Association between allergic responses and Schistosoma mansoni infection in residents in a low-endemic setting in Brazil

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ABSTRACT

Introduction: Schistosomiasis is endemic in 76 countries and territories. Several studies have found an inverse correlation between parasitic disease and the development of allergies. The purpose of the present study was to determine whether infection with Schistosoma mansoni in subjects with a low parasite load is protective against allergy. The final sample consisted of 39 S. mansoni-positive and 52 S. mansoni-negative residents of a small community in northeastern Brazil. Methods: All subjects were submitted to the Kato-Katz test, anti-S. mansoni IgG measurement, the prick test for aeroallergens, eosinophil counts and serum IgE measurement. Results: Subjects who reacted to one or more antigens in the prick test were considered allergic. Only 7 S. mansoni-positive subjects (17.9%) reacted to one or more antigens, whereas 20 S. mansoni-negative subjects (38.5%) tested positive for allergy. Conclusions: Our findings suggest that, in areas of low endemiocity, infection with S. mansoni significantly reduces the risk of the development of allergy in subjects with a low parasite load.

Keywords: Schistosomiasis. Schistosoma mansoni. Allergy.

INTRODUCTION

Schistosomiasis is endemic in 76 countries and territories. An estimated 200-250 million people are currently infected with schistosomiasis, and another 600-780 million people are at risk of infection1,2.

This disease is caused when granulomatous inflammatory lesions form around host tissues infected by Schistosoma mansoni eggs3. Eggs deposited in the liver stimulate cluster of differentiation 4+ (CD4+) T cells, which in turn activate macrophages and induce late hypersensitivity reactions leading to the formation of granulomas4. Initially, the immune response consists of an acute Th1 cell response directed at the adult worm; then, when eggs are deposited, the response is gradually shifted to a Th2 response. Failure to develop an effective Th2 response results in Th1- and Th17-mediated exacerbation of granulomatous inflammation5.

During the acute stage, the immune response to helminth infection is mediated by Th1 cells. This type of response generates heightened production of interferon-γ (IFN-γ)6 and tumor necrosis factor-α (TNF-α), which activate macrophages and induce the immunoglobulin G (IgG)-mediated opsonization and phagocytosis of antigens7. In the chronic stage, the immune response is mostly mediated by Th2 cells, leading to elevated interleukin 4 (IL-4) and interleukin 5 (IL-5) levels and interleukin 10 (IL-10) secretion and the subsequent reduction of IFN-γ8.

Several researchers have reported an inverse correlation between parasitic disease and the development of allergic diseases, leading to the hypothesis that parasites may have a protective (immunomodulatory) effect against atopy9. For instance, a study conducted in Ecuador reported an inverse correlation between helminth infection and allergy skin test results in children10.

Recent studies have also shown that asthma and allergic diseases are less prevalent in rural settings11, possibly due to protective effects associated with low socioeconomic status reflected in life style, food habits, large families, the prevalence of helminth infection and lack of good hygiene12. Likewise, it has been suggested that allergic diseases are less prevalent in developing countries because populations are more likely
to be exposed to bacteria and helminth infections, which are associated with poverty and the lack of basic sanitation. Helminth infections and allergic diseases are both associated with Th2 cytokines and high levels of immunoglobulin E (IgE) and eosinophilia. Although the two conditions appear to involve similar immune responses, a negative correlation has been demonstrated between helminth infection and allergic diseases.

Many epidemiologists investigating the increasing prevalence of allergic diseases subscribe to the hygiene hypothesis, which asserts that the lack of exposure to infectious agents and parasites, improvements in hygiene and the use of vaccines and antibiotics (especially in developed countries) can compromise the ability of the immune system to respond adequately to certain challenges, conceivably due to an imbalance between the Th1 and Th2 cell-mediated immune responses.

The lower frequency of positive allergic tests and decreased asthma severity in populations infected with helminths may be due to several factors, such as enhanced polyclonal IgE production, reduced levels of antigen-specific IgE, high concentrations of antigen-specific IgG4, the activation of regulatory cells and the production of regulatory cytokines. For instance, IL-10 can inhibit the release of histamine and other mast cell mediators. Because infection with *S. mansoni* is associated with increased IL-10 production, this may be the main mechanism by which allergic response is suppressed in infected individuals.

In general, studies evaluating the relationship between helminth infection, sensitivity to allergy skin tests and symptoms of allergic diseases have shown that the allergic response is reduced following infection with *Schistosoma* sp. Likewise, cross-sectional studies indicate that subjects infected with *S. mansoni* and *Schistosoma haematobium* are less frequently positive for aeroallergens on skin testing. It should be noted that these studies were conducted in regions where schistosomiasis is considered highly endemic. The purpose of the present study was to determine whether infection with *S. mansoni* in subjects with a low parasite load is also protective against allergic diseases.

### METHODS

#### Study area and population

The study was carried out in Planalto do Cajueiro, a locality in the municipality of Maranguape (State of Ceará, Northeastern Brazil). The prevalence of schistosomiasis in the region rose from 8.53% in 2006 to 13.76% in 2007.

Located 30km from Fortaleza (the State capital), Maranguape covers an area of 591km² and has 113,561 inhabitants. All members of the community (n=903) were invited to participate in the study, but only 357 agreed to undergo parasite examination, and 250 were submitted to serological tests. Subjects fulfilling the following criteria were excluded from the study: I) not a permanent resident of Planalto do Cajueiro, II) using anti-allergic drugs, such as corticoids or immunosuppressants, III) a history of immunodepression, IV) pregnant, V) a history of anaphylactic shock, VI) needle or skin test phobia, or VII) under 2 years of age.

#### Egg detection (Kato-Katz technique):

Egg detection was performed according to the Kato-Katz technique, using a Helm-Test® kit. To increase the sensitivity of the test, three slides of each fecal sample (rather than one slide, as recommended by the manufacturer) were used for the detection of eggs from *S. mansoni* and other helminths.

#### Anti-Schistosoma mansoni IgG antibodies:

Using adult worm antigen in conjunction with a protocol slightly adapted from Colley et al., an ELISA was performed to test for anti-*Schistosoma mansoni* IgG antibodies. The optical density was measured with an automatic ELISA reader (BioTeck®) using a 490nm filter.

Reactions with an optical density above 0.283 were considered positive. This cut-off value was based on the average optical density plus two standard deviations of 35 control serum samples collected from *S. mansoni*-negative individuals from a region where schistosomiasis is not endemic.

#### Prick test:

The preferred site for the prick test is the inner forearm. Following antisepsis with 70% alcohol, a 25µL aliquot of each allergenic extract was introduced into the skin surface with a standard disposable pricker according to a predefined grid.

The allergens included three species of dust mites (*Dermatophagoides pteronissimus*, *Dermatophagoides farinae* and *Blomia tropicalis*), cockroaches (mix) and fungi (mix). The reaction was measured after 20 min of exposure.

#### Hemogram:

A complete hemogram was performed with an automated hematology analyzer (Sysmex KX-21N). To quantify eosinophils, a smear of the sample was submitted to rapid panoptic staining for a differential leukocyte count under the microscope.

#### Serum IgE concentrations:

The total serum IgE concentration was measured with a commercially available kit (Monobind IgE AccuLite™ CLIA), following the manufacturer’s instructions. The reading was performed by ELISA, at 450nm.

#### Statistical analysis

Demographic, clinical and laboratory data were organized using Microsoft Office Excel 2007. Using GraphPad Prism software, the data distribution was analyzed for normality, and the differences were analyzed with Fisher’s exact test, the Kruskal-Wallis test followed by Dunn’s multiple comparison test and the Mann-Whitney test.
Ethical considerations

The study protocol had been previously approved by the Research Ethics Committee of the School of Medicine of the Federal University of Ceará [Universidade Federal do Ceará (UFC)] on March 4, 2010 (filed under #329/09).

RESULTS

A sample of 250 residents from a community with an 11.2% prevalence of schistosomiasis was examined. Thirty-nine of these subjects were S. mansoni-positive by Kato-Katz and ELISA. According to the results of ELISA for anti-S. mansoni IgG antibodies, 118 samples (47.2%) were positive and 132 (52.8%) were negative. A positive result by Kato-Katz or ELISA indicates current infection and/or an antibody immune response to the parasite, respectively. The 91 selected participants (39 Sm+ and 52 Sm−) were submitted to the prick test, and those reacting to one or more antigens were considered allergic. Only 7 Sm+ (14.0-461.5) for the Sm− group (Figure 2).

When analyzed with Fisher’s exact test, the findings shown in Figure 1 yielded the following results: p=0.0394, relative risk=0.5185, IC95%=0.2619-1.027 (confidence interval at 95%) and odds ratio=0.350, IC95%=0.130-0.9426. The fact that 18 of the 91 participants presented co-infection with other helminths or protozoa is not likely to have affected the results, considering the diversity of species and the negligible incidence of each.

Based on the results of the Kato-Katz and prick tests, the following groups were defined: Sm+PT+ (n=7), Sm+PT− (n=32), Sm PT+ (n=20) and Sm PT− (n=32). The median IgE concentrations were 434.0 UI/mL (38.5-452.5) for the Sm+PT+ group, 416.0 UI/mL (42.5-457.5) for the Sm+PT− group, 442.8 UI/mL (34.5-463.0) for the Sm PT+ group, and 154.8 UI/mL (0-1,044) for the Sm PT− group. When submitted to the Mann-Whitney test, significant differences were found between the Sm+PT- and SM-PT- groups (p=0.0094) (Figure 2).

The median eosinophil counts were 21,504 cells/µL (12,702-39,865) for the Sm PT+ group, 18,630 cells/µL (2,522-47,244) for the Sm PT− group, 464 cells/µL (52-1,200) for the Sm+PT+ group and 147 cells/µL (0-1,044) for the Sm+PT− group. When submitted to the Kruskal-Wallis test followed by Dunn’s multiple comparison test, significant differences were found between the Sm+PT+ and Sm+PT− (p<0.01), Sm+PT+ and Sm PT− (p<0.01), Sm+PT+ and Sm PT+ (p<0.01), and Sm+PT− and Sm PT− groups (p<0.001) (Figure 3).

DISCUSSION

Since 1976, when interventions to control schistosomiasis began, the epidemiological profile of the disease has changed in many regions of Brazil. In particular, control measures have had widely documented beneficial impacts on disease prevalence, severity and morbidity. However, these infections are still transmitted throughout Ceará state, causing mostly mild infections.

In tropical regions, chronic helminth infection and allergic diseases are known to be inversely correlated. Helminth infection is an environmental factor that may skew the T cell response to reduce the risk of allergic disease, and S. mansoni has been associated with this protective effect. Interleukins play an important role in immune defense against this parasite. In particular, IL-4 stimulates the production of helminth-specific IgE antibodies, which cover the worms as a result of antibody-dependent cytotoxicity, while IL-5 activates eosinophils that attach to the IgE-covered helminths through γ heavy chain-specific Fc receptors.
Eosinophils are proinflammatory cells associated with allergic diseases and parasite infections\(^1\) and are believed to play an important role in the immune response to \emph{S. mansoni} infection\(^3\). This hypothesis is based primarily on the histopathological evidence of eosinophils clustering around worms in biopsied tissues\(^3\) as well as the observation that, in the presence of antibodies and/or the complement system, eosinophils mediate the destruction of schistosomula \emph{in vitro}\(^4\).

When the Kruskal-Wallis test, followed by Dunn’s multiple comparison test, was used to analyze the differences in eosinophil counts between the four groups, significantly higher numbers were observed for \emph{S. mansoni}-positive subjects. However, there was no significant association between high eosinophil counts and prick test reactivity, likely because eosinophilia is a common reaction to infections and allergies alike\(^3\).

However, eosinophil counts differed significantly between the \emph{Sm}‒PT\(^+\) (18,630 cells/\mu L, 2,522-47,244) and \emph{Sm} PT\(^+\) (464 cells/\mu L, 52-1,200) groups, possibly because large numbers of these cells are recruited during the immune response to \emph{S. mansoni}.

Given the heterogeneity of exposure and the diversity of parasite species, helminth infection is not invariably protective against aeroallergens\(^9\). However, the results of the present study suggest that, in areas of low endemicity, low-level \emph{S. mansoni} infection significantly reduces the risk of the development of allergy.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

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**REFERENCES**


