

Schistosoma mansoni Antigens Modulate Allergic Response In Vitro in Cells of Asthmatic Individuals

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ABSTRACT *Schistosoma mansoni* infection is associated with a low prevalence of asthma and a less severe form of the disease. The mechanisms underlying this association may include the production of regulatory cells and cytokines. The aim of this study was to evaluate the immune response induced by the *S. mansoni* antigens, Sm22.6, PIII, and Sm29 and their ability to suppress allergen-specific IL-5 production by peripheral blood mononuclear cells (PBMC) from asthmatic individuals. PBMCs were stimulated in vitro with *S. mansoni* antigens in the presence or absence of antigen-1 of the mite *Dermatophagoides pteronyssinus* (Der p1). Cytokines were measured in PBMC supernatants by enzyme-linked immunosorbent assay (ELISA), and the phenotype of cells producing IL-10 was assessed using flow cytometry. High production of *S. mansoni* antigen-specific IL-10 was observed not only in cells of *S. mansoni*-infected individuals, but also in cells of noninfected asthmatic individuals. In the former group, the main cellular sources of IL-10 were CD4⁺ CD25⁺, and CD14⁺ cells. The levels of IFN- γ , IL-5, and IL-13 in the noninfected asthmatic group were \sim 100 pg/ml in response to the antigens. Moreover, when *S. mansoni* antigens were added to cultures stimulated with Der p1, levels of IL-10 were increased (Der p1 = 234 ± 118 ; Der p1+Sm22.6 = 1189 ± 595 ; Derp1+PIII = 799 ± 331 ; Derp1+Sm29 = 652 ± 288 pg/ml) with reduced levels of IL-5 (Der p1 = 286 ± 219 ; Der p1+Sm22.6 = 93 ± 153 ; Derp1+PIII = 132 ± 188 ; Derp1+Sm29 = 96 ± 86 pg/ml). The *S. mansoni* antigens evaluated in the present study induced the production of the regulatory cytokine IL-10 and down-modulated the Th2 immune response that participates in the pathology of asthma. Drug Dev Res 72:538–548, 2011. © 2011 Wiley-Liss, Inc.

Key words: asthma; *Schistosoma mansoni* antigens; interleukin-10

INTRODUCTION

Allergic diseases have been increasing in prevalence in developing countries around the world over the past few decades [Sears, 1997; Yazdanbakhsh et al., 2002]. A complex mechanism involving genetic and environmental factors is responsible for the pathogenesis of allergic atopic disorders such as asthma and

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rhinitis. The immunopathogenesis of these diseases involves type 2 helper cells (Th2), with production of interleukin (IL)-4, IL-5, IL-9, and IL-13 [Soroosh and Doherty, 2009; Wills-Karp, 1999]. However, the Th1- and Th17- type immune responses are also thought to play important roles in this process [Ballantyne et al., 2007; Cho et al., 2005; Smart and Kemp, 2002].

Asthma represents one of the most prevalent and severe manifestations of atopy. Current treatment is mainly based on corticosteroid therapy, and new alternatives could be developed from a better understanding of the mechanism underlying the inflammatory process. The induction of regulatory mediators capable of down-modulating both the Th1 and Th2-type immune responses would be one way to prevent this disease. Thus, induction of T-regulatory cells that produce immunomodulatory molecules, such as IL-10, might offer a rational strategy to prevent immune-mediated allergic inflammatory diseases [Araujo et al., 2010; Vissers et al., 2004].

It has been demonstrated that helminth infections modulate Th1-cytokines involved in autoimmune diseases and Th2-cytokines responsible for allergic diseases [Elliott et al., 2007]. This suppression appears not to be strictly dependent on parasite infection, but can be extended to pathogen-derived antigens [Cardoso et al., 2010; Elliott et al., 2003; Pacifico et al., 2009].

Among helminths associated with protection against allergies, *Schistosoma mansoni* appears to induce particularly strong down-modulation of the inflammatory response that mediates atopic disorders [Araujo and Carvalho, 2006]. Although the immune response in both allergies and *S. mansoni* infection is predominantly of the Th2 type, in *S. mansoni* infection, a high production of IL-10 has been demonstrated [Araujo et al., 1996; Malaquias et al., 1997]; however, in asthma there is an impairment in production of this cytokine [Araujo et al., 2004; Borish et al., 1996]. This is important, as IL-10 has a number of anti-inflammatory effects and appears to be protective against allergy [Adachi et al., 1999; Akdis and Blaser, 2001; Araujo et al., 2004; Kitagaki et al., 2006; Marinho et al., 2010; van den Biggelaar et al., 2000].

The aim of the present study was to evaluate the immune response induced by the *S. mansoni* antigens Sm22.6, PIII, and Sm29 in peripheral blood mononuclear cells (PBMC) of asthmatic individuals. We also assessed the ability of these *S. mansoni* antigens to induce regulatory cells and cytokines to suppress the production of the Th2-cytokine, IL-5 released by PBMCs of asthmatic individuals in response to the *Dermatophagoides pteronyssinus* antigen-1 (Der p1), one of the major allergens known in Brazil [Araujo et al., 2004].

The Sm22.6 antigen is a soluble protein associated with the tegument of *S. mansoni* and is present throughout its life cycle, with the exception of the egg stage [Jeffs et al., 1991]. In an experimental model of *S. mansoni* infection, Sm22.6 when used together with Freund's adjuvant induced partial protection (34.5%) against reinfection [Pacifico et al., 2006]. PIII is a multivalent antigen obtained from *S. mansoni* adult worms that modulates granuloma size in mice infected with *S. mansoni* [Hirsch and Goes, 1996; Hirsch et al., 1997]. We have previously shown that Sm22.6 and PIII are able to induce IL-10 by cells of *S. mansoni*-infected individuals [Cardoso et al., 2006b]. The Sm29 antigen is a membrane-bound glycoprotein located on the tegument of the adult worm and lung stage schistosomula [Cardoso et al., 2006a]. This protein induces a Th1 cytokine profile in mice and it increased by 50% the protection against infection [Cardoso et al., 2008].

We previously demonstrated that Sm22.6, PIII, and Sm29 antigens suppressed the Th2-inflammatory response in an experimental model of allergic asthma [Cardoso et al., 2010]. These antigens were tested in this study regarding their ability to induce IL-10 production and suppress the Th2 response in vitro in cells of asthmatic individuals.

MATERIAL AND METHODS

Study Design

In this study we enrolled individuals with mild asthma without helminth infection attending the allergy clinic of the Federal University of Bahia in Salvador, Bahia, Brazil. Asthmatic patients were selected if their responses to the ISAAC questionnaire corresponded with a personal history of asthma during the previous 12 months, if they had a mild form of disease according to results of the pulmonary function tests, and if the results of physical examinations performed by 2 physicians, noted abnormal findings, such as dyspnea and wheezing [Medeiros et al., 2003]. Patients selected were 5–50 years old. Children <5 years old were not included because of the difficulty in performing a pulmonary function test, whereas subjects >50 years of age were not included because of their increased rates of chronic obstructive pulmonary disease. Current smokers and those using antihistamines drugs or corticosteroids were also not included in the study. We included the first 20 individuals to fit the inclusion criteria and agreed to participate in this study. All participants were submitted to three stool parasite exams. To confirm that subjects were not infected by *S. mansoni*, we measured *S. mansoni*-soluble adult worm antigen (SWAP)-specific IgE and IgG4 in serum.

As control groups, we included individuals chronically infected with *S. mansoni* living in the endemic area of Conde-BA and asthmatic individuals infected with *S. mansoni* living in the same endemic area. All groups of individuals underwent an in vitro immune response evaluation, which included measurement of IL-10, IL-5, IL-13, and interferon- γ (IFN- γ) production by PBMCs in response to *S. mansoni* antigens. In the noninfected asthmatic group, we evaluated the main cellular source of IL-10 production by flow cytometry, as well as the effect of the addition of *S. mansoni* antigens to Der p1-specific IL-5 and IL-10 production.

Human experimentation guidelines of the US Department of Health and Human Service were followed in the conduction of this study, and the Ethical Committee of the Maternidade Climério de Oliveira, Federal University of Bahia approved the study (License number: 71/2004). Informed consent was obtained from all participants or their legal guardians.

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, to estimate parasite load [Katz et al., 1970].

Schistosoma mansoni Antigens

The *S. mansoni* recombinant proteins, Sm22.6 and Sm29, and a fraction of *S. mansoni* soluble adult worm antigen (SWAP), termed PIII, were used in this study. SWAP and SEA (soluble egg antigen) were used as control antigens. The recombinant proteins were produced in *Escherichia coli* and were tested for contamination with LPS using a commercially available LAL Chromogenic Kit (CAMBREX). The level of LPS in Sm22.6 was 0.132 ng/ml; in Sm29 it was 0.126 ng/ml. The antigen PIII was also tested for LPS contamination; the level was 0.002 ng/ml.

Cell Culture and Cytokine Measurements

PBMCs from individuals of the study were obtained via a Ficoll-Hypaque gradient method and adjusted to a concentration of 3×10^6 cells/ml in complete RPMI medium (Life Technologies GIBCO-BRL, Gaithersburg, MD). Cells were cultured in vitro with the antigens Sm22.6, PIII, and Sm29 in the presence or absence of Der p1 antigen. We also used SWAP and SEA (10 μ g/ml) and the mitogen phytohemagglutinin (PHA; 2 μ g/ml). Polymyxin B (final concentration 30 μ g/ml) was added to the cell cultures stimulated with recombinant proteins to abrogate the cytokine response to LPS as described by Cardoso et al. [2007]. Cultures were incubated for 72 hr and

supernatants were collected for cytokine measurements. Levels of IL-10, IFN- γ , IL-5, and IL-13 were determined by ELISA using commercially available kits (R&D Systems).

SWAP-Specific IgE and IgG4 Measurements in Human Serum

Levels of SWAP-specific IgE and IgG4 were measured in serum from all studied individuals using an indirect ELISA technique, as previously described [Ribeiro de Jesus et al., 2000; Souza-Atta et al., 1999].

Intracellular Staining for IL-10 Expression

Surface marker and intracellular cytokine expression were assessed by immunofluorescent staining of T cells, B cells, and monocytes. Intracellular staining was performed with a PE-labeled monoclonal antibody against human IL-10 in saponin buffer (phosphate-buffered saline [PBS], supplemented with 0.5% bovine serum albumin [BSA] and 0.5% saponin). Briefly, PBMCs (3×10^5) obtained by a Ficoll-Hypaque gradient were incubated with the antigens Sm22.6, PIII, and Sm29 (10 μ g/ml) for 20 hr, at 37°C and in 5% CO₂. The antibodies used for staining were immunoglobulin isotype controls FITC (clone MOPC-21, BD Pharmingen), PE (clone R35-95, BD Pharmingen), and PeCy5 (clone G155-178, BD Pharmingen), anti-CD14-FITC (clone M5E2, BD Pharmingen), anti-CD19-FITC (clone HIB19, BD Pharmingen), anti-CD3-FITC (clone OKT3, eBioscience), anti-CD4-PeCy5 (clone RPA-T4, BD Pharmingen), anti-CD8-PeCy5 (clone RPA-T8, BD Pharmingen), anti-CD25-FITC (clone M-A251, BD Pharmingen), and anti-GITR-FITC (clone 110416, R&D Systems). Intracellular staining was performed with PE-labeled monoclonal antibody against human IL-10 (clone JES3-19F1, BD Pharmingen) in saponin buffer (PBS, supplemented with 0.5% BSA and 0.5% saponin). During the last 4 hr of culture, Brefeldin A (10 μ g/ml; Sigma, St. Louis, MO), which impairs protein secretion by the Golgi complex, was added to the cultures. The cells were then washed in PBS and fixed in 4% formaldehyde for 20 min at room temperature. Data were collected on a FACScan flow cytometer (FACSort, BD Biosciences, San Jose, CA).

Analysis of FACS Data

The frequency of positive cells was analyzed using the program CellQuestTM in two regions. The lymphocyte region was determined using granularity (SSC) \times size (FSC) plot. Monocytes were selected based on their granularity and expression of CD14. Limits for the quadrant markers were always set based on negative populations and isotype controls. For analysis of CD8-positive lymphocytes, quadrants were always set

for CD8 high populations in order to not to include CD8-low-positive NK cells.

Statistical Analysis

Statistical analysis was performed using the software GraphPad Prism (GraphPad Software, San Diego, CA). The differences between means were assessed using nonparametric analysis of variance (ANOVA). Fisher's exact test was used to compare proportions. The difference in mean age was assessed by the Kruskal–Wallis test. The frequency of positive cells was expressed as percentages. Statistical significance was established at the 95% confidence interval.

RESULTS

The demographic characteristics of the studied individuals, as well as the antibody levels and parasite burden, are shown in Table 1. There was no statistically significant difference in the mean age or gender between groups. The levels of SWAP-specific IgE and IgG4 were above the cutoff in individuals infected with *S. mansoni*, for both nonasthmatics (group I) and asthmatics (group II). The levels of these antibodies were below the cutoff in the noninfected asthmatics (group III) included in the study, who were also negative to *S. mansoni* infection by three stool samples (Table 1).

Cytokine Profile Induced by *S. mansoni* Antigens in the PBMCs of the Studied Population

In PBMCs of *S. mansoni*-infected individuals from group I and group II, all antigens evaluated induced significant levels of IL-10, compared with nonstimulated cultures (Fig. 1A and B, respectively). An exception was observed in cultures stimulated with Sm22.6 from group II (Fig. 1B). As expected, we observed higher

levels of IL-10 in cultures stimulated with SWAP and SEA from group I (389 ± 420 and 340 ± 370 pg/ml, to SWAP and SEA, respectively; Fig. 1A) and group II (655 ± 469 and 742 ± 527 pg/ml, to SWAP and SEA, respectively; Fig. 1B) than from group III, whose mean levels were <50 pg/ml to these two antigens.

Surprisingly, the production of IL-10 in group III was higher in response to the *S. mansoni* antigens Sm22.6, PIII, and Sm29 (451 ± 350 , 364 ± 289 , and 712 ± 368 pg/ml, respectively; Fig. 1C) compared with nonstimulated cultures.

The levels of the Th2 cytokines IL-5 and IL-13 were also measured in supernatants of PBMC cultures (Fig. 2). In groups I and II, cultures stimulated with PIII had significant levels ($866 \pm 1,286$ and $825 \pm 1,216$ pg/ml, respectively) of IL-5 compared with nonstimulated cultures (34 ± 14 and 36 ± 19 pg/ml, respectively). In these groups there was a significant level of IL-5 production when PBMC cultures were stimulated with SWAP or SEA (Fig. 2A and B). In group III, levels of IL-5 were below the limit of detection in response to most *S. mansoni* antigens (15.6 pg/ml), being detected only in cell cultures stimulated with SEA in three individuals (mean 187 ± 142 pg/ml; Fig. 2C).

Similar to the IL-5 profile, the levels of IL-13 in groups I and II were higher in cultures stimulated with SWAP, SEA, and PIII, compared with cultures without stimulation ($P < 0.05$; Fig. 2D,E). The levels of IL-13 in response to *S. mansoni* antigens in the noninfected asthmatic individuals were ~ 100 pg/ml (Fig. 2F).

We also evaluated the production of the Th1-signature cytokine, IFN- γ in supernatants of PBMC cultures stimulated with *S. mansoni* antigens. These antigens induced IFN- γ production in cell of chronically infected individuals, with levels higher in cultures stimulated with Sm22.6 ($1,708 \pm 1,478$ pg/ml; $P < 0.001$), PIII ($729 \pm 1,134$ pg/ml; $P < 0.05$) and Sm29 ($870 \pm 1,242$ pg/ml; $P < 0.01$) compared with nonstimulated cultures (97 ± 192 pg/ml; Fig. 3A). There was no difference in IFN- γ production among nonstimulated and *S. mansoni* antigen-stimulated cultures in group II. All the *S. mansoni* antigens, however, induced production of this cytokine (Fig. 3B). We observed that in group III, IFN- γ was detected at levels of <100 pg/ml in response to all tested antigens (Fig. 3C). There was higher production of IL-10, IL-5, IL-13, and IFN- γ in cultures stimulated with the mitogen PHA compared with nonstimulated cultures ($P < 0.05$; data not shown).

Phenotype of Cells Producing IL-10 After *S. mansoni* Antigen Stimulation of PBMC In Vitro

Because IL-10 appears to play an important role in modulating the inflammatory response in asthma, in

TABLE 1. Demographic Data and *Schistosoma mansoni* Infection Status in Individuals Included in the Study

Subjects	Group I (n = 20)	Group II (n = 22)	Group III (n = 18)
Age, y ^a mean \pm SD	21 \pm 8	19 \pm 10	24 \pm 9
Gender, % male ^b	63.6	48.0	50.0
SWAP-specific IgG4 (OD) mean \pm SD	0.34 \pm 0.20	0.40 \pm 0.20	0.02 \pm 0.03
SWAP-specific IgE (OD) mean \pm SD	0.14 \pm 0.20	0.11 \pm 0.07	0.01 \pm 0.03
<i>S. mansoni</i> burden, eggs/g of stool mean \pm SD	203 \pm 297	112 \pm 134	0

^a $P > 0.05$; Mann-Whitney test.

^b $P > 0.05$; Fisher's exact test.

Group I: *S. mansoni*-infected individuals living in an endemic area; Group II: Asthmatic infected with *S. mansoni* living in an endemic area; Group III: Asthmatics without helminth infections living outside the endemic areas. Cutoff: IgG4: 0.18, IgE: 0.05.

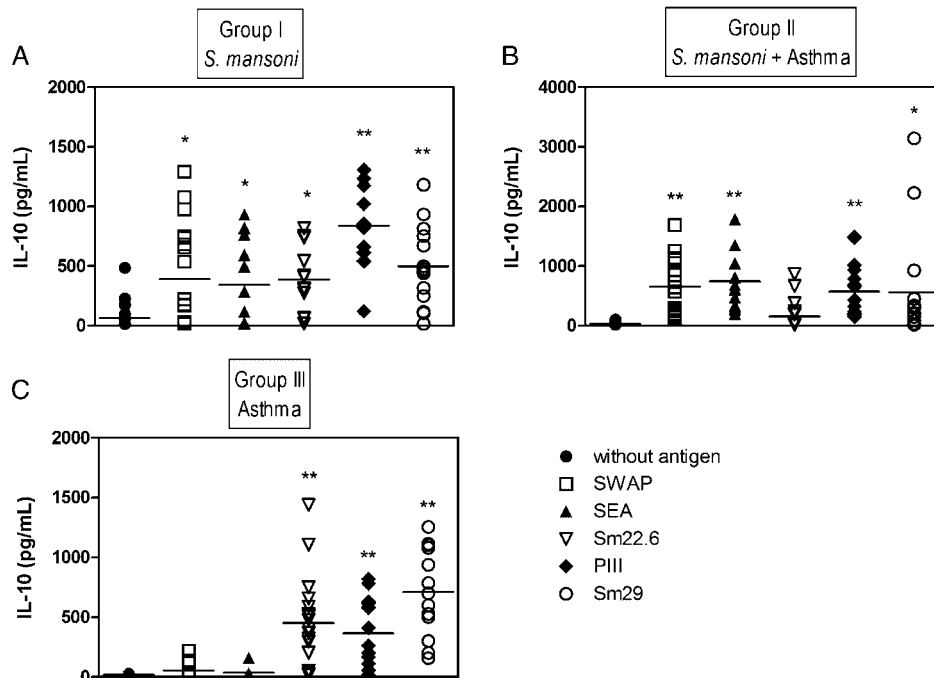


Fig. 1. Levels of IL-10 produced by peripheral blood mononuclear cells (PBMCs). Levels of IL-10 produced by peripheral blood mononuclear cells (PBMCs) from group I, patients chronically infected with *S. mansoni* (A); group II, *S. mansoni*-infected asthmatics (B); and group III, noninfected asthmatics (C). Cultures were stimulated *in vitro* with 10 μ g/ml of the *S. mansoni* antigens Sm22.6, PIII, and Sm29, besides the soluble adult worm antigen (SWAP) and the soluble egg antigen (SEA). Polymyxin B (30 μ g/ml) was added to the cultures stimulated with the recombinant antigens Sm22.6 and Sm29 to block the effect of LPS. Levels of antigen-specific IL-10 were measured in supernatants of PBMC cultures using a sandwich ELISA, as described under Materials and Methods. Dots represent levels of IL-10 of each individual. Horizontal lines represent the median values. Asterisks indicate statistically significant differences between antigen-stimulated cultures and culture without antigen stimulation. * $P < 0.05$ and ** $P < 0.01$; Kruskal–Wallis test.

addition to the measurement in PBMC cultures, we evaluated the main cell source of this cytokine.

The phenotype of IL-10-expressing cells in response to the antigens Sm22.6, PIII, and Sm29 was performed using the flow cytometry technique in PBMCs of group III (Table 2). After stimulation with Sm22.6, the frequency of CD14⁺ and CD4⁺CD25⁺ cells expressing IL-10 was higher compared with unstimulated cultures ($P < 0.05$). In cultures stimulated with Sm29, the main cell sources of IL-10 were TCD4⁺, CD4⁺GITR⁺, and CD4⁺CTLA-4⁺ cells ($P < 0.05$). There was no significant difference in IL-10-producing cells when the cultures were stimulated with PIII (Table 2).

Effect of the Addition of *S. mansoni* Antigens on IL-10 and IL-5 Production in Response to the Allergen Der p1

Because the *S. mansoni* antigens Sm22.6, PIII, and Sm29 induced IL-10 production by PBMCs of uninfected asthmatics, whereas they did not induced the production of the Th2 cytokine IL-5, we decided to assess the ability of these antigens to alter the response to the allergen Der p1 *in vitro* in cells from these individuals. The addition of Sm22.6, PIII, and Sm29 to the cultures stimulated with

Der p1 antigen led to an increase in the levels of IL-10 to all tested antigens (1,190 \pm 595 to Sm22.6+Derp, 799 \pm 331 to PIII+Derp and 652 \pm 288 pg/ml to Sm29+Derp; $P < 0.001$), compared with cultures stimulated with Der p1 alone (234 \pm 118 pg/ml; Fig. 4A–C). In contrast, there was a decrease in the levels of Der p1-specific IL-5 in cultures to which Sm22.6 and Sm29 were added (Der p1 = 286 \pm 219, Der p1+Sm22.6 = 93 \pm 153, Der p1+Sm29 = 95 \pm 86 pg/ml; Fig. 4A,C). The addition of PIII to the culture did not change the mean levels of IL-5 production in response to Der p1 (Fig. 4B).

DISCUSSION

Chronic helminth infections, or their products, induce the production of T regulatory cells and molecules, such as IL-10. This response has been associated with a down-regulation of allergic inflammatory mediators, such as Th2-cytokines, eosinophils, and histamine in murine models of allergic asthma [Cardoso et al., 2010; Lima et al., 2002; Pacifico et al., 2009; Royer et al., 2001].

In this study, we characterized *in vitro* the immune response by PBMCs from asthmatic patients to the *S. mansoni* antigens Sm22.6, PIII, and Sm29. We

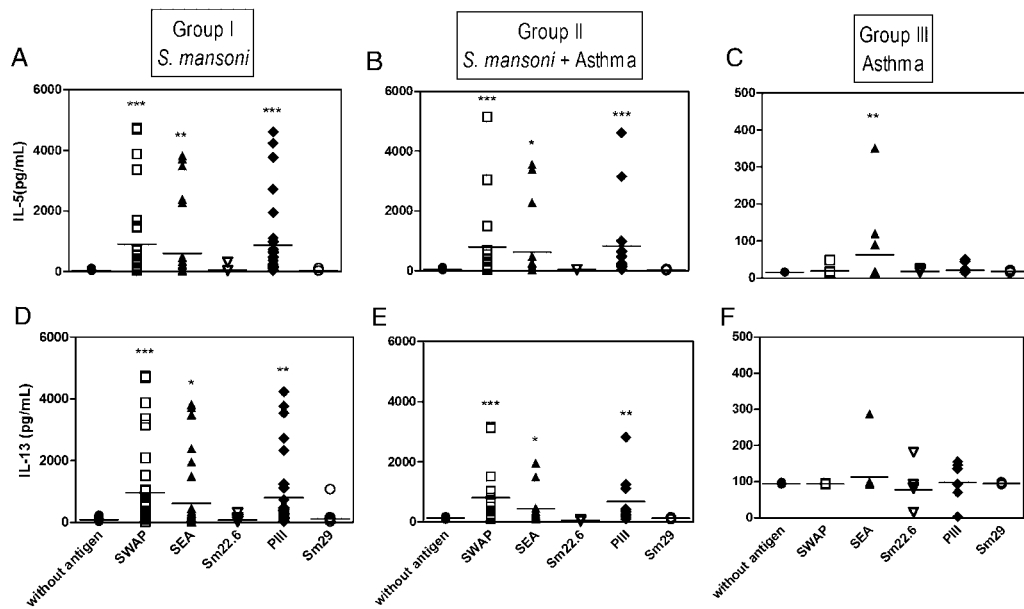


Fig. 2. Production of Th2 cytokines IL-5 and IL-13 by peripheral blood mononuclear cells (PBMCs). Production of Th2 cytokines IL-5 and IL-13 by peripheral blood mononuclear cells (PBMCs) of patients from group I (A and D, respectively), group II (B and E, respectively) and group III (C and F, respectively). Cultures were stimulated *in vitro* with 10 $\mu\text{g}/\text{ml}$ of the *S. mansoni* antigens Sm22.6, PII, and Sm29, besides the soluble adult worm antigen (SWAP) and the soluble egg antigen (SEA). Polymyxin B (30 $\mu\text{g}/\text{ml}$) was added to the cultures stimulated with the recombinant antigens Sm22.6 and Sm29 to block the effect of LPS. Levels of antigen-specific IL-5 and IL-13 were measured in supernatants of PBMC cultures using a sandwich ELISA, as described under Materials and Methods. Dots represent levels of cytokine of each individual. Horizontal lines represent the median values. Asterisks indicate statistically significant differences between antigen-stimulated cultures and culture without antigen stimulation. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.005$; Kruskal–Wallis test.

also evaluated whether these antigens have the ability to down-modulate the production of the cytokine IL-5, which is a key inflammatory cytokine in asthma. We demonstrated that these antigens induce production of the regulatory cytokine IL-10 in cells of noninfected asthmatics without inducing significant levels of Th1 or Th2 inflammatory cytokines. This is desirable, as it is well known that IL-4 and IL-5 are key cytokines involved in the inflammatory response in asthma and IFN- γ and TNF- α are associated with asthma severity [Cho et al., 2005; Stephens et al., 2002].

It has been documented that extracts of helminths and other pathogens can stimulate cells of noninfected individuals to produce cytokines [Van der Kleij et al., 2002]. A study evaluating the immune response to the *S. haematobium* toll-like ligand antigen, lyso-phosphatidyl serine (PS), demonstrated that this antigen induced the production of IL-10 by the innate immune system in children without infection [Van der Kleij et al., 2004].

Moreover, it has been demonstrated that *S. mansoni* phosphatidylserine (PS) also has the ability to stimulate antigen-presenting cells from naive individuals to produce IL-10 via toll-like receptor (TLR)-2 stimulation, and promotes T-regulatory cell

maturation. The cytokine production in response to TLR-stimulation differed between infected and uninfected children, being higher in uninfected than in infected ones [Van der Kleij et al., 2004]. The ability of parasite antigens to interact with TLRs and promote differentiation of cells from the innate immune system suggests that there are molecular patterns (PAMPs) associated with helminths that are involved in the down-regulation of the immune response. For instance, Thomas et al. [2003] defined a pathogen-associated molecule, LNFPIII-Dex that has the ability to drive the differentiation of naive DCs to a DC2 phenotype *in vitro* via a mechanism dependent on TLR4 and independent of MyD88. LNFPIII-Dex induces the production of anti-inflammatory mediators, suggesting that it can be used to prevent autoimmune and allergic diseases [Thomas et al., 2003]. Parasites are often long-lived and inhabit immunocompetent hosts for extended periods; therefore, it is not surprising that they induce modulatory molecules that modify host immune responses to allow for their survival [Van der Kleij et al., 2004].

The main sources of IL-10 in cells of noninfected asthmatic individuals stimulated with the Sm22.6, PIII, and Sm29 antigens in this study were CD4⁺ CD25⁺ cells, and monocytes. It was previously demonstrated

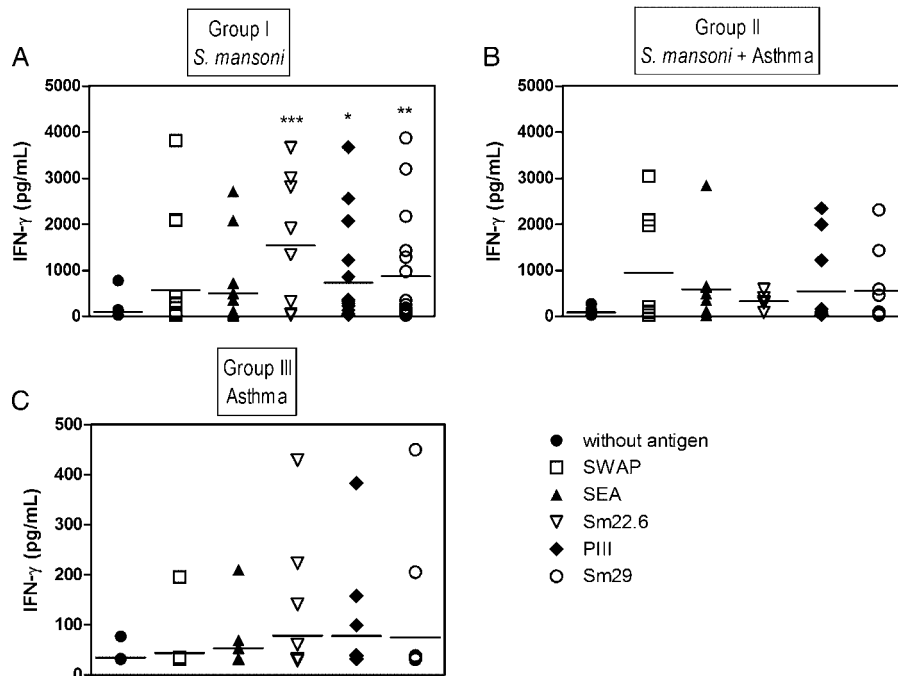


Fig. 3. Levels of IFN- γ produced by peripheral blood mononuclear cells (PBMCs). Levels of IFN- γ produced by peripheral blood mononuclear cells (PBMCs) of patients from group I (A), group II (B), and group III (C). Cultures were stimulated *in vitro* with 10 μ g/ml of the *S. mansoni* antigens Sm22.6, PIII, and Sm29, besides the soluble adult worm antigen (SWAP) and the soluble egg antigen (SEA). Polymyxin B (30 μ g/ml) was added to the cultures stimulated with the recombinant antigens Sm22.6 and Sm29 to block the effect of LPS. Levels of antigen-specific IFN- γ were measured in supernatants of PBMC cultures using a sandwich ELISA, as described under Materials and Methods. Dots represent levels of IFN- γ of each individual. Horizontal lines represent the median values. Asterisks indicate statistically significant differences between antigen-stimulated cultures and culture without antigen stimulation. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.005$; Kruskal–Wallis test.

TABLE 2. Frequency of Cells Producing IL-10 After *In Vitro* Stimulation With *S. mansoni* Antigens Sm22.6, PIII, and Sm29 ($n = 8$)[†]

	Without antigen	Sm22.6	PIII	Sm29
CD3 ⁺ CD4 ⁺	27.1 \pm 5.7	30.8 \pm 10.1	29.7 \pm 7.5	29.5 \pm 7.8
IL-10 in CD3 ⁺ CD4 ⁺	0.20 \pm 0.06	0.37 \pm 0.21	0.25 \pm 0.15	0.47 \pm 0.2
CD3 ⁺ CD8 ⁺	14.7 \pm 6.3	17.4 \pm 6.5	13.9 \pm 6.3	12.9 \pm 6.0
IL-10 in CD3 ⁺ CD8 ⁺	0.54 \pm 0.26	0.52 \pm 0.39	0.40 \pm 0.21	0.68 \pm 0.45
CD14 ⁺	16.7 \pm 6.0	7.3 \pm 3.6*	13.6 \pm 4.4	10.7 \pm 2.9
IL-10 in CD14 ⁺	0.64 \pm 0.56	3.09 \pm 1.87*	0.89 \pm 0.76	0.94 \pm 0.27
CD19 ⁺	2.8 \pm 1.6	2.9 \pm 1.2	2.0 \pm 0.9	2.0 \pm 0.8
IL-10 in CD19 ⁺	0.92 \pm 0.82	1.95 \pm 1.70	0.85 \pm 0.99	2.25 \pm 1.90
CD4 ⁺ CD25 ⁺	4.5 \pm 1.0	4.6 \pm 1.5	3.4 \pm 1.2	3.1 \pm 1.0
IL-10 in CD4 ⁺ CD25 ⁺	2.75 \pm 0.81	4.68 \pm 1.85*	1.26 \pm 0.63	3.57 \pm 1.26
CD4 ⁺ GITR ⁺	0.71 \pm 0.31	0.83 \pm 0.54	0.55 \pm 0.42	0.69 \pm 0.37
IL-10 in CD4 ⁺ GITR ⁺	3.27 \pm 2.88	7.38 \pm 4.90	4.30 \pm 4.17	14.7 \pm 10.4*
CD4 ⁺ CTLA-4 ⁺	2.4 \pm 0.59	2.4 \pm 0.8	2.4 \pm 1.3	2.6 \pm 1.0
IL-10 in CD4 ⁺ CTLA-4 ⁺	1.22 \pm 0.58	3.85 \pm 3.55	1.56 \pm 0.87	3.42 \pm 2.43*

[†]Values represent mean percentage \pm standard deviation.

Frequency of different cell types expressing IL-10 in PBMC cultures stimulated with the *S. mansoni* antigens Sm22.6, PIII, and Sm29. * $P < 0.05$; unpaired *t*-test. Asterisks indicate statistically significant differences between frequency of cell producing IL-10 in cultures stimulated with *S. mansoni* antigens versus cultures without antigen stimulation.

that IL-10 is produced by both the innate and adaptive immune responses following *S. mansoni* infection, and most of the CD4⁺ T cells that produce IL-10 also express the CD25 marker [Hesse et al., 2004]. In

support of the present results, studies have shown that CD4⁺CD25⁺ T cells, through the production of IL-10, protect mice treated with *Schistosoma japonicum* egg against experimental asthma [Yang et al., 2007].

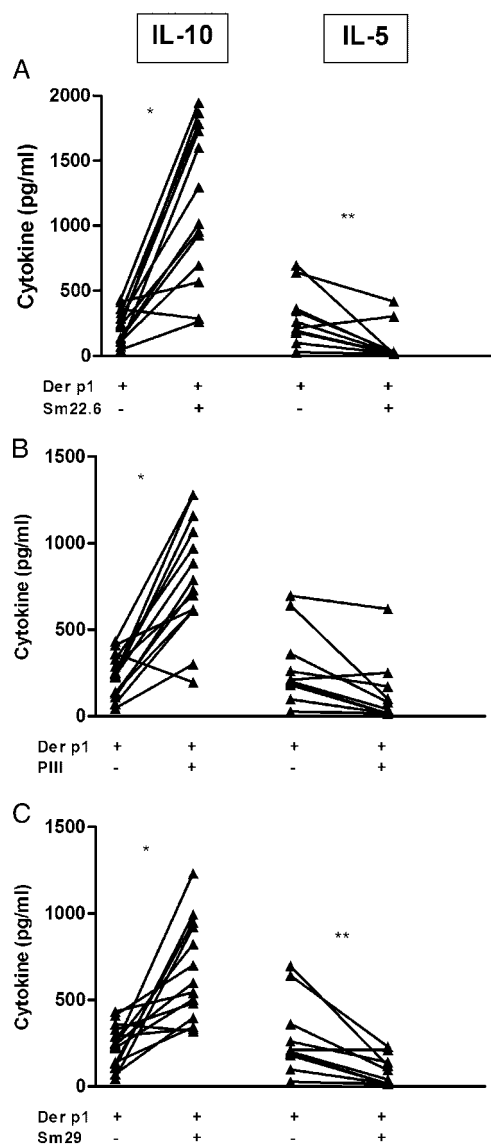


Fig. 4. Levels of *Dermatophagoides pteronyssinus* antigen 1 (Der p1)-specific production of IL-10 and IL-5 in supernatants of PBMCs. Levels of *Dermatophagoides pteronyssinus* antigen 1 (Der p1)-specific production of IL-10 and IL-5 in supernatants of PBMC cultures of noninfected asthmatic individuals (group III, $n = 14$) in the presence or absence of the *S. mansoni* antigens Sm22.6 (A), PIII (B), and Sm29 (C). Cytokines were measured by ELISA, as described under Materials and Methods. Asterisks indicate statistically significant differences between levels of cytokine in cultures stimulated with Der p1 versus Der p1 plus *S. mansoni* antigens. * $P < 0.001$ and ** $P < 0.05$; Wilcoxon matched-pairs signed-rank test.

Monocytes are important cells in linking the innate and adaptive immune response. The production of IL-10 by these cells in response to Sm22.6 antigen corroborates our previous studies showing alternatively activated monocytes as important source of IL-10 in PBMC cultures from *S. mansoni*-infected asthmatics [Oliveira et al., 2009]. Sm22.6 is a surface membrane

molecule that belongs to the family of EF-hand containing proteins with sequence similarity to dynein light chain (DLC) and with major nonparasite allergens [Fitzsimmons et al., 2007]. Herein, we hypothesize that Sm22.6 protein motifs may bind to a receptor in human monocytes leading to the production of IL-10. There are limited data in the literature evaluating the role of TLRs in protein recognition. Vabulas et al. [2001] showed that human HSP60 protein is recognized by the TLR-2 from innate immune cells.

We also demonstrated that Sm22.6 and Sm29 antigens have an important immunomodulatory effect on IL-5 production, as demonstrated by the lower levels of this cytokine in cultures stimulated with Der p1 in the presence of the *S. mansoni* antigens. The cytokine IL-5 induces the synthesis of eosinophils and activates these cells and provides additional evidence that the antigens used in this study are able to down-modulate the Th2-mediated inflammatory response.

The three antigens tested in this study were also able to induce the production of IL-10 by PBMCs of uninfected asthmatic individuals in response to Der p1 antigen. Previously, we demonstrated impairment in Der p1-specific IL-10 production by PBMCs of asthmatic uninfected individuals [Araujo et al., 2004].

We also showed in a murine model of OVA-induced allergic asthma that Sm22.6, PIII, and Sm29 antigens down-modulated the Th2-inflammatory response. Such down-regulation included a decrease in lung inflammation, number of eosinophils in bronchoalveolar lavage fluid (BALF), levels of EPO in lung tissue and serum OVA-specific IgE levels, in comparison with nonimmunized mice. IL-10 appears unlikely to be the only molecule responsible for this modulation, as this cytokine was not induced by all tested antigens in our experimental model [Cardoso et al., 2010].

Other regulatory mechanisms may contribute to the suppression of allergic inflammation induced by helminths. Indeed, Pacifico et al. [2009] showed that T CD4⁺CD25⁺ cells protect mice against allergen-induced airway inflammation via an IL-10 independent mechanism. This differs from other studies that have demonstrated that IL-10 is a key cytokine in suppressing the inflammatory response in OVA-induced asthmatic mice infected with helminths. In mice infected with *H. polygirus*, 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 [Kitagaki et al., 2006].

It has also been demonstrated that cytotoxic T-lymphocyte antigen 4 (CTLA-4), a molecule rapidly up-regulated after T-cell activation and which provides a negative feedback signal limiting the immune response as reviewed by Deurloo and van Oosterhout

[2004], is involved in the suppression of allergic response in asthma. In murine models of asthma, treatment with CTLA-4-Ig was able to reduce IL-4 and IL-5 production in response to allergen challenge [Tsuyuki et al., 1997]. Furthermore, treatment of mice infected with *Trichinella spiralis* with anti-CTLA-4 resulted in high levels of IL-4, suggesting that the presence of CTLA-4 is important for inhibition of the Th2 response [Furze et al., 2006]. Indeed, we previously demonstrated that the lower levels of Th2-cytokines in asthmatics infected with *S. mansoni* compared with noninfected asthmatics was associated with a higher frequency of TCD4 cells expressing CTLA-4 [Oliveira et al., 2009].

The most important source of IL-10 in cells of asthmatic individuals stimulated with Sm29 antigen in our study were the TCD4⁺ cells expressing CTLA-4 and glucocorticoid-induced TNF receptor family-related protein (GITR). GITR is a molecule constitutively expressed on the cell surface of natural T-regulatory cells [McHugh et al., 2002; Shimizu et al., 2002]; it delivers a strong co-stimulatory signal allowing IL-2 dependent proliferation of Tregs [Shevach and Stephens, 2006].

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 [Araujo et al., 2004], T-regulatory cells [Pacífico et al., 2009], and other mechanisms such as the expression of CTLA-4 [Oliveira et al., 2009].

In the present study, IL-10 induced by the *S. mansoni* antigens, Sm22.6 and Sm29 suppressed the Th2-specific allergic response as demonstrated by the down-regulation of Der p1-specific IL-5 production in the presence of these antigens.

Taken together, we conclude that the *S. mansoni* antigens used in this study possess the ability to induce IL-10 production in vitro and to down-regulate the inflammatory allergic response to aeroallergen. These antigens therefore may have therapeutic potential in allergic diseases.

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Disclosures

The authors have no conflict of interest.

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