for the development of therapeutics relying on helminth products to overcome allergic responses, since induction of allergic responses as well as the effector mechanisms maybe tightly regulated, and the effector responses they elicit may have been selected for redundancy.

10. Convergence of Allergic and Antiparasite Responses

There is current interest in determining the common features in the induction of immune responses by allergens and by helminths as well as the evolutionary advantages of maintaining allergic responses. As illustrated above, several studies have suggested that similarities in antigens may underlie the commonality of Th2 responses elicited by allergens and by helminths. A recent review [246] of allergic responses indicated that there is relatively little structural similarity between different allergens (e.g., house dust mite, food allergens, and haematophagous fluids) and between allergens and helminth parasites. Instead, this paper suggests that the relationship between allergic and antiparasite Th2 responses arises from a common response to different classes of environmental challenges which include helminth parasites, venoms and haematophagous fluids, and environmental irritants such as carcinogens and noxious xenobiotics, so that this diverse group of stimuli activates responses collectively known as “allergic host defences” [246]. Within this paradigm, these environmental challenges are characterised only by the type of response they elicit with multiple pathways leading to the activation of Th2 responses with the result of protecting against environmental challenges by either reduced exposure to, or elimination of the “irritant.” In this scenario, allergic reactivity is believed to have evolved as an important and essential mechanism against harm rather than a harmful overreaction of a misdirected immune system [247]. Studies in cancer patients also show a negative association between cancer and atopy which has led to the suggestion that allergy protects against some types of cancer [248, 249]. This suggests that the Th2 responses protecting against allergens, carcinogens, and helminths are complex. This presents a challenge for the development of therapeutics relying on helminth products to overcome allergic responses, since induction of allergic responses as well as the effector mechanisms maybe tightly regulated, and the effector responses they elicit may have been selected for redundancy.

11. Conclusions

We have shown similarities in the immunological responses to schistosome parasites and to allergens. Studies continue to determine the aetiology of the similar responses and the evolutionary pathways that may have led to the development and maintenance of allergic responses which are paradoxically harmful to the host [246, 247], but may be essential to protect against harm from environmental challenges [246]. The clinical manifestation of atopy is complex with several studies from helminth endemic areas having shown that allergic sensitisation and clinical manifestation of allergy can be dissociated [222]. Furthermore, allergic disease and parasitic infections exist as comorbidities in many patients and are not mutually exclusive [250]. The role of impaired serological allergy diagnosis in parasitized allergy patients as well as under diagnosis in developing countries needs to be addressed to inform future studies. Detailed longitudinal and mechanistic studies relating atopy and clinical disease to schistosome infection and disease in human populations will be valuable to inform on not only the immunological process occurring, but more importantly on clinical management of allergy and schistosomiasis patients.

Acknowledgments

This paper contains some of our work which was funded by the World Health Organization (Grant no. RPC264), The Welcome Trust (Grant no. WT082082MA), the University of Edinburgh, and the Government of Rwanda. F. Mutapi is funded by the Thrasher Foundation. The authors are grateful to Laura Appleby for providing comments on the manuscript.

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Research Article

Risk Factors for Asthma in a Helminth Endemic Area in Bahia, Brazil

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Received 14 April 2012; Revised 16 June 2012; Accepted 5 July 2012

Academic Editor: William Harnett

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Protective factors associated with atopy or asthma in rural areas include socioeconomic level, overcrowding, and helminth infection. However, little epidemiological information was originated from schistosomiasis areas. This study aimed to investigate factors associated with asthma in a schistosomiasis endemic area. A questionnaire was used to obtain information on demographics, socioeconomic, and environmental features. The ISAAC questionnaire was used to identify individuals with asthma. Parasitological exam was done in all participants and skin prick test to aeroallergens in all asthmatics. Prevalence of Schistosoma mansoni infection was 57.4% and Ascaris lumbricoides, 30.8%. Asthma was found in 13.1% of the population, and 35.1% of them had a positive SPT. Active and passive smoking was positively associated with asthma, whereas A. lumbricoides was negatively associated. In a schistosomiasis hyperendemic region, current infection with A. lumbricoides is protective against asthma. However, we cannot rule out the involvement of S. mansoni in this process.

1. Introduction

Asthma is the most outstanding allergic disease especially in developed countries [1, 2]. However, in developing countries the frequency of asthma is much lower, particularly in rural areas, where the prevalence of parasite infection is high [3–5]. Factors associated with protection against atopy in rural populations include reduced socioeconomic level, poor living conditions, and increased overcrowding [6]. Moreover, several studies have shown a protective role of different parasites in the development of asthma or atopy [6–10].

Studies conducted in Venezuela have shown that geo-helminth infections are associated with high levels of total IgE and low levels of allergen-specific IgE, as well as suppression of allergic reactivity, which is reversible by anthelminthic treatment [11, 12]. Other authors have also shown low prevalence of positive skin prick test (SPT) for aeroallergens in endemic areas for geo-helminths [7, 13] or Schistosoma spp. [9, 14]. We have previously reported that asthmatic individuals living in regions with high frequency of S. mansoni infection have a mild course of asthma [15], and that treatment for schistosomiasis leads to a worsening of the clinical manifestations of asthma [3]. It is noteworthy that in these previous studies conducted with S. mansoni infected patients, symptoms of asthma or frequency of positive SPT was compared to individuals from outside schistosomiasis endemic areas.

The immunological mechanisms involved with protection against the development of asthma are not well known, although it has been proposed the participation of regulatory molecules [3, 14, 16–20]. Moreover, risk or protective factors associated with asthma in helminth endemic areas remain
still not well established. Despite a fair amount of epidemiological information is available on risk factors for asthma in developed countries, scant attention has been given to risk factors associated with asthma in rural communities from developing countries, especially in regions endemic for schistosomiasis.

We conducted a cross-sectional study in a rural area endemic for helminths, including S. mansoni, to evaluate possible risk and protective factors associated with the presence of asthma. In addition to demographic, socioeconomic and environmental factors, we also evaluated the influence of current S. mansoni, A. lumbricoides, Trichuris trichiura, and hookworm infections with the presence of asthma in individuals living in the region.

2. Material and Methods

2.1. Subjects and Endemic Area. This study was carried out at Agua Preta, a rural community in the district of Gandu, south of Bahia Brazil, 300 km distant from Salvador, the capital of the state. This village is composed of about 500 inhabitants, who live in poor sanitary conditions. Using a standard questionnaire, age, sex, income, housing conditions, and exposure to pollutants were registered. Additionally, information on water contact was obtained individually or from guardians in case of children under 10 years old. The International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire, which assess the personal history of bronchial asthma and rhinitis in the past 12 months, as well as family history of atopy and concomitant pathologies, was applied to each individual aging from 6 to 50 years old. In order to exclude individuals with chronic obstructive pulmonary disease (COPD), a clinical evaluation, chest X-ray and spirometry were performed in all asthmatic individuals. None of the asthmatic individuals included in this study had COPD. Subjects who presented personal history of asthma in the past 12 months according to ISAAC questionnaire underwent a skin prick test (SPT) for aeroallergens.

Interviews were carried out initially at the health post of the region, located at the center of Agua Preta. The recruitment strategy consisted in household visits to invite subjects to the health post. During the last days of the recruitment, when the number of subjects who spontaneously went to the health post were lower, new household visits were done in order to invite and perform a home-based interview for each individual who agreed in participate in this study.

The ethical Committee of the Maternity Climério de Oliveira, Federal University of Bahia, approved the present study and informed consent was obtained from individual or their legal guardians. All participants who tested positive for parasites were treated at the end of the study. Those who had complaints of allergy were properly instructed regarding indoor environmental control and drug treatment.

2.2. Assessment of Water Contact. The water contact questionnaire was developed based on previous observations carried out on site about habits of the population regarding the use of local rivers. Each participant was asked to provide the frequency and duration of the exposure to the main seven activities (farming, bathing, washing clothes, washing hair, washing dishes, fishing, and playing). Subjects were classified across all activities into four categories to reflect the level of exposure: no exposure, low exposure (<1 h/week), medium exposure (1–3 h/week), or high exposure (1–3 h/day) [21].

2.3. Fecal Examinations for Parasites. All participants received a plastic container for fecal samples at three time points 2–30 days apart. Participants were instructed to deposit stool sample and return the container immediately to the collection point, where samples were stored at 4 °C. Each stool sample had a slide prepared and tested by using the Kato-Katz method to estimate the number of S. mansoni, A. lumbricoides and T. trichiura eggs per gram of feces (EPG) [22]. Additionally, the Hoffmann-Pons-Janer method was used to qualitatively investigate the presence of Hookworm infection [23].

2.4. Skin Prick Test. SPTs were performed on all individuals identified as asthmatic according to the ISAAC criteria. Glycerinated allergens tested included Dermatophagoides pteronyssinus, D. farinae, Blomia tropicalis, Periplaneta Americana, and Blatella germanica (IPI-ASAC). We also used histamine (1:1000) and saline as positive and negative controls, respectively. SPT results were obtained 20 minutes after application, and a positive skin reaction was defined as a wheal with a mean diameter greater than 3 mm.

2.5. Statistical Analyses. The variable age are expressed as arithmetic mean and standard deviation (SD), whereas parasite load are expressed as geometric mean and 95% of confidence interval. Helminth infections were classified into low, moderate, or high intensity of infection according to World Health Organization (WHO) criteria [24]. Parasite load ranging from 1 to 99 epg for S. mansoni is classified as low, 100 to 300 epg moderate, and equal or greater than 400 epg high intensity of infection. For A. lumbricoides low intensity of infection is defined as 1 to 4,999 epg, moderate 5,000 to 49,999 epg, and high intensity of infection is equal or greater than 50,000 epg. Light T. trichiura infection is considered as parasite load ranging from 1 to 999 epg, moderate 1,000 to 9,999, and high intensity of infection equal or greater than 10,000 epg. The intensity of hookworm infection was not assessed since the Kato-Katz technique is not adequate for quantifying hookworm eggs.

Statistical analyses were performed by using the STATA statistical package version 11.2 (Stata Corp., College Station, TX). A logistic regression model was used to test association between asthma and covariates in a univariate and multivariate way. The alpha level for statistical significance was established as 0.05 for all analyses and odds ratio (OR) was obtained at a 95% of confidence interval (CI). In order to reduce the chance of type I error a P value adjustment was performed by the Bonferroni correction in all significant associations. Multivariate analyses were adjusted for age, gender, number of stool samples evaluated, and/or smoking status. Adjustment for age and gender was performed because these could be possible confounding
factors, since depending on gender or age lifestyle could be significantly different. The adjustment for number of stool samples analyzed was done because 82.8% of subjects who had three samples evaluated were asthmatics, and it is well known that the Kato-Katz sensitivity is influenced by the number of samples evaluated, reaching a sensitivity of 91.7% for the diagnosis of schistosomiasis when four samples are analyzed [25]. For investigating the influence of helminth infections on asthma, the smoking status was taken into account since this was positively associated with asthma in this region, which could be responsible for the underestimation of the real effect that helminth infections have on asthma.

3. Results

3.1. Baseline Characteristics of the Studied Subjects. A total of 427 individuals were included in this study, being 53.2% male. The mean age was 26.9 ± 19.0 years, ranging from 1 to 92 years. Approximately 95% of participants reported an average income less than the Brazilian national minimum wage (≤$250.00) per month. Regarding the presence of pollutants 94.4% of the population uses wood burning stove and 14.5% were active smokers.

The frequency of intestinal parasites was investigated in 406 (95.1%) individuals, 214 (52.7%) of whom had only one sample analyzed, whereas 160 (39.4%) and 32 (7.9%) subjects had two and three samples evaluated, respectively. The helmminth most frequently identified in the region was S. mansoni, affecting 57.4% of the population, followed by T. trichiura (36.9%), hookworm (31.8%), and A. lumbricoides (30.8%). Intensity of infection was assessed for S. mansoni (89 [75–105] epg), A. lumbricoides (1,946 [1,278–2,963] epg), and T. trichiura (120 [97–149] epg).

History of exposure to contaminated water was reported by 332 (77.8%) subjects, 34.3% of whom had a low level of exposure, 18.7% medium, and 47.0% were highly exposed. The group highly exposed was composed mainly of female (67.9%), whereas the group with low level of exposure was represented mostly by men (68.4%). Despite the evident influence of gender on the intensity of exposure, age is a factor that apparently did not affect the level of exposure. The group classified as highly exposed had a mean age of 27 ± 17 years, while the groups of medium and low level of exposure had a mean age of 26 ± 18 and 27 ± 20 (P > 0.05), respectively. As expected, the group that reported higher level of exposure also showed higher frequency of schistosomiasis, when compared to the nonexposed group (Table 1).

The prevalence of asthma and rhinitis was evaluated by the ISAAC questionnaire in 335 individuals, representing all subjects who were 6 to 50 years old. The prevalence of self-reported wheezing in the past 12 months (here designated as “asthma”) was 13.1%. Skin prick test for aeroallergens was performed on 37 out of 44 (84.1%) asthmatic individuals. False positive result, defined as presence of skin reaction in the negative control saline, was found in only one subject (2.3%). On the other hand, false negative result, defined as no skin reaction detected, even to histamine, was found in four (9.1%) asthmatic individuals. Among those 32 individuals who had a validated skin prick test result, 13 (35.1%) was positive for at least one allergen, whereas 19 (64.9%) of them had a negative SPT result.

3.2. Risk Factors Associated with Asthma. Univariate and multivariate analysis for the association between asthma and possible risk or protector factors are presented in Table 2. Evaluated variables included gender, age, income, passive or active smoking, family history of allergy, housing quality, number of people per household, use of wood burning stoves, and level of exposure to fresh water. Multivariate analyses were adjusted for age and gender.

The frequency of asthmatic individuals living in the endemic area who are active smokers was higher (26.2%) than the prevalence of active smokers among those nonasthmatic subjects (11.8%). In order to obtain the power of this difference, we performed a sample size calculation based on these data and found that 42 individuals in the “Asthmatic” group and 272 in the “nonasthmatic” group have 76.1% power to detect a difference with a significance alpha level of 0.05.

Only active or passive smoking was positively associated with the presence of personal history of asthma in the past 12 months, even after adjustment for confounding factors (Table 2). After controlling for age and gender the prevalence of asthma was 5.45 (CI 95%: 2.09–14.24; P = 0.001) and 3.67 (CI 95%: 1.83–7.34; P < 0.001) times higher among active and passive smokers, respectively, than nonsmokers.

3.3. Association between Asthma and Helminth Infections. The association between helminth infection or intensity of infection and asthma is shown in Table 3. Univariate analysis indicates that hookworm infection (OR = 2.54 [1.32–4.91]; P = 0.005), moderate intensity of A. lumbricoides infection (OR = 4.01 [1.74–9.28]; P = 0.001), T. trichiura (OR = 2.18 [1.13–4.20]; P = 0.020), and S. mansoni (OR = 2.89 [1.29–6.48]; P = 0.010) infection were positively associated with asthma. However when adjusting for the confounding factors age, gender, number of stool samples evaluated, and smoking status, no significant association was observed between hookworm (OR = 1.41 [0.54–3.72]; P = 0.486), T. trichiura (OR = 0.95 [0.36–2.54]; P = 0.918), S. mansoni (OR = 1.44 [0.48–4.29]; P = 0.517), or any helminth infection (OR = 0.94 [0.19–4.56]; P = 0.935) and asthma. Moreover, no significant association was found between number of helmminth infections and asthma (data not shown).

Multivariate analysis showed that A. lumbricoides infection is negatively associated with asthma (OR = 0.26 [0.07–0.94]; P = 0.041). When the Bonferroni correction was performed this association became nonsignificant (P = 0.984). Nevertheless, we found a significant association between A. lumbricoides infection and asthma when controlling for other helmminth infections (data not shown). The association between asthma and parasite load was also assessed and we found that low (OR = 0.27 [0.07–0.94] P = 0.071) or moderate (OR = 0.29 [0.05–1.83]; P = 0.187) intensity of A. lumbricoides infection was not associated with asthma. Since only three individuals, all nonasthmatic, showed high intensity of A. lumbricoides infection in the region, we were
### Table 1: Frequency of *S. mansoni* infection according to the level of exposure.

<table>
<thead>
<tr>
<th>Level of exposure</th>
<th><em>S. mansoni</em></th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>No contact</td>
<td>26 (29.5%)</td>
<td>62 (70.5%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Low</td>
<td>62 (55.9%)</td>
<td>49 (44.1%)</td>
<td>2.18 (1.13–4.22)</td>
</tr>
<tr>
<td>Medium</td>
<td>38 (67.9%)</td>
<td>18 (32.1%)</td>
<td>3.17 (1.41–7.15)</td>
</tr>
<tr>
<td>High</td>
<td>107 (70.9%)</td>
<td>44 (29.1%)</td>
<td>4.00 (2.11–7.61)</td>
</tr>
</tbody>
</table>

Note: Multivariate analysis adjusted by age, gender, and number of stool samples analyzed.

### Table 2: Univariate and multivariate analysis for the association between asthma and possible risk or protector factors.

<table>
<thead>
<tr>
<th></th>
<th>Nonasthmatic</th>
<th>Asthmatic</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 291)</td>
<td>(n = 44)</td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>141 (85.4%)</td>
<td>24 (14.6%)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Male</td>
<td>150 (88.2%)</td>
<td>20 (11.8%)</td>
<td>0.78 (0.41–1.48)</td>
<td>0.452</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6–10</td>
<td>44 (83.0%)</td>
<td>9 (17.0%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>11–15</td>
<td>48 (81.4%)</td>
<td>11 (18.6%)</td>
<td>1.12 (0.42–2.96)</td>
<td>0.819</td>
</tr>
<tr>
<td>16–20</td>
<td>41 (91.1%)</td>
<td>4 (8.9%)</td>
<td>0.48 (0.14–1.67)</td>
<td>0.247</td>
</tr>
<tr>
<td>21–30</td>
<td>82 (88.2%)</td>
<td>11 (11.8%)</td>
<td>0.66 (0.25–1.70)</td>
<td>0.386</td>
</tr>
<tr>
<td>31–40</td>
<td>43 (89.6%)</td>
<td>5 (10.4%)</td>
<td>0.57 (0.18–1.83)</td>
<td>0.345</td>
</tr>
<tr>
<td>41–50</td>
<td>33 (89.2%)</td>
<td>4 (10.8%)</td>
<td>0.59 (0.17–2.09)</td>
<td>0.416</td>
</tr>
<tr>
<td>Income ($)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;250</td>
<td>271 (86.9%)</td>
<td>41 (13.1%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>250–500</td>
<td>19 (86.4%)</td>
<td>3 (13.6%)</td>
<td>1.04 (0.30–3.68)</td>
<td>0.947</td>
</tr>
<tr>
<td>&gt;500</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Active smoker</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>240 (88.6%)</td>
<td>31 (11.4%)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Yes</td>
<td>32 (74.4%)</td>
<td>11 (25.6%)</td>
<td>2.66 (1.22–5.81)</td>
<td>0.014</td>
</tr>
<tr>
<td>Passive smoker</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>190 (92.7%)</td>
<td>15 (7.3%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>97 (78.2%)</td>
<td>27 (21.2%)</td>
<td>3.53 (1.79–6.94)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Family history of allergy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>180 (87.0%)</td>
<td>27 (13.0%)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Yes</td>
<td>65 (84.4%)</td>
<td>12 (15.6%)</td>
<td>1.23 (0.59–2.57)</td>
<td>0.581</td>
</tr>
<tr>
<td>Housing quality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brick house</td>
<td>103 (92.0%)</td>
<td>9 (8.0%)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Adobe house</td>
<td>107 (84.3%)</td>
<td>20 (15.7%)</td>
<td>2.14 (0.93–4.92)</td>
<td>0.073</td>
</tr>
<tr>
<td>Mud house</td>
<td>48 (87.3%)</td>
<td>7 (12.7%)</td>
<td>1.67 (0.59–4.77)</td>
<td>0.337</td>
</tr>
<tr>
<td>Number of inhabitants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–3</td>
<td>95 (87.2%)</td>
<td>14 (12.8%)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>4–6</td>
<td>148 (88.1%)</td>
<td>20 (11.9%)</td>
<td>0.92 (0.44–1.90)</td>
<td>0.816</td>
</tr>
<tr>
<td>&gt;6</td>
<td>43 (81.1%)</td>
<td>10 (18.9%)</td>
<td>1.58 (0.65–3.83)</td>
<td>0.314</td>
</tr>
<tr>
<td>Wood burning stove</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>19 (95.0%)</td>
<td>1 (5.0%)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Yes</td>
<td>271 (86.3%)</td>
<td>43 (13.7%)</td>
<td>3.01 (0.39–23.1)</td>
<td>0.288</td>
</tr>
<tr>
<td>Exposure to fresh water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No contact</td>
<td>57 (87.7%)</td>
<td>8 (12.3%)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Low</td>
<td>86 (93.5%)</td>
<td>6 (6.5%)</td>
<td>0.50 (0.16–1.51)</td>
<td>0.217</td>
</tr>
<tr>
<td>Medium</td>
<td>41 (85.4%)</td>
<td>7 (14.6%)</td>
<td>1.22 (0.41–3.62)</td>
<td>0.725</td>
</tr>
<tr>
<td>High</td>
<td>107 (82.3%)</td>
<td>23 (17.7%)</td>
<td>1.53 (0.64–3.64)</td>
<td>0.335</td>
</tr>
</tbody>
</table>

Note: Multivariate analysis adjusted by age and gender.
Table 3: Univariate and multivariate analysis of the association between asthma and helminth infections.

<table>
<thead>
<tr>
<th></th>
<th>Nonasthmatic (n = 291)</th>
<th>Asthmatic (n = 42)</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33%</td>
<td>45%</td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Hookworm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>189 (90.9%)</td>
<td>19 (9.1%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>90 (79.6%)</td>
<td>23 (20.4%)</td>
<td>2.54 (1.32–4.91)</td>
<td>0.005</td>
</tr>
<tr>
<td>A. lumbricoides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>187 (89.0%)</td>
<td>23 (11.0%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>92 (82.9%)</td>
<td>19 (17.1%)</td>
<td>1.68 (0.87–3.24)</td>
<td>0.122</td>
</tr>
<tr>
<td>Intensity (epg)</td>
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<td></td>
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</tr>
<tr>
<td>0</td>
<td>193 (89.4%)</td>
<td>23 (10.6%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>1–4999</td>
<td>60 (88.2%)</td>
<td>8 (11.8%)</td>
<td>1.12 (0.48–2.63)</td>
<td>0.797</td>
</tr>
<tr>
<td>5000–49999</td>
<td>23 (67.6%)</td>
<td>11 (32.4%)</td>
<td>4.01 (1.74–9.28)</td>
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<tr>
<td>≥50000</td>
<td>3 (100%)</td>
<td>0</td>
<td>—</td>
<td>—</td>
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<tr>
<td>T. trichiura</td>
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<td></td>
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<tr>
<td>Negative</td>
<td>173 (90.6%)</td>
<td>18 (9.4%)</td>
<td>1.00</td>
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<tr>
<td>Positive</td>
<td>106 (81.5%)</td>
<td>24 (18.5%)</td>
<td>2.18 (1.13–4.20)</td>
<td>0.020</td>
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<tr>
<td>Intensity (epg)</td>
<td></td>
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</tr>
<tr>
<td>0</td>
<td>175 (90.7%)</td>
<td>18 (9.3%)</td>
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<tr>
<td>1–999</td>
<td>96 (80.7%)</td>
<td>23 (19.3%)</td>
<td>2.33 (1.20–4.53)</td>
<td>0.013</td>
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<td>1000–9999</td>
<td>7 (87.5%)</td>
<td>1 (12.5%)</td>
<td>1.39 (1.61–11.9)</td>
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<td>≥10000</td>
<td>1 (100%)</td>
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<td>—</td>
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<tr>
<td>S. mansoni</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Negative</td>
<td>113 (93.4%)</td>
<td>8 (6.6%)</td>
<td>1.00</td>
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<tr>
<td>Positive</td>
<td>166 (83.0%)</td>
<td>34 (17.0%)</td>
<td>2.89 (1.29–6.48)</td>
<td>0.010</td>
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<tr>
<td>Intensity (epg)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>114 (93.4%)</td>
<td>8 (6.6%)</td>
<td>1.00</td>
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</tr>
<tr>
<td>1–99</td>
<td>104 (86.7%)</td>
<td>16 (13.3%)</td>
<td>2.19 (0.90–5.33)</td>
<td>0.084</td>
</tr>
<tr>
<td>100–399</td>
<td>34 (73.9%)</td>
<td>12 (26.1%)</td>
<td>5.03 (1.90–13.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>≥400</td>
<td>27 (81.8%)</td>
<td>6 (18.2%)</td>
<td>3.17 (1.01–9.89)</td>
<td>0.047</td>
</tr>
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<td>Any helminth</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>49 (94.2%)</td>
<td>3 (5.8%)</td>
<td>1.00</td>
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</tr>
<tr>
<td>Positive</td>
<td>230 (85.5%)</td>
<td>39 (14.5%)</td>
<td>2.77 (0.82–9.33)</td>
<td>0.100</td>
</tr>
</tbody>
</table>

Note: Multivariate analysis adjusted by age, gender, number of stool samples analyzed, and smoking status.

not able to test the association between high ascariasis parasite load and asthma.

4. Discussion

This study assessed possible risk or protective factors associated with presence of asthma in a population living in a rural poor area of Bahia, Brazil. Our results showed that the main risk factor associated with presence of asthma is smoking. We found no association between socioeconomic variables and the development of asthma. Additionally, we demonstrated that current A. lumbricoides infection is associated with protection against asthma when adjusting for confounding factors.

The cross-sectional design, the use of questionnaires to access information on the presence of asthma, and risk factors were the major limitations of this study. The study design did not allow us to make causal inferences, and confounding factors may not be equally distributed between groups. However, we performed statistical analysis taking into account the confounding factors, and reverse causality is not possible for most of the risk factors evaluated. We are also aware that the use of questionnaires makes the study open to information bias since it depends on the interviewed response. Therefore, misclassification of exposure may have occurred in the present study. Also, we call attention that we did not evaluate past helminth infections and that the parasitological technique used here is not sensitive for very low parasite burden.

The differentiation between asthma and COPD was one of our major concerns when planning this study. One way to reduce this possible misdiagnose was the exclusion of...
individuals over 50 years of age. Many authors have reported that COPD rarely presents clinically before the 5th decade of life and that the prevalence of COPD occurs primarily in smokers over 40 years of age [26, 27]. According to the PLATINO study the prevalence of COPD in individuals aging from 40 to 49 years in a metropolitan area of Brazil is 8.4% [28]. In our study only 4 (9%) asthmatic individuals aged between 41 and 50 years old. Therefore, 91% of subjects included in this study were under 41 years old. Moreover, only 1 out of 4 (25%) asthmatic individual over 40 years of age was active smoker.

Although we did not observe association between socioeconomic variables and asthma, other authors have demonstrated that reduced socioeconomic level and overcrowding are associated with protection against atopy in a rural region [6]. This phenomenon was probably not detected in our study because the overall population was homogeneous in regard to socioeconomic level, being identified only subjects with an average income less than $500 per month. Additionally, several other studies have showed an association between smoking and risk of asthma, which is consistent with our data [29–32]. Even though this association is well known, its interpretation must be made cautiously, especially when using a questionnaire-based diagnosis of asthma. Because smokers have been reported to have more severe asthma than nonsmokers, it is expected that the use of self-reported questionnaires, such as the ISAAC questionnaire, may overestimate the risk of asthma among smokers [33]. However, this bias probably did not interfere in our study since it has been shown that individuals from endemic areas for schistosomiasis have mild asthma [15]. In order to evaluate the effect of helminth infections in the presence of asthma statistical analyses were adjusted for smoking status, given that this is an independent determinant factor for asthma.

In the last years researchers showed an increased interest in evaluating the role of helminth infections in the development of allergic diseases. Although several studies show that in endemic areas for helminths, infection with A. lumbricoides or other helminths has a negative association with atopy, possibly acting as a protective factor against the development of asthma, none of these studies was conducted in endemic area for schistosomiasis [6–8, 12, 34]. Studies carried out with individuals from endemic areas infected with S. mansoni have showed low reactivity to the skin prick test (SPT) to aeroallergens and fewer symptoms of asthma compared to asthmatics without infection [10, 15], and an inverse correlation between SPT and presence of infection [9]. It is worth noting that none of these previous studies evaluated the association between SPT or symptoms of asthma and current S. mansoni infection within asthmatic subjects living in the same region. The prevalence of positive SPT to aeroallergens in asthmatic individuals included in this study was 35.1%, which is far below what is found in urban regions from Brazil [10, 35, 36]. Nevertheless, we found no significant difference in the frequency of positive SPT among asthmatics currently infected or not by any helminth (data not shown). Despite the frequency of positive skin test has been low, asthmatic subjects from these regions probably have atopic asthma, demonstrated by the presence of aeroallergen-specific IgE [10].

The presence of asthma was not associated with current infection by S. mansoni in the studied population. However, the prevalence of asthma in the site was only 13.1%, which is much lower than the frequency observed in adolescents of 13 and 14 years old from urban areas of Salvador (24.6%), Feira da Santana (21.5%) or Vitoria da Conquista (30.5%), Bahia, when using the same criteria for defining asthma [5]. The prevalence of asthma was representative of the entire study population, including individuals from 6 to 50 years old. However, when analyzing only the age of 13 and 14 years of age, the prevalence was 15%, which is very close to the frequency observed in the general population in the endemic area and still far from that observed in other centers evaluated in Bahia, Brazil [5]. This is in agreement with other study conducted in different regions of Brazil, which showed lower prevalence of asthma and rhinitis in rural than in urban areas [4].

Thus, the presence of a protective factor for the development of asthma in an endemic community for helminths including schistosomiasis is evident, which could not be explained only by the presence of current S. mansoni infection. The fact that these individuals inhabit a region of high endemicity, constantly exposed to S. mansoni reinfection, might be responsible for the low prevalence of asthma observed on site, with no significant difference in the frequency of asthma among currently infected or not. On the other hand, we found in this schistosomiasis endemic area that individuals currently infected with A. lumbricoides have a lower prevalence of asthma when compared to noninfected subjects, which was not observed for the presence of T. trichiura, hookworm, any helminth infection, or even for different intensity of infection. Although this association has been lost when performing the Bonferroni correction, it is well established that reducing type I error by P value adjustments increases the probability of type II error for those association that are not null. The formal premise for such adjustments is the much wider hypothesis that there is no association between any variables under observation, and that only random processes govern the variability of all observations. This “universal” null hypothesis presumes that all observed associations reflect only random variation that could be obtained particularly in empiric situations [37, 38]. Once this study evaluated associations that are not empiric, but instead evidence-based, such as the association between A. lumbricoides infection and asthma, we are confident that this association was not due to random variation, and therefore it would be better to take the risk of making type I error and call attention to a possible beneficial association for asthma.

Studies conducted in urban areas in Brazil have showed controversial results for the association between A. lumbricoides infection and atopy [8, 39]. Although no association between SPT response and A. lumbricoides infection was found in asthmatic individuals [39], a recent study showed that the presence of A. lumbricoides infection is able to reduce the prevalence of positive SPT response but do not affect the prevalence of asthma [8]. Overall, helminth infection is
associated with reduced risk of asthma or atopy in regions with high frequency of parasite infections [7, 11, 13], whereas in areas with low endemcity, *A. lumbricoides* infection is associated with increased risk of atopy and asthma [40, 41]. Although *A. lumbricoides* is a recognized cause of tropical pulmonary eosinophilia, chronic infection induces a regulatory immune response which includes interleukin-10- and transforming growth factor-β-secreting cells [42, 43]. In addition to the suppression of allergic inflammation, activation of the regulatory immune response induced by helminths is responsible for preventing the elimination of parasites and protects the host from damage that could be caused by excessive inflammatory response [44]. Our findings support the hypothesis that exposure to *A. lumbricoides* induces a generalized suppression on the immune response, which seems to be able to reduce the risk of asthma without additive effect by helminth coinfection. This effect was not observed for other helminth infections probably because *A. lumbricoides* was the less frequently helminth found in the region, which results in less exposure and lower rates of reinfection, allowing thereby the detection of differences in asthma prevalence between individuals currently infected or not.

In conclusion, our findings indicate that smoking is the major risk factors associated with asthma in a rural population endemic for schistosomiasis. However, in rural endemic areas for helminth infections the prevalence of asthma is much lower than what is observed in urban regions, and *A. lumbricoides* infection is negatively associated with the presence of asthma within the population. Although we were unable to show a protective association between current *S. mansoni* infection and asthma, we cannot rule out the possible participation of *S. mansoni* in modulating the immune response of allergic diseases, probably because this is a hyperendemic region where individuals are constantly reinfected. This study contribute to a better understanding of the role of helminth infections in allergies.

**Conflict of Interests**

The authors have no conflict of interests concerning the work reported in this paper.

**Acknowledgments**

The authors thank Leda Maria Alcântara for the collaboration in reading parasitological slides; all individuals from the region of Água Preta-Gandu, Bahia, Brazil, for participating in the study; the Health Agent Irene de Jesus for collaborating in the field work.

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Clinical Study

The Effect of Antihelminthic Treatment on Subjects with Asthma from an Endemic Area of Schistosomiasis: A Randomized, Double-Blinded, and Placebo-Controlled Trial

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Received 14 April 2012; Revised 15 June 2012; Accepted 21 June 2012

Academic Editor: Elena Pinelli

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This is a prospective, double-blinded, and placebo-controlled trial evaluating the influence of antihelminthic treatments on asthma severity in individuals living in an endemic area of schistosomiasis. Patients from group 1 received placebo of Albendazole or of Praziquantel and from group 2 received Albendazole and Praziquantel. Asthma severity was assessed by clinical scores and by pulmonary function test. There was no significant difference in the asthma scores from D0 to D1–D7 after Albendazole or Praziquantel and from D0 to D30–90 after Albendazole or Praziquantel in both, group 1 and 2. It was observed, however, a clinical worsening of the overall studied population after 6 months and 12 months of antihelminthic treatments. Additionally, we observed increased frequency of forced expiratory volume in 1 second (FEV1) < 80% on 12 and 18 months after treatment. The worsening of asthma severity after repeated antihelminthic treatments is consistent with the hypothesis of the protective role conferred by helminths in atopic diseases.

1. Introduction

Helminthic infections and allergic diseases are highly prevalent in many parts of the world, and both lead to type 2 immune response with secretion of IL-4, IL-5, and IL-13, with a consequent increase in the production of IgE and eosinophilia. Lynch et al. [1] studying the skin prick test (SPT) response in children from an ascariasis endemic area observed a decreased reactivity to the test and subsequently an increased response after treatment of intestinal helminths [2].

Studies conducted by the Immunology Service (SIM) of the Federal University of Bahia, Brazil, demonstrated a negative association between the cutaneous immediate hypersensitivity response to the skin prick test to aeroallergens and Schistosoma mansoni parasite load, measured by a quantitative assessment of the number of eggs per gram of stool [3]. Over a year-long follow-up study in the state of Bahia, a group of researchers from SIM compared three populations from impoverished areas of the state, including one group from an endemic area of S. mansoni, one group from a rural nonendemic area, and a third group from a slum area in Salvador, the capital of the state. In the study, it was observed that asthma severity indicators were lower in individuals living in the endemic areas of schistosomiasis when compared to the other two areas in which there were no recorded cases of S. mansoni transmission [4].
Several factors may explain the lower frequency of positive SPT and lower asthma severity in populations infected with helminths. The possible hypothesis includes high production of polyclonal IgE and reduced levels of allergen-specific IgE [1, 2], high concentrations of antigen-specific IgG4 [5], activation of regulatory cells, and regulatory cytokine production [6]. For instance, IL-10 can promote a decrease in the release of histamine and other mast cell mediators [7]. Since infection by S. mansoni induces high production of IL-10, it is possible that this is the main mechanism by which the allergic response is suppressed in infected individuals. This hypothesis is reinforced by studies of van den Biggelaar et al. [6], which showed that reduced reactivity to SPT in African children infected with S. haematobium was associated with an increase in IL-10 production in vitro by cells of these individuals. Our hypothesis for the current study is that treatment of helminth infections, including a drug to treat schistosomiasis, alters the immune response leading to worsening in asthma outcomes. The aims of this study were, firstly, evaluating early events associated to asthma severity resulting from treatment of helminth infections, and secondly, determining the degree of interference of antihelminthic treatment in the clinical course of asthma in a randomized, double-blinded, and placebo-controlled trial.

2. Materials and Methods

2.1. Subjects and Study Design. This is a randomized, double-blinded, and placebo-controlled trial with two groups of asthmatics living in a S. mansoni endemic area. The study was carried out in Agua Preta, a small village near the city of Gandu in the State of Bahia in Brazil. Gandu is located 280 km south of Salvador, the capital of the State of Bahia. Agua Preta is composed of a residential area in the center of the village and surrounding farms. There are approximately 800 people living in that community. They live in poor sanitary conditions and agriculture is their predominant occupation. The Agua Preta community was identified by our group as a community with a frequent infection of Schistosoma mansoni (49.5% in a survey of 427 residents in 2006) and helminthic infection in general (prevalence of Ascaris lumbricoides, Trichuris trichiura, and hookworm at that time was 24.2%, 33.8%, and 22.2%, resp.). Six hundred one individuals from Agua Preta agreed to participate in this study. They were screened for asthma through a direct questionnaire on the basis of the International Study of Asthma and Allergies in Childhood (ISAAC) [8]. In addition, questions concerning concomitant illnesses, socioeconomic status, and living conditions were asked in a complementary questionnaire. Patients were selected as having asthma if their responses to the ISAAC questionnaire were considered by a physician to be indicative of a personal history of asthma in the past 12 months, and if they were 5 to 50 years old. Children under 5 years old were not included due to difficulties in performing the pulmonary function test. Subjects over 50 years old were not included due to increased rates of chronic obstructive pulmonary disease in this age group. Only fifty asthmatic individuals from the community met the inclusion criteria and they were randomized either as group 1 (who would receive initially placebo of antihelminthic treatments; n = 25) or group 2 (who would receive Albendazole in a split dose of 400 mg, followed by Praziquantel 50 mg/Kg of body weight a week later; n = 25). Five individuals from group 1 left the study in the first week after the beginning of the study for personal reasons, remaining 20 individuals in this group. The loss of these individuals did not alter significantly demographic features of group 1, such as the age and gender distribution. At each evaluation, every patient underwent a physical examination, always performed by the same physician (blind to the type of treatment). At the evaluations, common asthma symptoms and signs were checked, such as cough, dyspnea, and wheezing. In addition, a questionnaire elicited information on asthma (i.e., presence and frequency of asthma attacks, type of treatment received during the attack at home, emergency department, or hospital) and the use of prophylactic or symptomatic antiasthma drugs (e.g., antihistamine, inhaled, or oral beta-2-agonist, and inhaled, oral, or parenteral corticosteroid) since the previous evaluation. These parameters were scored as follows: physical examination as 0 (normal examination) and 1 (at least one abnormal finding); asthma exacerbations as 0 (no), 1 (yes, if treated at home), and 2 (yes, if treated at an emergency department or at a hospital); use of antiasthma drugs as 0 (no), 1 (yes, except for oral or parenteral corticosteroid use), and 2 (yes, if oral or parenteral corticosteroid had been used) as reported in a previous study [4].

Human subject study guidelines of the US Department of Health and Human Service were followed in the conduction of this study. The study was approved by the Ethics Committee of Professor Edgard Santos Hospital. Informed consent was obtained from all patients or their legal guardians.

In the initial assessment, patients responded to a questionnaire to assess the clinical score of asthma [4] underwent a physical examination, chest radiograph, pulmonary function test, and blood sampling for evaluation of immune response (IL-5, IL-10, and IFN-γ in supernatants of cultures stimulated with SWAP and Der p1). Stool exams for parasites were also performed on each patient. The two groups were then treated with antihelminthics or placebo and reevaluated after the completion of treatment as shown in the study design flowchart (Figure 1). Chest radiographs and pulmonary function tests were performed at the time of enrolment and at day 7 after treatment. Spirometry test was repeated 30 days after treatment. Immunological evaluation was performed at enrolment and repeated at 7 and 90 days after treatment. After 90 days of enrolment, both placebo and treatment groups were treated with Albendazole and Praziquantel. From there, the two groups were assessed monthly by clinical examination, questionnaire, and pulmonary function test for a total period of 18 months according to the study design (Figure 1).

2.2. Pulmonary Function Tests, Chest X-Ray, and Skin Prick Test to Aeroallergens (SPT). Pulmonary function tests were performed on all subjects at enrollment and at each visit thereafter. The parameter used was Forced Expiratory...
Volume in 1 second (FEV1). Results were considered as normal when FEV1 value was ≥80% [9].

Chest X-ray was performed in a specialized clinic in Gandu, at baseline and at D7 after Praziquantel treatment. SPTs were performed on the right forearms of all individuals at enrolment using *Dermatophagoides pteronyssinus* (Der p), *D. farinae* (Der f), *Blomia tropicalis* (Blo t), *Periplaneta americana* (Per a), and *Blattella germanica* (Bla g) glycerinated allergen extracts (FDA Allergenic). Histamine (1:1000) and glycerinated saline were used as positive and negative controls, respectively. A positive skin reaction was defined as formation of a wheal with a mean diameter greater than 3 mm. The SPT results were read 20 minutes after application, and a SPT response was considered as positive if there was at least one positive test of the five tested allergens.

2.3. Immune Response—Cell Culture and Cytokine Measurements. All enrolled individuals had their blood taken for immunological studies. Peripheral blood mononuclear cells (PBMC) from the blood samples of the two groups were analyzed for in vitro immune response, which included measurement of IL-10, IL-5, and IFN-γ production by PBMCs in response to soluble S. mansoni adult worm antigen (SWAP, kindly provided by Dr. Alfredo Góes from the Federal University of Minas Gerais, Brazil) and to the antigen 1 from *D. pteronyssinus* (Der p1 extract, cosmo Bio Co., Ltd.).

PBMCs from individuals of the study were obtained through the Ficoll-Hypaque gradient and adjusted to the concentration of $3 \times 10^6$ cells/mL in complete RPMI medium (Life technologies GIBCO BRL, Gaithersburg, MD). Cells were cultured in vitro with the antigens Der p1 (25 μg/mL) and SWAP (10 μg/mL). The mitogen phytohemagglutinin (PHA) in the final concentration of 2 μg/mL was also used in the cultures. The cultures were incubated for 72 hours at 37°C and 5% CO2 and the supernatants were collected for cytokine measurements. Levels of IL-5, IL-10, and IFN-γ were determined by ELISA sandwich technique, using commercially available kits (R&D Systems) and the results were expressed in picograms per milliliter based on a standard curve.

2.4. Fecal Examinations for Parasites. Three stool samples from each individual were examined using the Hoffman sedimentation method to identify helminths and enteric protozoa, and the Kato-Katz method was used to estimate parasite load [10].

2.5. Sample Size and Statistical Analysis. Only fifty asthmatic individuals from the village where the study was carried out met the inclusion criteria. The power of the study was calculated taking into account the results of a previous study from our group [4] which demonstrated that the frequency of asthma symptoms in asthmatic individuals living in an endemic area of schistosomiasis is 18.6%, whereas the prevalence of symptoms in a worm-free population is 58.7%. Based on these data, a sample size of 20 in the placebo group and 25 in the treated group has 76% power to detect difference between proportions with a significance alpha level of 0.05 (two-tailed).

Statistical analyses were performed using the software Statistical Package for Social Science (version 9.0 for Windows; SPSS). Fisher’s exact test was used to compare proportions. The Mann-Whitney U test was used to compare levels of cytokines between groups, and the Wilcoxon matched-pairs signed rank test was used to compare the levels of cytokines intragroup before and after anthelminthic treatments. Statistical significance was established at the 95% confidence interval.


3. Results

3.1. Features of the Studied Subjects. The study included 45 asthmatic patients. They were divided into two groups: one group received placebo of antihelminthic treatments (placebo of Albendazole and placebo of Praziquantel (group 1 or placebo group), while the other group received Albendazole to treat geohelminths and Praziquantel to treat *S. mansoni* infection (group 2 or Praziquantel group). The demographic data of subjects enrolled in the study are shown in Table 1. *S. mansoni* parasite burden and infection with other helminths as well as the frequency of positive skin prick test to aeroallergens are also shown in Table 1.

Gender distribution did not differ significantly between groups who received placebos (G1; 50% male) and those who received antihelminthic treatment (G2; 32% male, \( P > 0.05 \); Table 1).

The mean age of patients included in the study was 17.6 ± 13.1 years. There was no significant difference in the mean age between groups (21.3 ± 15.4 and 14.7 ± 10.3 years in Group 1 and Group 2, resp.) with age ranges from 6 to 20 years found in 65% of G1 and in 72% of G2 (\( P > 0.05 \)) and 21 to 50 years in 35% and 28% of G1 and G2, respectively (\( P > 0.05 \); Table 1).

The frequency of rhinitis did not differ significantly between patients from group 1 and 2, being 50% and 72%, respectively (\( P > 0.05 \); Table 1). The frequency of active smokers was similar between the placebo and Praziquantel groups (30% and 28%, resp.; \( P > 0.05 \)), while the frequency of second-hand smoking exposure was higher in patients from group 1 (90%) then in group 2 (36%; \( P < 0.0005 \); Table 1).

There was no significant difference in the frequency of FEV1 ≤80% between the placebo and Praziquantel groups at baseline (5% and 12%, resp.; \( P > 0.05 \)). A positive response to the skin prick test to aeroallergens was found in 36% of patients from the Praziquantel group compared to 45% of placebo group at baseline (\( P > 0.05 \); Table 1). The frequency of positive response to the different aeroallergens such as Der p, Der f, Blo t, Per a and Bla g also did not differ between the two groups of patients (\( P > 0.05 \)). A positive response to histamine was found in 100% of patients from group 1 and 90.9% of those from group 2 (\( P > 0.05 \); Table 1).

The frequency of *S. mansoni* infection did not differ between groups (\( P > 0.05 \)). The *S. mansoni* parasite burden also did not differ significantly between group 1 and group 2 (115 ± 49 and 427 ± 228 eggs/g feces, resp.; \( P > 0.05 \)). Patients were also infected with other helminths, such as *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm. However, there was no significant difference in the frequency of other helminthic infection between groups (Table 1; \( P > 0.05 \)). Co-infection with *S. mansoni* and one or more other helminths were observed in 90% and 100% of patients from G1 and G2, respectively (\( P > 0.05 \); Table 1).

Clinical scores for asthma were initially evaluated at baseline (day 0 or pretreatment) and for seven consecutive days during the first week after treatment with placebo or Albendazole (D1 to D7). At day 7 after Albendazole treatment, patients from group 1 and group 2 were treated with placebo of Praziquantel and Praziquantel, respectively. They were clinically evaluated and had their asthma score recorded during the following seven days. The results of clinical scores of asthma after treatment with placebo of Albendazole and placebo of Praziquantel are shown in Figure 2(a). There was no significant difference in frequency of asthma scores at D1 to D7 compared to D0 (\( P > 0.05 \)) in patients from group 1 who received placebos of Albendazole and Praziquantel (Figure 2(a)).

There was also no significant difference in the frequency of asthma scores from D0 to D1–D7 after Albendazol and Praziquantel in group 2 (\( P > 0.05 \); Figure 2(b)).

The mid-term effect of Praziquantel treatment on the asthma severity was evaluated during the first 90 days of posttreatment. Three monthly consecutive evaluations (D30 to D90) were performed in each patient in this time period. The mean frequencies of asthma clinical scores are shown in Figure 3(a). There was no significant difference in the frequency of clinical scores 0, 1, 2, 3, or 4 between D0 (80%, 5%, 15%, 0%, 0%) and D30 to D90 (73 ± 8.8%, 6.7 ± 6.2%, 19 ± 5.7%, 0%, 0%) in the placebo group (Figure 3(a)). In the group treated with Praziquantel, the frequency of different scores (0, 1, 2, 3, and 4) also did not differ from D0 (55%, 25%, 20%, 0%, 0%) to D30–D90 (68 ± 5%, 15.7 ± 6.9%, 16 ± 5.4%, 0%, 0%; \( P > 0.05 \); Figure 3(b)).

The long-term effect of antihelminthic treatment on asthma severity was evaluated in three different time periods, 6, 12, and 18 months after-treatments. The placebo group was treated with Albendazole and Praziquantel at day 90, when the Praziquantel group received the second treatment with these two drugs. Since, after day 90, both groups were treated with antihelminthics, the groups were combined into one group of treated patients thereafter. The frequency of score zero in the studied population was lower after 6 months of treatment (58%) compared to D0 (73%; \( P < 0.05 \); Figure 3(c)), being the frequency of score one higher (20%) compared to baseline (6%; \( P < 0.05 \)). Likewise, the frequency of score 2 was higher after 12 months of treatment (30%) compared to D0 (15%; \( P < 0.05 \); Figure 3(c)).

There was, however, no significant difference in the frequency of different asthma clinical scores at 18 months after treatments (65%, 15%, 15%, 3%, 0%, to scores 1, 2, 3, and 4, resp.) compared to baseline (73%, 6%, 15%, 2% and 2% to scores 1, 2, 3, and 4, resp.; \( P > 0.05 \); Figure 3(c)).

The severity of asthma was also evaluated through pulmonary function test (PFT). The result of FEV1 in patients from group one and two are shown in Figure 4. There was no significant difference in the frequency of FEV1 <80% either in Praziquantel or in the placebo groups when the baseline values were compared to D7 post Albendazole, D7 after Praziquantel and also to D90 after Praziquantel (\( P > 0.05 \), Figure 4(a)). On the other hand, the frequency of FEV1 <80% among all the subjects with asthma (\( n = 45 \)) was higher at 12 months (22.2%) and subsequently at 18 months after treatment (34.8%) compared to the frequency at baseline (9%; \( P < 0.05 \), Figure 4(b)).
Table 1: Baseline characteristics of studied subjects.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 placebo n = 20</th>
<th>Group 2 Praziquantel n = 25</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male n (%)</td>
<td>10 (50)</td>
<td>8 (32)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Female n (%)</td>
<td>10 (50)</td>
<td>17 (68)</td>
<td></td>
</tr>
<tr>
<td>Age group n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child/teenagers (6–20 years old)</td>
<td>12 (65)</td>
<td>18 (72)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Adult (21–50 years old)</td>
<td>8 (35)</td>
<td>7 (28)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Rhinitis n (%)</td>
<td>10 (50)</td>
<td>18 (72)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Smoking n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>6 (30)</td>
<td>7 (28)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Passive</td>
<td>18 (90)</td>
<td>9 (36)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Positive SPT response n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dermatophagoides pteronyssinus</em></td>
<td>2 (10)</td>
<td>5 (20)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><em>D. farinae</em></td>
<td>2 (10)</td>
<td>5 (20)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><em>Blomia tropicalis</em></td>
<td>4 (20)</td>
<td>7 (28)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><em>Periplaneta americana</em></td>
<td>1 (5)</td>
<td>3 (12)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><em>Blatella germanica</em></td>
<td>2 (10)</td>
<td>3 (12)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Positive total</td>
<td>9 (45)</td>
<td>9 (36)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Current helminth infections n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Schistosoma mansoni</em></td>
<td>16 (80)</td>
<td>19 (76)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>10 (50)</td>
<td>14 (56)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hookworm</td>
<td>7 (35)</td>
<td>10 (40)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><em>Trichuris trichiura</em></td>
<td>10 (50)</td>
<td>13 (52)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Coinfection (S. mansoni /1 or + helminths)</td>
<td>14 (90)</td>
<td>19 (100)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Figure 2: Frequency of clinical scores of asthma in patients from Group 1 at day zero (D0) and one to seven days (D1–D7) after treatment with placebo (Plac) of Albendazole (Alb) and placebo of Praziquantel (PZQ) (a); and at day zero (D0) and one to seven days (D1–D7) after treatment with Albendazole or Praziquantel (Group 2) (b). Praziquantel was given seven days after the treatment with Albendazole in the Group 2. Data were represented as mean ± SD. There was no significant difference in the frequency of asthma scores between D0 versus D1–D7 in none of groups (P > 0.05; Chi-square Test for Independence).
3.2. Cytokine Profile Induced by *S. mansoni* and *D. pteronyssinus* Antigens in PBMCs of the Studied Population. We measured the cytokines IFN-γ, IL-5, and IL-10 in the supernatants of PBMC cultures stimulated with the *S. mansoni* soluble adult worm antigen (SWAP) and the antigen 1 of the *D. pteronyssinus* (Der p1). There was no significant difference in the levels of IFN-γ between D0 and D7 or D0 and D90 in response to SWAP and Der p1 (P > 0.05; Table 2). The baseline of IL-5 mean levels in response to Der p1 in cultures was lower in the placebo group (150 pg/mL) than in Praziquantel group (463 pg/mL; P < 0.05). There was, however, no significant difference between the baseline mean values and those found at D7 or D90 after treatment in response to SWAP or Der p1 in either group (P > 0.05; Table 2). On the other hand, in the group of Praziquantel, the mean levels of IL-10 in response to SWAP decreased from 642 pg/mL at D0 to 175.6 pg/mL at D90 after treatment (P < 0.05; Table 2). There was no significant difference in the mean level of IL-10 in response to Der p1 comparing baseline values with those from D7 and D90 in both placebo and Praziquantel groups (Table 2). There was a high production of IL-10, IL-5, and IFN-γ in cultures stimulated with the mitogen PHA compared to nonstimulated cultures (P < 0.05) in both groups of patients (data not shown).
**Table 2:** Levels of cytokines in supernatants of PBMC cultures in the studied subjects.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Antigen</th>
<th>D0</th>
<th>G1/placebo</th>
<th>D7</th>
<th>G2/PZQ</th>
<th>D90</th>
<th>G1/placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>G1/placebo</td>
<td></td>
<td>G2/PZQ</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>G1/placebo</td>
<td>G2/PZQ</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G1/placebo</td>
<td>G2/PZQ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G1/placebo</td>
<td>G2/PZQ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Without stimulus</td>
<td>32.7 (31.2–276.0)</td>
<td>31.6 (31.2–91.0)</td>
<td>31.2 (31.2–46.7)</td>
<td>39.7 (31.2–583.6)</td>
<td>31.2 (31.2–130.7)</td>
<td>32.7 (31.2–2563.0)</td>
</tr>
<tr>
<td></td>
<td>SWAP</td>
<td>149.5 (42.0–10337.0)</td>
<td>88.0 (31.2–2092.0)</td>
<td>56.0 (43.8–1415.0)</td>
<td>137.8 (37.4–15543.0)</td>
<td>70.0 (15.6–3329.0)</td>
<td>130.7 (31.2–2563.0)</td>
</tr>
<tr>
<td></td>
<td>Der p1</td>
<td>338.5 (102.4–7475.0)</td>
<td>710.0 (149.0–2568.0)</td>
<td>116.7 (42.0–420.2)</td>
<td>333.9 (32.7–9669.0)</td>
<td>201.0 (31.2–4818.0)</td>
<td>149.4 (15.6–1275.0)</td>
</tr>
<tr>
<td>IL-5</td>
<td>Without stimulus</td>
<td>15.6 (15.6–112.0)</td>
<td>15.6 (15.6–15.6)</td>
<td>15.6 (15.6–15.6)</td>
<td>15.6 (15.6–40.7)</td>
<td>15.6 (15.6–40.7)</td>
<td>15.6 (15.6–40.7)</td>
</tr>
<tr>
<td></td>
<td>SWAP</td>
<td>468.5 (15.6–5156.0)</td>
<td>310.2 (15.6–5904.0)</td>
<td>542.0 (15.6–4444.0)</td>
<td>1863.0 (15.6–4606.0)</td>
<td>1670.0 (21.5–4444.0)</td>
<td>2850 (136.0–4444.0)</td>
</tr>
<tr>
<td></td>
<td>Der p1</td>
<td>150.0 (15.6–630.0)</td>
<td>463.0 (207.0–4606.0)</td>
<td>28.8 (15.6–407.4)</td>
<td>60.0 (15.6–4606.0)</td>
<td>28.7 (15.6–1720.0)</td>
<td>352.0 (15.6–3509.0)</td>
</tr>
<tr>
<td>IL-10</td>
<td>Without stimulus</td>
<td>27.5 (15.6–475.0)</td>
<td>28.0 (15.6–102.0)</td>
<td>57.0 (16.8–589.0)</td>
<td>23.1 (15.6–1144.0)</td>
<td>36.0 (15.6–446.9)</td>
<td>15.6 (15.6–127.0)</td>
</tr>
<tr>
<td></td>
<td>SWAP</td>
<td>264.5 (26.0–1688.0)</td>
<td>642.0 (125.0–1132.0)</td>
<td>288.0 (29.4–641.0)</td>
<td>667.3 (15.6–2447.0)</td>
<td>240.0 (21.1–638.4)</td>
<td>175.6 (60.3–1062.0)</td>
</tr>
<tr>
<td></td>
<td>Der p1</td>
<td>451.0 (56.0–1060.0)</td>
<td>349.0 (177.0–1539.0)</td>
<td>924.0 (297.1–2063.0)</td>
<td>288.0 (15.6–2086.0)</td>
<td>390.0 (54.3–1744.0)</td>
<td>204.0 (60.0–638.0)</td>
</tr>
</tbody>
</table>

Data showed as median (minimum and maximum values) pg/ml;

*Placebo D0 versus PZQ D0 P < 0.05 (Mann-Whitney t-test);

*PZQ D0 versus PZQ D90 P < 0.05 (Wilcoxon matched-pairs signs ranks test).
4. Discussion

The aim of this study was to determine in a randomized, double-blinded, and placebo-controlled trial whether antihelminthic treatment would interfere with the clinical course of asthma in individuals living in a *S. mansoni* endemic area. Additionally, we evaluated the *in vitro* cytokine profile in response to an aeroallergen and to *S. mansoni* antigen before and after antihelminthic treatment. There was no change in the asthma score during the first weeks after treatment in both groups, neither was there any variation after 30 to 90 days. At day 90, group 1 and group 2 were combined into one group who received Albendazole and Praziquantel each three months thereafter. When comparing the baseline score (D0) of this group, we found a higher frequency of score one and two at 6 and 12 months after treatments. The most likely straightforward explanation for these findings is that early after treatment the antigens released during the parasite killing maintain the protective effect on asthma severity. The effect of the antihelminthic treatments on the clinical score of asthma was perceived, however, only six to 12 months after repeated treatments. Considering that patients remained living in the same area using the contaminated water in their activities, we believe that only after sequential treatments, the loss of the protective effect of parasite infection over asthma symptoms could be observed. Although the asthma clinical score scale used in this study is not validated, against other measures of asthma severity or control, it gives an idea of the clinical course of the disease, when obtained repeatedly. It considers the use of antiasthmatic drugs and corticosteroids, as well as the need for the patient to visit emergency rooms or the hospital due to asthma attacks. The relevance of this score categorization was supported by the results of pulmonary function test which showed a significantly higher frequency of patients with abnormal lung function 12 and 18 months after antihelminthic treatments. For medical and ethical reasons, the group of placebo could not be left without antihelminthic treatment for more than 90 days. Hence, the two groups of patients received Albendazole and Praziquantel thereafter. One may ask if the adverse effect of long-term treatment on asthma severity we observed in this study would be due some seasonal differences during the follow-up study. We are aware that it is a limitation in this type of study design; however, as the study was conducted in a region with a tropical climate with no defined seasons and low weather variations during the year, this factor may not affect significantly the asthma severity.

Although our initial hypothesis in this study was that *S. mansoni* infection protects against asthma, the observation herein does not allow us to rule out the effect of other helminth infections in this protection, as have been proposed by other authors in experimental models of OVA-induced asthma [11–13]. In a polyhelminth endemic area, it is difficult to establish which parasite is protecting the host against a harmfully Th2-mediated pathological process. Previous systematic reviews and meta-analysis studying the effect of geohelminth infections on the risk of asthma showed that these parasites in general do not protect against asthma, but hookworm was shown to reduce the risk of the disease [14, 15]. A clinical trial using *Trichuris suis* ova resulted in no significant changes in symptom scores of allergic rhinitis [16]. Furthermore, treatment of hookworm
infection, which leads to a reduction in the worm burden, increased the risk of allergen skin sensitization but did not interfere with the symptoms of allergic diseases [17], symptom scores of allergic rhinitis [16] and asthma [18].

In a systematic review and meta-analysis of epidemiological studies that researched the association between intestinal parasite infection and the presence of atopy, the authors found a consistent protective effect on allergic sensitization in patients with *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, or *Schistosoma* sp, infection [18]. There is evidence that in *S. mansoni* infection, the large number of regulatory cells and high levels of modulators molecules in the host system lead to a downmodulation not only of the parasite immune response, but also the immune response to bystander antigens as revised by Dunne and Cooke [19].

A possible explanation for these conflicting observations may include the time of exposure to the worm (if acute or chronic) and also the helminthic species. For instance, it was demonstrated in a randomized, double-blinded and placebo controlled trial that Albendazole and Praziquantel treatment of infected women during pregnancy was associated with increased risk of eczema and wheeze in the offspring [20]. New data presented here reinforce the idea that deworming may be associated with asthma worsenings.

Regarding the effect of antihelminthic treatments on cytokine production, we were able to measure IFN-y, IL-5, and IL-10 in response to Der p 1 and to SWAP in supernatants of PBMC cultures before treatment and 7 and 90 days after treatment. Although Th2 molecules such as IL-4, IL-5, and IL-13 are key cytokines involved in the inflammatory response in asthma, IFN-y has also been associated with asthma severity [21, 22]. The baseline levels of IL-5 in response to Der p 1 in the present study were higher in the Praziquantel group compared to the placebo group. However, there was no significant difference in the pre-versus posttreatment levels of this cytokine in the two studied groups. On the other hand, the production of IL-10 in response to SWAP decreased 90 days after antihelminthic treatment in the Praziquantel group. This result suggests that the regulatory mechanisms exerted by the parasites begin to diminish early after treatment.

In the past few years, it has been shown that chronic helminth infections or parasite products induce the production of T-regulatory cells and molecules such as IL-10. This response has been associated with a down-modulation of allergic inflammatory mediators, such as Th2-cytokines, eosinophils, and histamine in murine models of allergic asthma [7, 12, 23]. We have characterized the *in vitro* immune response of asthmatic patients to some *S. mansoni* antigens and found a high production of *S. mansoni* antigen-specific IL-10 not only in cells of *S. mansoni* infected individuals but also in cells of noninfected asthmatic individuals [24]. There was also a significantly higher production of IL-10 and lower production of Th2-cytokine IL-5 in response to Der p 1 in PBMC cultures of asthmatic patients infected with *S. mansoni* compared to non-infected asthmatic patients [25]. Our findings of decreased levels of IL-10 production after antihelminthic treatment in the present study, therefore, are in agreement with our’s [25] and other authors, [26] previous studies.

Other regulatory mechanisms may contribute to the suppression of allergic inflammation induced by helminths. For instance, Pacifico et al. showed that T CD4+CD25+ cells protect mice against allergen induced airway inflammation through an IL-10 independent mechanism [12]. This finding differs from other studies, which have demonstrated that IL-10 is a key cytokine that suppresses the inflammatory response in OVA-induced asthma in mice infected with helminths. In mice infected with *H. polygyrus*, for example, the reduction in the number of eosinophils and in the levels of IL-5 was associated with IL-10 production and migration of regulatory cells to the draining lymph nodes [27].

It has also been demonstrated that cytotoxic T-lymphocyte antigen 4 (CTLA-4), a molecule rapidly upregulated after T-cell activation that provides negative feedback signaling, and limits the immune response as reviewed by Deurloo et al. [28], is involved in the suppression of allergic response in asthma [29]. We previously demonstrated that the lower levels of Th2-cytokines in asthmatics infected with *S. mansoni* compared to non-infected asthmatics were associated with a higher frequency of CD4+ T-cells expressing CTLA-4 [30]. Based on these findings, it is likely that the mechanisms underlying the regulation of inflammatory responses in asthma by *S. mansoni* antigens involve IL-10 [31], T-regulatory cells [12], and other mechanisms such as the expression of CTLA-4 [29, 30].

In the present study, where the number of asthmatic individuals who filled out the inclusion criteria and who agreed to participate was small, there was a significant worsening in the clinical scores of asthma as well as in the pulmonary function after repeated antihelminthic treatments. These findings are consistent with the hypothesis of the protective role of helminths on atopic diseases. Changes in clinical scores of asthma and in the pulmonary function tests were observed later after sequential antihelminthic treatments. Based on these results, we argue that the antigens released by the parasite when it dies can maintain protection against atopic diseases. After the clearance of antigens and repeated antihelminthic treatments to maintain a low parasite load, a loss of protection of parasite infection in the clinical course of asthma is observed. The data suggests that IL-10 could be implicated in this protection. However, a better understanding of immune events following antihelminthic treatment is still required and may have practical consequences in the development of future therapies of allergic diseases.

**Acknowledgments**

We are very grateful to all volunteers from the community of Agua Preta - Gandu who agreed to participate in this study and to the local health agent Irene Jesus for assistance with the field work. We thank Dr. Irisâma Souza for performing the Chest-X-ray evaluation and Cristina Toledo for the review of the manuscript. This work was supported by the Brazilian National Research Council (CNPq). MIA, AAC and EMC are investigators supported by CNPq.
References


