Schistosoma mansoni antigens modulate the allergic response in a murine model of ovalbumin-induced airway inflammation


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**Summary**

Schistosoma mansoni infection has been associated with protection against allergies. The mechanisms underlying this association may involve regulatory cells and cytokines. We evaluated the immune response induced by the S. mansoni antigens Sm22·6, PIII and Sm29 in a murine model of ovalbumin (OVA)-induced airway inflammation. BALB/c mice were sensitized with subcutaneously injected OVA-alum and challenged with aerolized OVA. Mice were given three doses of the different S. mansoni antigens. Lung histopathology, cellularity of bronchoalveolar lavage (BAL) and eosinophil peroxidase activity in lung were evaluated. Immunoglobulin (Ig)E levels in serum and cytokines in BAL were also measured. Additionally, we evaluated the frequency of CD4+ follicular helper (Tfh) cells in cultures stimulated with OVA and the expression of interleukin (IL)-10 in mice immunized with PIII and Sm22·6 were reduced, while the levels of IL-10 were higher in mice immunized with Sm22·6 compared to the non-immunized mice. The frequency of CD4+FoxP3+ T cells was higher in the groups of mice who received Sm22·6, Sm29 and PIII, being the expression of IL-10 by these cells only higher in mice immunized with Sm22·6. We concluded that the S. mansoni antigens used in this study are able to down-regulate allergic inflammatory mediators in a murine model of airway inflammation and that the CD4+FoxP3+ T cells, even in the absence of IL-10 expression, might play an important role in this process.

Keywords: allergy, asthma, vaccines

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**Introduction**

Allergic atopic disorders, such as asthma and rhinitis, result from genetic and environmental factors; there is a deregulated immune response, involving the T helper type 2 (Th2) cytokines interleukin (IL)-4, IL-5 and IL-13 and the Th1/ proinflammatory cytokines interferon (IFN)-γ and tumour necrosis factor (TNF)-α. Asthma is a chronic inflammatory disease with high morbidity and mortality, characterized by recurrent episodes of airway obstruction and wheezing [1,2]. Currently, more than 300 million people have asthma worldwide, and the numbers are increasing [3].

Human populations with high rates of parasitic helminth infections have a low prevalence of allergic disorders [4–7]. Also, treatment with anti-helminthic drugs leads to an increase in the skin prick test response to aeroallergens [8,9].

Among helminths associated with protection against allergies, Schistosoma mansoni appears to induce particularly strong down-modulation of the inflammatory response that mediates atopic disorders [10]. In a 1-year follow-up study, we reported that asthmatics from a rural area endemic for schistosomiasis had fewer asthma symptoms when compared to those from a rural area in which there was no transmission of S. mansoni [11]. We also demonstrated that peripheral blood mononuclear cells (PBMC) from asthmatic individuals infected with S. mansoni produce higher levels of the anti-inflammatory cytokine IL-10 and lower levels of IL-4 and IL-5 after restimulation in vitro with the allergen Dermatophagoides pteronyssinus antigen 1 (Der p1), compared to asthmatics without helminthic infections [12].

Although the immune responses in both allergies and S. mansoni infection are predominantly of the Th2 type, high
IL-10 production has been found in *S. mansoni* infection [13,14], while there is reduced IL-10 production in asthma patients [15]. A number of anti-inflammatory effects have been reported for IL-10; it appears to protect against allergy [12,16–19]. Support for this idea was provided by the observation that immunotherapy success is associated with increased IL-10 levels [20,21]. The induction of regulatory responses and disease prevention by helminths or their products has been observed not solely for allergic diseases, but also for autoimmune disorders [19,22–24].

Several *S. mansoni* antigens have been tested as vaccines to prevent *S. mansoni* infection and to prevent liver pathology, including Sm22·6, PIII and Sm29 [25,26]. We tested the potential of these three antigens to down-modulate the inflammatory response in an ovalbumin (OVA)-induced model of airway inflammation. The Sm22·6 antigen is a soluble protein associated with the tegument of *S. mansoni*, present throughout the life cycle of this helminth, with the exception of the egg stage [27]. Pacifico et al. found that recombinant Sm22·6 induces partial protection (34.5%) against experimental *S. mansoni* infection and also induces high levels of IL-10 production [28]. PIII is a multivalent antigen obtained from the *S. mansoni* adult worm antigen (SWAP); it modulates granuloma size in mice infected with *S. mansoni* [29,30]. The third antigen used in this study, Sm29, is a membrane-bound glycoprotein found on the tegument of the adult worm during the lung stage of *S. mansoni* infection [31]. This protein induces a Th1 cytokine profile in mice and provides 50% protection against infection [32].

We have shown previously that Sm22·6 and PIII are able to induce IL-10 production in *S. mansoni*-infected individuals [33]; in the current study, we investigated whether these two antigens, as well as Sm29, are able to down-modulate the inflammatory allergic response in an experimental murine model of OVA-induced airway inflammation. We used the antigen IL-4-inducing principle of *S. mansoni* eggs (IPSE), which is a bioactive glycoprotein present in the soluble egg antigen (SEA), as a positive control because it induces activation of basophils and production of IL-4 and IL-13 [34], which are involved in the allergic inflammatory process.

**Materials and methods**

**S. mansoni** antigens

The *S. mansoni* recombinant proteins, Sm22·6 and Sm29, and an *S. mansoni* soluble adult worm antigen fraction, PIII, were tested. The recombinant protein IPSE was used as control antigen. The recombinant proteins were produced in *Escherichia coli* and were tested for lipopolysaccharide (LPS) using a commercially available chromogenic LAL end-point assay kit (Cambrex, Charles City, IA, USA). The levels of LPS in Sm22·6, Sm29 and IPSE were below 1·2 endotoxin units (EU)/mg of protein. The antigen PIII were also tested for LPS contamination; the levels were under the detection limit of 0·01 EU/ml.

**Animals**

We used 6–8-week-old female BALB/c mice obtained from the Federal University of Minas Gerais (UFMG) animal facility. All protocols were reviewed and approved by the Ethics Committee on Animal Experiments of the Federal University of Minas Gerais.

**Immunization protocol**

To promote allergic airway inflammation, mice (five per group) were sensitized with 10 μg of OVA (Sigma-Aldrich, St Louis, MO, USA) in 1 mg of aluminium hydroxide gel (alum) by subcutaneous injection (days 0 and 15). On days 22–27, they were challenged with aerosolized OVA (1% solution for 30 min). The phosphate-buffered saline (PBS) group received PBS-alum instead of OVA-alum. The mice were immunized with 25 μg of the *S. mansoni* antigens Sm22·6, PIII, Sm29 and IPSE or PBS in 1 mg of alum through subcutaneous injection 2 days before and 8 and 18 days after injecting OVA (Fig. 1a). They were euthanized at day 28 and the immune response evaluated. The different groups of mice were designated according to the immunization protocol, as follows: OVA-sensitized non-immunized mice (OVA), OVA-sensitized Sm22·6-immunized mice (Sm22·6), OVA-sensitized PIII-immunized mice (PIII), OVA-sensitized Sm29-immunized mice (Sm29) and OVA-sensitized IPSE-immunized mice (IPSE).

**Fig. 1.** Induction of airway inflammation in mice and immunization with *Schistosoma mansoni* antigens. BALB/c mice were sensitized with subcutaneous injections of 10 μg of ovalbumin (OVA) in 1 mg of alum on days 0 and 15, and then challenged with OVA aerosol (1% OVA for 30 min) from days 22 to 27. The animals were euthanized on day 28 for the analyses (a). Different groups of mice were immunized with the antigens Sm22·6, PIII, Sm29 and IL-4-inducing principle of *S. mansoni* eggs (IPSE). The negative control group was immunized with phosphate-buffered saline (PBS)/alum (b). Five mice were included in each group and two independent experiments were performed.
Brochoalveolar lavage

The lungs were washed by cannulating the trachea and gently injecting/recovering (3×) 1·0 ml of PBS. The bronchoalveolar lavage fluid (BAL) was centrifuged at 300 g at 4°C for 5 min and the supernatants were stored at −20°C for cytokine analysis. The cell pellet was resuspended in 0·1 ml of 3% bovine serum albumin (BSA) and cells counted using a haemocytometer. The cells were then cytocentrifuged and stained with haematoxylin and eosin (H&E) for differential counting based on cell morphology and staining patterns. The means of three independent counts of 100 cells in a randomized field were shown.

Lung histopathology

Following bronchoalveolar lavage, the lungs were fixed with formalin. Serial sagittal sections of whole lung (3–4 μm thick) were cut and stained with Gomori trichome for light microscopy. At least 10 fields were selected randomly and examined. The severity of the inflammatory process in the lungs was scored by two pathologists who were blinded to group identity. The scale varied from 0 to 5 as follows: 0, no inflammation; 1, minimal; 2, mild; 3, medium; 4, moderate; and 5, marked [35,36].

Eosinophil peroxidase (EPO) assay

The EPO assay was performed as described previously [37]. Briefly, a 100-mg sample of tissue from each lung was homogenized in 1·9 ml of PBS and centrifuged at 12 000 g for 10 min. The supernatant was discarded and the erythrocytes were lysed. The samples were centrifuged, the supernatant discarded and the pellet resuspended in 1·9 ml of 0·5% hexadecyltrimethyl ammonium bromide in PBS saline. The samples were then lysed with 0·15 M NH₄Cl, 10 mM KHCO₃, 0·1 mM Na₂EDTA, pH 7·2; and the red blood cells were lysed with ACK buffer (0·15 M NH₄Cl, 10 mM KHCO₃, 0·1 mM Na₂EDTA, pH 7·2; Invitrogen, CA, USA). The cell suspension was washed twice in RPMI-1640 and adjusted to 1·0 ml/well of PBS, 0·2% FCS, and 2 mM NaN₃.

The cell suspension (1·0 ml/well) was centrifuged at 300 g for 5 min and the supernatants were frozen in liquid nitrogen and centrifuged at 4°C for 30 min at 12 000 g for 10 min. The supernatant was used for the enzymatic assay. Briefly, o-phenylenediamine (OPD) (10 mg) was dissolved in 5·5 ml distilled water, and then 1·5 ml of OPD solution was added to 8·5 ml of Tris buffer (pH 8·0), followed by addition of 7·5 μl H₂O₂. In a 96-well plate, 100 μl of substrate solution was added to 50 μl of each sample. After 30 min, the reaction was stopped with 50 μl of 1 M H₂SO₄, and the absorbance was read at 492 nm.

Cytokine measurements

Levels of IL-4, IL-5, IL-10, TNF-α and IFN-γ were determined by bronchoalveolar lavage (BAL) of the different groups of mice with an enzyme-linked immunosorbent assay (ELISA) sandwich technique using commercially available kits (OptEIA; BD Bioscience, San Jose, CA, USA), according to the manufacturer’s protocol. The optical density (OD) values were read at 450 nm. The results were expressed as picograms per millilitre, compared to a standard curve.

Determination of OVA-specific immunoglobulin (IgE) levels

The levels of OVA-specific IgE in serum were determined by ELISA, as described previously [38,39]. Briefly, Maxisorp 96-well microtitre plates (NUNC, Roskilde, Denmark) were coated with rat anti-mouse unlabelled IgE (1 : 250; Southern Biotechnology, AL, USA) in pH 9·6 carbonate-bicarbonate buffer for 12–16 h at 4°C and then blocked for 1 h at room temperature with 200 μl/well of 0·25% PBS-casein. Fifty microlitres of each serum was added per well and incubated for 2 h at room temperature. The samples were then incubated with 50 μl/well of OVA-biotin (1 mg/ml; Sigma, St Louis, MO, USA) at room temperature for 1 h. Plate-bound antibody was detected by treatment with 50 μl/well of streptavidin–horseradish peroxidase (1 : 10 000; Southern Biotechnology) for 1 h at room temperature. The colour reaction was developed by adding 100 μl/well of 200 pmol of OPD (Sigma) in pH 5·0 citrate phosphate buffer plus 0·04% H₂O₂ for 10 min and stopped with 50 μl of 5% sulphuric acid per well. The plates were read at 492 nm in an ELISA reader (Bio-Rad, Hercules, CA, USA).

Cell staining and flow cytometry

The lungs of five mice per group were removed and treated with 100 U/ml of collagenase from Clostridium histolyticum (Sigma) for 30 min at 37°C. Subsequently, the digested lung tissue was filtered through a 70 micrometre cell strainer and the red blood cells were lysed with ACK buffer (0·15 M NH₄Cl, 10 mM KHCO₃, 0·1 mM Na₂EDTA, pH 7·2; Invitrogen, CA, USA). The cell suspension was washed twice in RPMI-1640 and adjusted to 1×10⁶ cells per well for surface staining and to 2×10⁵ cells for the intracellular cytokine experiment. For CD4 and forkhead box P3 (FoxP3) staining, the cells were generally blocked with anti-mouse CD16/CD32 monoclonal antibodies (mAbs) (Fc-block) and stained for surface marker using fluorescein isothiocyanate (FITC)-labelled anti-mouse CD4 (BD Bioscience) mAb or isotype control, which were incubated for 20 min at 4°C with antibody dilution solution (PBS 0·15 M, 0·5% BSA, 2 mM Na₃citrate). The cells were then washed with 0·15 M PBS and incubated with streptavidin–phycocyanin–cyanine 5 (PE-Cy5) (1 : 200) for an additional 20 min at 4°C. Surface-stained cells were washed twice with 0·15 M PBS and incubated with fixation/permeabilization buffer (eBioscience) for 30 min at 4°C. Anti-FoxP3-PE labelled antibodies in permeabilization buffer (eBioscience) were added to cells and then incubated for 30 min at 4°C. Cells were washed twice with 150 μl of permeabilization buffer (eBioscience) and fixed with 2% paraformaldehyde. For IL-10 and FoxP3 intracellular staining, the cells were cultured for 14 h in medium or OVA (25 μg/ml). After this stimulation period, 1 mg/ml of brefeldin A was
added to the cell culture, which was incubated for an additional 4 h in a CO₂ incubator at 37°C. Before CD4 staining, the cells were treated with anti-CD16/CD32 (Fc-block). Cell surface and intracellular staining were performed as described above for surface experiments; however, the cells were stained for CD4, IL-10 and FoxP3 using anti-CD4 FITC-labelled, anti-IL-10 PE-labelled, and anti-FoxP3 biotin-labelled plus streptavidin–PE-Cy5 antibodies. Data acquisition was performed using fluorescence activated cell sorter (FACScan) (Becton Dickinson, San Jose, CA, USA). Data analysis was performed using a FlowJo interface (Becton Dickinson).

**Statistical analysis**

Statistical analysis was performed using the software GraphPad Prism (GraphPad Software, San Diego, CA, USA). The mean ± standard deviation (s.d.) of the number of cells and the antibody, EPO and cytokine concentrations were calculated. A one-way analysis of variance (ANOVA) was used to compare the levels of cytokines, IgE and EPO between groups. Fisher’s exact test was used to compare proportions. The alpha level for statistical significance was established as 5%.

**Results**

**Immunization with S. mansoni antigens Sm22-6, PIII and Sm29 results in modulation of OVA-induced airway inflammation in mice**

The severity of the inflammatory response to OVA was evaluated in the lungs of mice immunized with S. mansoni antigens and in control mice. A dense mixed-cellular infiltrate surrounding the airway was observed in the sensitized non-immunized mice (Fig. 2b) and in the IPSE-immunized group (Fig. 2f). Comparatively, much less peribronchial airway inflammation was observed in OVA-sensitized mice immunized with Sm22-6, PIII and Sm29, and in non-sensitized mice that were treated with PBS (Fig. 2c,d,e,a, respectively).

**Modulatory effect of S. mansoni antigen immunization on cellularity in BAL**

Mice immunized with the S. mansoni antigens Sm22-6, PIII and Sm29 had significantly fewer total cells and eosinophils in the BAL fluid than did non-immunized mice and mice immunized with IPSE, while there was no significant difference in the number of neutrophils, lymphocytes and macrophages between groups (Table 1).

**Immunization with S. mansoni antigens down-regulates OVA-specific IgE production and peroxidase activity in eosinophils**

The serum levels of OVA-specific IgE were measured in sensitized non-immunized mice and in those immunized with the different S. mansoni antigens. The levels of this isotype were markedly lower in S. mansoni antigen-immunized mice than in sensitized non-immunized mice (Fig. 3a).

The levels of eosinophil peroxidase (EPO) were also significantly lower in the lungs of mice immunized with Sm22-6 and PIII than in the non-immunized group (Fig. 3b).

**Cytokine response induced by S. mansoni antigens**

We measured the cytokines IL-4, IL-5 and IL-10 in BAL fluid. The levels of IL-4 and IL-5 were lower in mice immunized with Sm22-6 and PIII compared to non-immunized mice.
The frequency of CD4+FoxP3+ T cells and CD4+FoxP3+IL-10+ expressing cells

The frequency of CD4+FoxP3+ T cells and of CD4+FoxP3+IL-10+ T cells in cultures stimulated with OVA was evaluated in the different groups of mice. We found that the frequency of the CD4+FoxP3+ T cells was significantly higher in mice immunized with Sm22·6 and PIII. There was a tendency of higher expression of these cells in mice immunized with Sm29 (P = 0·06) (Fig. 5a). Conversely, the frequency of these cells expressing IL-10 was higher only in mice immunized with Sm22·6 when compared to the OVA group (Fig. 5b).

Discussion

There is evidence that Schistosoma sp. infection protects humans [11] and mice against allergic asthma [36,38,40]. We evaluated whether the S. mansoni antigens Sm22·6, PIII and Sm29 could down-modulate the inflammatory allergic response in a murine model of OVA-induced airway inflammation. We found that immunization with these S. mansoni antigens protected mice against allergic inflammation; there was a strong reduction in the number of inflammatory cells and eosinophils recruited to the airways. Moreover, the levels of OVA-specific IgE production were also decreased in mice immunized with Sm22·6, PIII and Sm29, and the levels of EPO in the lungs were lower in mice immunized with Sm22·6 and PIII, compared to the non-immunized mice. Additionally, we found that these two antigens have an important immunomodulatory effect on the production of the Th2 cytokines IL-4 and IL-5, evidenced by the lower levels of these cytokines in the BAL of immunized compared to non-immunized mice. IL-5 is a well-known cytokine that induces the production of eosinophils and activates these cells, while IL-4 acts on B cells, inducing IgE synthesis. This antibody participates in the pathogenesis of asthma by binding to mast cells, basophils and eosinophils, which release inflammatory mediators upon contact with the allergen. We observed lower levels of allergen-specific IgE in the groups of S. mansoni antigen-immunized mice compared to non-immunized mice. This is additional evidence that these antigens are able to prevent Th2-mediated inflammation.

![Fig. 3. Levels of ovalbumin (OVA)-specific immunoglobulin (Ig)E in the serum (a). The data are presented as the mean optical density (OD) at 492 nm ± standard deviation (s.d.). Levels of eosinophil peroxidase (EPO) in the lungs of non-immunized mice and mice immunized with the antigens Sm22·6, PIII, Sm29 and phosphate-buffered saline (PBS), respectively (b). The results are expressed as mean ± s.d. Five mice per group were evaluated and two separate experiments were performed. *P < 0·05, **P < 0·01 and #P < 0·005 versus OVA group.](https://example.com/fig3.png)
Fig. 4. Effect of *Schistosoma mansoni* antigen immunization on cytokine production in bronchoalveolar lavage (BAL) fluid. Levels of interleukin (IL)-4 (a), IL-5 (b), IL-10 (c) and interferon (IFN)-γ (d) in the different groups of mice. Lower levels of IL-4 and IL-5 were observed in the BAL of mice immunized with Sm22·6 and PIII, while higher levels of IL-10 were found in Sm22·6-immunized mice when compared to the non-immunized group. We observed that in mice immunized with Sm22·6 and with PIII the ratio IL-10/IL-4 in BAL was higher than in non-immunized mice (e). The figures represent the mean ± standard deviation of five mice per group. Two separate experiments were performed. *P < 0.05, **P < 0.01 and ***P < 0.005 versus ovalbumin (OVA) group.

Fig. 5. Frequency of CD4+forkhead box P3 (Foxp3)+ T cells in cultures stimulated with ovalbumin (OVA) (a) and of CD4+FoxP3+ T cells expressing interleukin (IL)-10 (b) in mice immunized with the different *Schistosoma mansoni* antigens. Cell suspensions isolated from lungs of mice were stained for surface expression of CD4 and for intracellular expression of FoxP3 and IL-10 and then analysed by flow cytometry in the lymphocyte region of the forward/side-scatter plot. (a) Represents the gating strategy for the analysis of CD4+FoxP3+ frequency. The lymphocyte region was determined in the forward/side-scatter plot and then a dot-plot was accessed to determine CD4+FoxP3+ T cells frequency. The frequency of CD4+FoxP3+ T cells was higher in mice immunized with Sm22·6 and PIII compared to non-immunized mice (b), while expression of IL-10 was seen only in CD4+FoxP3+ T cells of Sm22·6 mice (c). The figures represent the mean and standard deviation from five mice in each group. *P < 0.05 versus OVA group.
In mice immunized with Sm29, despite the fact that there was a reduction in the number of inflammatory cells, eosinophils and OVA-specific IgE compared to non-immunized mice, the decrease in the levels of IL-4 and IL-5 in BAL did not reach statistical significance. The slight decrease in IL-4 and IL-5 production might be effective to reduce IgE production and eosinophil growth or recruitment without, however, being sufficient to alter EPO activity in lung tissue.

We used the antigen IPSE as our positive control as this antigen is an inducer of the Th2 response in schistosomiasis [34]. As expected, in mice immunized with IPSE the Th2 parameters such as number of eosinophils, levels of EPO, IL-4 and IL-5 were comparable to asthmatic non-immunized mice; unpredictably, however, in IPSE-immunized mice there was a reduction in the levels of OVA-specific IgE.

This study examined inflammatory mediators responsible for lung airway inflammation in a murine model of OVA-induced allergic asthma, and found an eosinophil infiltration of the airway. Eosinophils play a pivotal role in the airway inflammation in asthma and they correlate with the extent of inflammatory process in the lung parenchyma [41]. Lung function could not be evaluated in the present study. Lung inflammation and airway hyperreactivity (AHR) are two distinct characteristics of asthma [42–46]. Indeed, it has been demonstrated that methacoline-induced AHR in mouse models correlates with an antigen-specific Th2 immune response [46–49], but not with severity of eosinophilic lung inflammation [47,50].

It has been reported that IL-10 is the main cytokine involved in suppression of Th2 allergic inflammation due to helminth infection [12,40]. We evaluated the levels of this cytokine in BAL of sensitized mice. Although the levels of this cytokine were higher only in mice immunized with Sm22·6, the ratio IL-10/IL-4 was higher in mice immunized with Sm22·6 and also with PIII compared to non-immunized mice. In fact, it is possible that IL-10 may not be the only mechanism involved in down-modulation of the allergic inflammatory response in S. mansoni antigen-immunized mice. Indeed, suppression of inflammatory cell migration to the airways and down-modulation of IgE production were seen in mice immunized with Sm29 compared to non-immunized mice, despite the low levels of IL-10 in BAL. The possibility that there are other modulatory mediators that act independently of IL-10 is supported by our previous demonstration that regulatory T cells of S. mansoni-infected mice protect against allergen-induced airway inflammation through an IL-10-independent mechanism [38]. While infection with Nipostrongylus brasiliensis has been found to suppress airway inflammation in an IL-10-dependent manner [51], other researchers have found that N. brasiliensis products inhibit an allergic response in the airways of mice, independently of the levels of IL-10 [52]. Therefore, for the same parasites, different modulatory mechanisms of the allergic response may exist. In this study the frequency of CD4⁺FoxP3⁺ T cells was significantly higher in mice immunized with Sm22·6 and PIII. There was a trend towards increased frequency of these cells in mice immunized with Sm29, compared to non-immunized mice. However, only in mice immunized with Sm22·6 was there a significantly higher frequency of CD4⁺FoxP3⁺ T cells expressing IL-10 compared to non-immunized mice. In agreement with these data, higher levels of IL-10 in BAL relative to non-immunized group was also observed only in mice immunized with Sm22·6. It is possible that the CD4⁺FoxP3⁺ T cells could be acting through cell–cell contact to inhibit Th2-like mediators in the other groups of mice. Indeed, in the group of mice immunized with Sm29 we did not observe an increase in IL-10 production; nevertheless, there was a reduction in eosinophil infiltration and in the OVA-specific IgE levels.

We found no increase in the levels of the Th1 cytokines IFN-γ and TNF in the BAL of immunized mice compared to non-immunized ones. These data argue in favour that down-modulation of the Th2 response by the parasite antigens was not due to an increase in Th1 response. This is an important finding, because Th1 cytokines could contribute to the inflammatory process in severe asthma [53]. Other studies have demonstrated that helminth infections or antigens down-regulate the allergic response through the action of regulatory T cells, rather than by altering the Th1/Th2 balance [23,38].

In conclusion, we showed that S. mansoni antigens Sm22·6, PIII and Sm29 are able to down-modulate the inflammatory response in a model of allergic airway inflammation and we suggest that the CD4⁺FoxP3⁺ T cells might be involved in this modulation. Studies evaluating other mechanisms underlying the modulatory effect of S. mansoni antigens on the allergic inflammation are in progress; they may contribute to the development of new strategies to prevent allergic diseases.

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Disclosure

The authors have no financial conflict of interest.

References

S. mansoni antigens modulate allergy


46 Epstein MM. Do mouse models of allergic asthma mimic clinical disease? Int Arch Allergy Immunol 2004; 133:84–100.