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IMMUNOLOGY AND HOST-PARASITE INTERACTIONS - ORIGINAL PAPER

Profile of T and B lymphocytes in individuals resistant to *Schistosoma mansoni* infection

Robson da Paixão de Souza[1] · Maria Ilma Araújo[1,2] · Diego Mota Lopes[1] · Sérgio Costa Oliveira[2,3] · Jamille Souza Fernandes[4] · Kelvin Edson M. de Jesus[5] · Edgar M. Carvalho[1,2,5] · Ricardo Riccio Oliveira[5] · Luciana Santos Cardoso[1,2,6]

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Abstract

The mechanisms involved in the development of resistance to infection/reinfection by *Schistosoma mansoni* still arouse great interest and controversy. Some authors demonstrate that resistance to infection is attributed to a mixed Th1 and Th2 response and resistance to reinfection after repeated treatments through mechanisms associated with the Th2 response. Through flow cytometry, the phenotypic characterization of B and T lymphocytes in individuals residing in endemic areas with low parasite loads over 10 years was evaluated for the first time in humans. In this study, individuals with low parasite loads for *Schistosoma mansoni* had a higher proportion of Th1 and Th2 cells. In addition, lymphocytes from these individuals showed a higher degree of expression of costimulatory molecules CD28 and CTLA-4 and regulatory molecules FoxP3 and IL-10, when compared to individuals with high parasite loads. Our data indicate that the control of the parasite load of *S. mansoni* must be associated with a Th1, Th2, and regulatory response, and that further studies are needed to elucidate the possibility of mechanisms associated with the hyporesponsiveness of lymphocytes from individuals with high parasite loads.

Keywords *Schistosoma mansoni* · T lymphocytes · B lymphocytes · Schistosomiasis

Introduction

Considered one of the most important parasitic diseases in the world, schistosomiasis affects more than 240 million individuals, and it is estimated that around 700 million are at risk of infection (WHO 2021). In Brazil, schistosomiasi*S. mansoni* affects about 6 million individuals, and approximately 25 million people are exposed to contamination, especially those individuals who live in rural and peri-urban areas (Brasil - Ministério da Saúde 2018).

The initial phase of *S. mansoni* infection progresses with a Th1 immune response, with production of IFN-γ, TNF, IL-6, and IL-1 (Lambertucci et al. 1997; de Jesus et al. 2002). This response may be associated with the control of the parasite load in the lung and liver through the activation of macrophages by IFN-γ and activation of $TCD8^+$ cells (Smythies et al., 1992; Wynn et Al. 1994). After oviposition, the acute phase is then modulated, so that in the chronic phase, the response is polarized to the Th2/regulatory type, characterized by the production of IL-4, IL-5, IL-13, and IL-10 (Gazzinelli and Colley 1992; Williams et al. 1994; Correa-Oliveira et al. 1998; Souza et al. 2012). This change

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✉ Luciana Santos Cardoso
lucianac@ufba.br

1 Serviço de Imunologia, Complexo Hospitalar Universitário Professor Edgard Santos, Universidade Federal da Bahia, Rua João das Botas s/n, Canela, Salvador, BA 40.110-160, Brazil

2 INCT-DT-CNPQ/MCT), Instituto Nacional de Ciência E Tecnologia Em Doenças Tropicais, Rua João das Botas s/n, Canela, Salvador, BA 40.110-160, Brazil

3 Departamento de Bioquímica E Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

4 Centro das Ciências Biológicas E da Saúde, Universidade Federal Do Oeste da Bahia, Barreiras, BA, Brazil

5 Instituto Gonçalo Moniz, Fundação Oswaldo Cruz (FIOCRUZ), Rua Waldemar Falcão No. 121, Candeal, Salvador, BA 40296-710, Brazil

6 Departamento de Análises Clínicas E Toxicológicas, Faculdade de Farmácia, UFBA, Avenida Adhemar de Barros s/n, Ondina, Salvador, BA 40.170-970, Brazil

in the response pattern has been related to the production of IL-4 and IL-10, induced by egg antigens and the increase in the number of regulatory cells — Treg (Grzych et al. 1991; Pearce et al. 1991; Silveira et al. 2004).

In addition to T lymphocytes, B cells also play several roles in the immune system, being able to participate in both protective mechanisms and physio pathogenic events of various diseases. They also participate in immune responses mediated by T cells through the presentation of antigens, contributing to the activation of virgin cells, cell proliferation, and self-reactive T cell activity (Rodríguez-Pinto and Moreno 2005; Yan et al. 2006). Combined with its well-defined role in the production of antibodies, B cells can regulate immune responses to different pathogens, acting as a source of cytokines (Harris et al. 2000).

In experimental model of Schistosomiasis, T cells in peripheral blood of susceptible mice were significantly declined and in nocaute mice to T cells ($CD3^{-}/^{-}B6$), the absence of T cells abolish the resistance to infection (Lu Liaoxun et al. 2020).

Despite this, the immune response of infected individuals with a low parasite load, regardless of exposure level, has not yet been characterized. It is possible that individuals residing in an endemic area and who have a low parasite load over time present a peculiar cytokine profile, which may confer some level of resistance to the infection. There are few studies on the characterization and participation of T and B lymphocytes in *Schistosoma mansoni* infection and the possible association of these cells in the development of resistance in humans. Therefore, the aim of this study was to evaluate the profile of T and B lymphocytes regarding the production of Th1, Th2, and regulatory profile cytokines with regard to the expression of regulatory molecules, such as CD25, CTLA4, and FoxP3. Additionally, hematological and biochemical parameters were evaluated in groups of individuals resistant and susceptible to infection by *Schistosoma mansoni.*

Material and methods

Endemic area in schistosomiasis

Individuals infected by *Schistosoma mansoni*, living in the municipality of Conde, Bahia, located about 200 km from the capital Salvador, co-infected or not by other helminths, were evaluated in this study. Sanitary conditions are precarious, which compromises the effective control of parasitic infections. The main source of income is based on fishing in rivers, and these places are also used as a source of leisure and conducting domestic activities; thus, the population is exposed to *S. mansoni* infection.

Individual selection strategy

The selection of individuals is presented in a previous publication by our group (Souza et al. 2019). Individuals with low parasite burden, named in this study as resistant to infection by *S. mansoni*, were performed using databases (DB) generated during previous studies carried out in the same area in the years 2010, 2008, and 2004 containing information about sex, age, parasite load, degree of exposure to contaminated water, period of stay in the endemic area, and history of treatment with praziquantel. All parasitological evaluations in this area used the quantitative Kato-Katz technique to measure the burden of *S. mansoni* (Katz et al. 1970).

Individuals who were selected as resistant to infection presented a low parasite burden (≤ 99 epg of feces) and met the following criteria: age between 10 and 65 years of either gender, some contact with contaminated water, and having resided in the region for at least 11 months during surveys in 2010, 2008, and 2004. The strategy for selecting those individuals with low parasite burden was initially carried out with information contained in the 2010 DB, which had 850 individuals. Of these, we selected 335 individuals with low parasite burden and some contact with contaminated water. Later, these individuals were re-evaluated with the same criteria by the 2008 DB, where 122 out of 335 individuals met the established criteria. Based on information obtained in 2004, of the 122 individuals previously selected, 45 were classified as resistant to infection by *S. mansoni*. This study considered high parasite load equal to or greater than 200 epg of feces. Susceptible group to *S. mansoni* infection were those living in endemic areas, of either gender, between 10 and 65 years old and who had a high parasite load (≥ 200 epg of feces) during the coproparasitological survey conducted in 2010. At this stage, 39 individuals with high parasite burden (≥ 200 epg of feces) were selected. All selected individuals were then subjected to further coproparasitological evaluation for confirmation of resistance or susceptibility to *S. mansoni* infection. Two stool samples from each participant were prepared, and two slides from each sample were examined. We randomly selected 20 individuals classified as resistant and 16 individuals classified as susceptible to assess immune response and laboratorial evaluations. In addition, were enrolled 5 individuals without helminth infection, of either gender and seronegative for *S. mansoni* antigens (SWAP and SEA) that never resided in endemic areas for schistosomiasis to compose the group of healthy controls. All subjects evaluated were negative for HIV 1/2, HTLV I/II, and B and C hepatitis (Souza et al. 2019).

All study participants received treatment for schistosomiasis using Praziquantel in a single dose of 50 mg/kg

for adults and 60 mg/kg for children after the diagnosis of schistosomiasis in this study.

Hematological and biochemical evaluation

About 15 mL of venous blood was collected and packed in appropriate tubes (Vacutainer®; Becton Dickinson) for hematological and biochemical analyses. The first sample was packed in a tube containing EDTA-K2 or EDTA-K3 for complete blood count with platelets. Slender smears were made for cellular morphological study and blood figurative elements. Cell count was performed using the flow cytometry principle with the Sysmex XS-1000i equipment. The sample for the coagulation study was stored in a tube containing buffered sodium citrate 0.109 mol/L (3.2%), in the proportion of nine parts of blood for one part of citrate solution. Platelet-poor plasma was obtained after centrifugation at 2000 *g* for 15 min, separated, and centrifuged again under the same conditions. Prothrombin time (TP) and INR determination (international normalized ratio) were performed using the Clot/Drake Coagulometer digital timer. A third sample was collected for biochemical evaluation. The hepatic profile was evaluated using the ALT, AST parameters GGT, ALP, albumin, and renal profile through urea and creatinine analytes. The dosages were performed in the Metrolab 2300®, Wiener Lab Group. All laboratory analyses were performed in the Clinical Analysis Laboratories from the Federal University of Bahia.

Coproparasitological evaluation

All selected individuals underwent a new coproparasitological assessment together with a new socioeconomic survey to determine resistance/susceptibility to infection by *S. mansoni*. Two stool samples from each participant were examined and two slides from each sample were analyzed using the Kato-Katz method (Katz et al. 1970).

Peripheral blood mononuclear cells

Approximately 30 ml of peripheral blood was collected and peripheral blood mononuclear cells (PBMC) were isolated by the Ficoll-Hypaque gradient, and the concentration of 3×10^5 cells/ml in RPMI 1640 medium was adjusted, containing 10% human serum (positive and inactivated AB), 100 U/ml streptomycin 100 mg/ml, 2 mM L-glutamine, and 30 mM HEPES (Life Technologies GIBCO, BRL, Gaithersburg, MS). The cells (3×10^5 cells/well) were stimulated by the soluble antigen of the adult worm (SWAP) and incubated for 16 h at 37 °C, 5% CO_2 in 96-well plate with bottom in "U," to perform the evaluation of the expression of surface and intracytoplasmic molecules, CD3, CD4, CD5, CD8, CD19, CD25, CD28, CTLA-4, and FoxP3. SWAP is the soluble antigen of the adult worm (SWAP) prepared as a soluble saline buffer-soluble, according to the methodology described by Hirsch et al. in 1996 (Hirsch and Goes 1996).

Evaluation of ex vivo PBMC phenotype

After incubation, the cells were labelled with 20µL of a solution containing the fluorochrome-conjugated antibodies, at a ratio of 2µL of antibodies per well, specific for CD3 (anti-CD3 PerCP-Cy5.5, clone SK7; eBioscience), CD4 (anti-CD4 PerCP-Cy5.5, clone RPA-T4; eBioscience), CD5 (anti-CD5-FITC, clone L17F12; eBioscience), CD8 (anti-CD8 Pe-Cy7, clone SK1; eBioscience), CD19 (anti-CD19 PerCP -Cy5.5, clone SJ25C1; eBioscience), CD25 (anti-CD25 PE-Cy7, clone BC96; eBioscience), CD28 (anti-CD28 APC, clone CD28.2; eBioscience), and CTLA-4 (CD152) (anti-CTLA-4 PE, clone 14D3; eBioscience). The plates were incubated and protected from light for 20 min at 4 °C. Three successive washes were performed using 1 × PBS and a wash solution of (1 × PBS, 1 M azide and BSA) to eliminate excess monoclonal antibodies, followed by resuspension of the cells in 100µL of 1 × PBS and 100µL of 2% formaldehyde. The plates were kept at 4 °C and protected from light until the moment of analysis in the FACSCanto® equipment (BD Biosciences, San Jose, CA).

Mononuclear cells were analyzed according to the frequency and intensity of expression of cell surface markers using the FlowJo program (Tree Star). Cell populations were defined by nonspecific fluorescence from forward scatter (FSC) and side scatter (SSC) as parameters of cell size and complexity, respectively. According to cell characteristics, lymphocyte populations were selected by windows in this population. The specific region corresponding to the window in the lymphocyte area was then delimited.

The strategy for selecting the regions of lymphocyte subpopulations and their markers was based on the use of isotypic controls specific for mice IgG1-PerCP-CY5.5 (clone P3.6.2.8.1, eBioscience), IgG1-Pe-Cy7 (clone P3.6.2.8.1, eBioscience), IgG1-PE (clone eBM2a, eBioscience), and IgG1-APC (clone P3.6.2.8.1, eBioscience) and IgG1-FITC (clone M1-14D12).

Analysis of the expression of intracellular markers

The expression of the cytokines IFN-γ, IL-5, IL-10 and the transcription factor FoxP3 was analyzed as described by Sornasse et al. (1996) (Sornasse et al. 1996). Suspensions with 3×10^5 cells/ml were incubated in 96-well plates for 16 h at 37 °C, 5% CO2 in the presence of SWAP. After incubation, 20 µl/well of brefeldin A (Sigma-Aldrich) diluted 10 times was added and another incubation was carried out under the same conditions for 4 h. The cells were then labelled with the monoclonal antibodies, washed

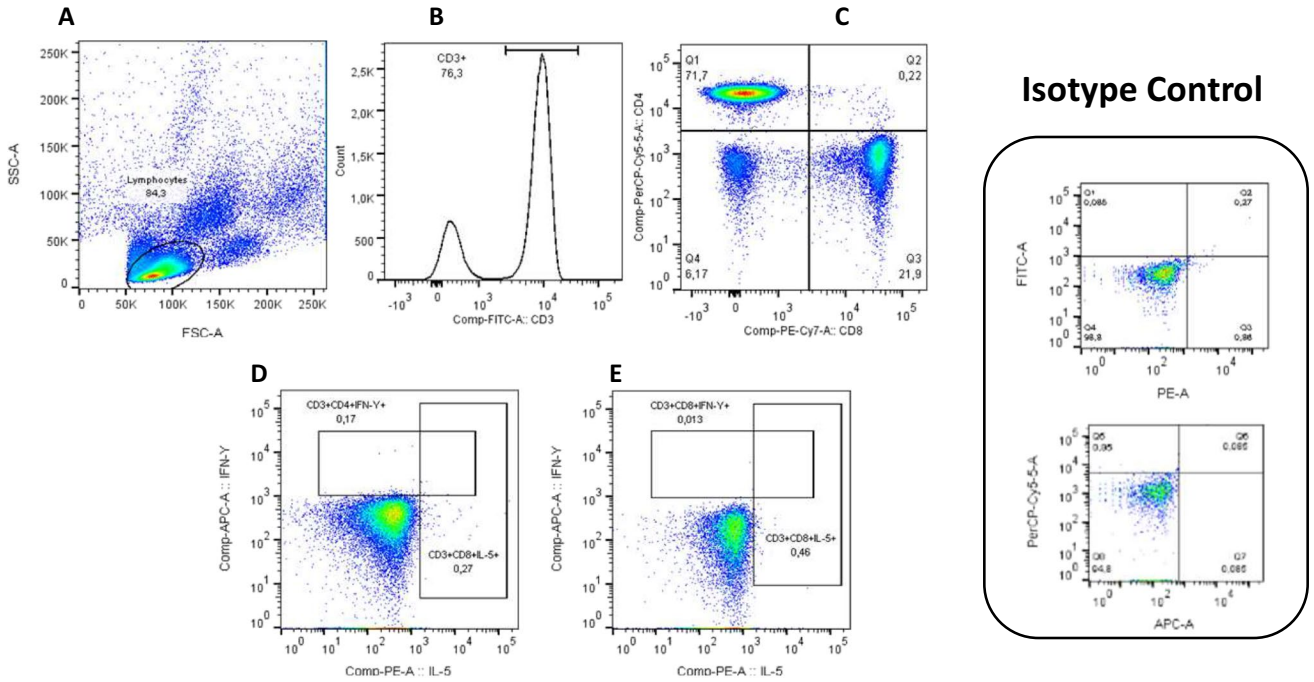


Fig. 1 Region of lymphocyte identification through the expression of SSC (size) and FSC (granulosity) parameters in CMSP of individuals with different parasitic loads of *Schistosoma mansoni* (A). Lymphocyte classification strategy through the expression of CD3 (B), classification of lymphocytes as to the expression of CD4 and CD8 (C). Histogram representative of the expression of IFN-γ and IL-5 cytokines in TCD4 (D) and TCD8 (E), respectively

with 1X PBS and sodium azide, fixed with 200 µl of 2% formaldehyde, and kept at 4 °C. After this step, the plates were centrifuged and incubated with 150 µl/well of permeabilization buffer (1X PBS, sodium azide, and 10% saponin) for 10 min at room temperature. After incubation, intracellular labelling was performed using monoclonal antibodies specific for IFN-γ (anti-IFN-γ APC, clone 4S.B3; eBioscience), IL-5 (anti-IL-5 PE, catalog IC605P, R&D Systems), IL-10 (anti-IL-10, clone JES3-19F1), and FoxP3 (anti-FoxP3 PE, clone PCH 101; eBioscience) followed by a 30-min incubation at room temperature, and protected from light. Successive washes were then performed using the permeabilization buffer, followed by resuspension of the cells in 200 µL of 2% formaldehyde and transfer to FACS reading tubes. The acquisition was performed using the FACSCanto device (Becton Dickinson), in a total of 100,000 events.

T cell selection strategies (CD4 and CD8) and the expression of intracellular molecules IL-5 and IFN-γ are shown in Fig. 1A–E. In Fig. 2, the selection strategies of T cells (CD4 and CD8) and the expression of CD25 molecules are shown. Strategies for selection of B cells (CD19) and CD5 expression are also shown. In addition, Fig. 2 shows the strategy for analyzing the regulatory profile of T and B cells through the expression of intracellular molecules FoxP3 and IL-10.

Statistical analysis

Data regarding the sample of the population studied were presented through the median (minimum–maximum limit). Flow cytometric cell phenotype results were expressed as percent positivity of total cells or percent positivity within a specific cell group. The values of intracellular cytokines, the FoxP3 transcription factor, and costimulatory molecules were expressed through the mean of fluorescence intensity (MIF). For comparison between groups, parametric and non-parametric tests were used according to the nature of the generated data, such as ANOVA and Kruskal–Wallis, respectively. All statistical tests were two-tailed, and *p* values lower than the pre-established significance level of 5% were considered statistically significant. Graphic representation was performed using the GraphPad Prism® version 5.0 program.

Ethical considerations

This work is part of the project approved by the Ethics Committee of the Gonçalo Moniz Research Center – CPqGM/Fiocruz/Ba (Opinion/Resolution n° 385.806 – 05/09/2013).

The methods used for the evaluations did not endanger patients, in addition to those inherent to the usual procedures for diagnosing the disease. Before entering the study, all

patients were informed about the nature of the research. Voluntary participation in this study was achieved by patients signing the free and informed consent form (FICF) for the participants or their legal representatives, and follows the norms of the National Health Council (CNS 466/2012). All collections and handling of biological material were carried out using standardized safety measures.

Results

Demographic characteristics and laboratory evaluation

Demographic characteristics, parasite load, contact with contaminated water, and treatment history of the different groups of individuals included in this study are shown in Table 1. No significant differences were observed between groups regarding age and sex distribution. There were also no differences between groups regarding the degree of contact with contaminated water in all individuals.

Regarding the history of treatment with Praziquantel, it was observed that individuals underwent a course of treatment during the evaluation period; however, no significant differences were observed.

The analysis of the coproparasitological profile of the individuals selected in this study demonstrates that individuals resistant to infection by *S. mansoni* also experience lower parasite loads and lower frequencies of coinfections for the helminths *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms (data not shown).

The results of the laboratory evaluation of the renal, hepatic, and hematological profiles are shown in Table 2. No differences were observed in the urea and creatinine levels. Regarding assessment of the liver profile, no significant differences were observed in the levels ALT, AST, GGT, or albumin among the groups. However, individuals with high parasite loads had higher levels of ALP than those observed in the group of individuals with low load and the control group ($p < 0.05$). However, of the 10 individuals who had increased serum ALP levels, 07 were aged between 10 and 18 years.

In the evaluation of the blood count, only 05 individuals (02 with low load and 03 with high load) had anemia (Hb < 11.5 g/dL). However, no significant differences were observed in all parameters that made up the erythrogram in the different groups.

The total white blood cell count did not differ among groups, but four individuals in each group had leukopenia or leukocytosis. Also, the concentrations of neutrophils, lymphocytes, and basophils did not differ among the evaluated groups. However, it was observed that individuals with a high parasite load had higher concentrations of eosinophils when compared to healthy controls. This same group also presented concentrations of monocytes lower than those observed in the group of healthy controls.

In the evaluation of the platelet count and the evaluation of coagulation, no differences were observed between platelet counts and INR among all evaluated groups.

Individuals with low and high *Schistosoma mansoni* parasite load had the same frequency of T and B lymphocytes in peripheral blood

Initially, we evaluated the frequencies of T lymphocytes ($CD4^+$ and $CD8^+$) and B lymphocytes ($CD3^-CD19^+$) in PBMC cultures from individuals infected with S. *mansoni*, with low and high parasite loads stimulated with the SWAP antigen. No significant differences were observed in the frequencies of $TCD4^+$, $TCD8^+$, or B cells between the groups of individuals with low and high parasite loads (not shown).

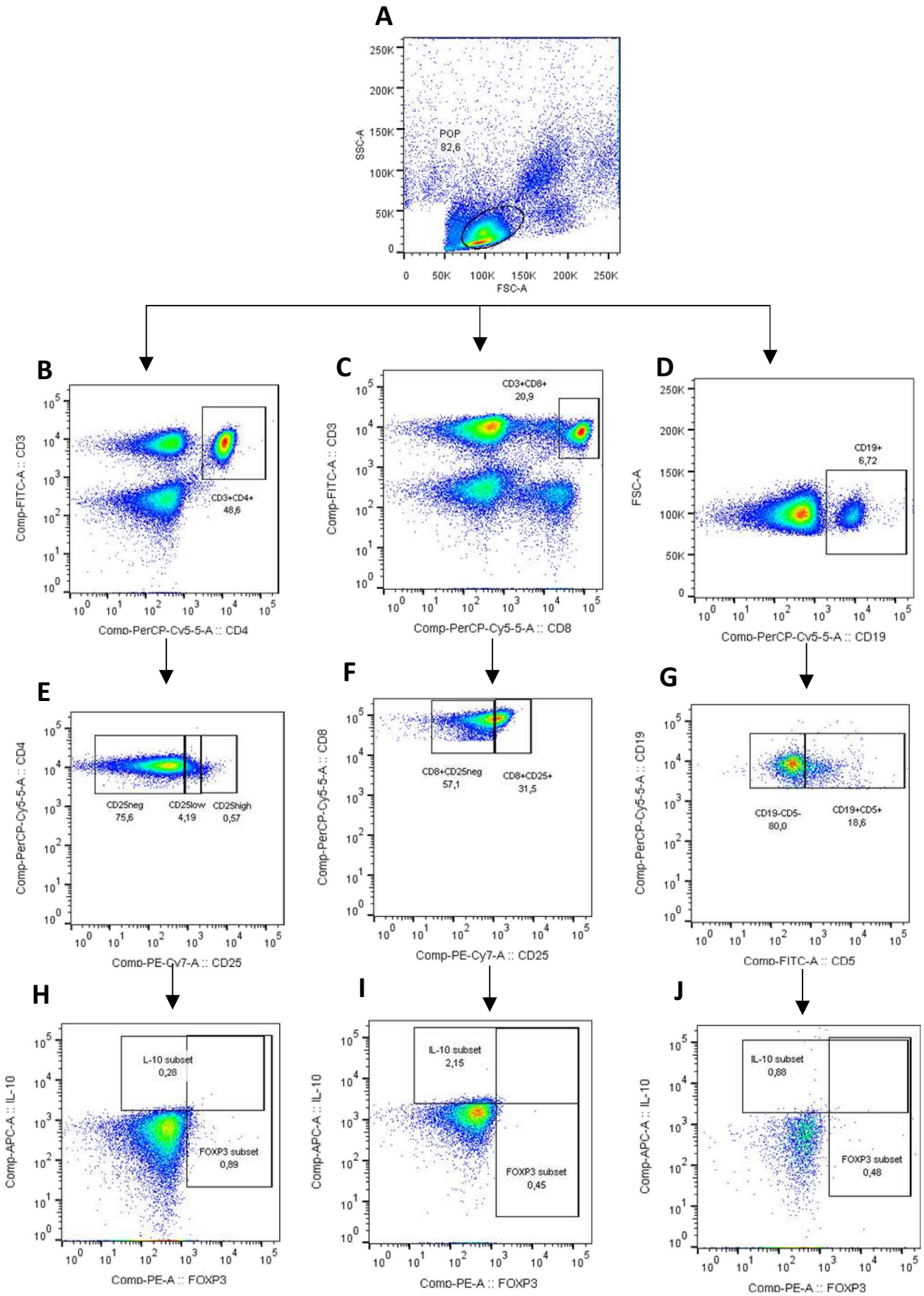
Low parasite load is associated with high production of IFN-γ and IL-5 by $TCD4^+$ and $TCD8^+$ lymphocytes

Although there is no difference in the frequency of lymphocytes, the production of cytokines and the activation status of cells seem to differ between the groups evaluated.

The expression of intracellular IFN-γ by $CD4^+$ T lymphocytes was higher in resistant individuals compared to susceptible individuals ($p < 0.05$; Fig. 3A). In $CD8^+$ T lymphocytes, the same profile was observed ($p < 0.05$; Fig. 3B). In assessing the frequency of $TCD4^+IL\text{-}5^+$ or $TCD8^+IL\text{-}5^+$ cells, it was observed that the group of individuals with low parasite load (resistant) had a higher mean fluorescence intensity (MFI) of $IL\text{-}5^+$ compared to individuals with high load ($p < 0$ 0.05), as shown in Fig. 3C and D.

High parasite load of *Schistosoma mansoni* is associated with decreased activation of CD4+ and CD8+ T lymphocytes

In this study, the expression of co-stimulatory molecules CD28 and CTLA-4 in $CD4^+$ and CD8 + T lymphocytes stimulated by SWAP antigens was also evaluated. It was observed that cells from individuals with a low parasite load of *S. mansoni* showed greater expression of the CD28 and CTLA-4 molecules, both in the population of $CD4^+$ T lymphocytes and in the population of $CD8^+$ T lymphocytes compared to individuals with a high load (Table 3).


A
POP
82,6
SSC-A
FSC-A
B
CD3+CD4+
48,6
Comp-FITC-A :: CD3
Comp-PerCP-Cy5-5-A :: CD4
C
CD3+CD8+
20,9
Comp-FITC-A :: CD3
Comp-PerCP-Cy5-5-A :: CD8
D
CD19+
6,72
FSC-A
Comp-PerCP-Cy5-5-A :: CD19
E
CD25neg
75,6
CD25low
4,19
CD25high
0,57
Comp-PerCP-Cy5-5-A :: CD4
Comp-PE-Cy7-A :: CD25
F
CD8+CD25neg
57,1
CD8+CD25+
31,5
Comp-PerCP-Cy5-5-A :: CD8
Comp-PE-Cy7-A :: CD25
G
CD19-CD5-
80,0
CD19+CD5+
18,6
Comp-PerCP-Cy5-5-A :: CD19
Comp-FITC-A :: CD5
H
L-10 subset
0,28
FOXP3 subset
0,89
Comp-APC-A :: IL-10
Comp-PE-A :: FOXP3
I
IL-10 subset
2,15
FOXP3 subset
0,45
Comp-APC-A :: IL-10
Comp-PE-A :: FOXP3
J
IL-10 subset
0,88
FOXP3 subset
0,48
Comp-APC-A :: IL-10
Comp-PE-A :: FOXP3

◂**Fig. 2** Region of lymphocyte identification through the expression of SSC (size) and FSC (granulosity) parameters in CMSP of individuals with different parasitic loads of *Schistosoma mansoni* (A). T lymphocyte classification strategy by the expression of CD4 and CD8 (B, C) and B lymphocytes through the expression of CD19 (D). Classification of T lymphocytes as the expression of CD25 in TCD4 and TCD8 (E, F) and B lymphocytes as to the expression of CD5 (G). Histogram representative of the expression of molecules associated with the regulatory profile in TCD4 (H), TCD8 (I), and BCD19 (J)

Resistance to infection is associated with a regulatory T and B cell profile

To assess whether *S. mansoni* infection induces a regulatory profile in $TCD4^+$ and $TCD8^+$ cells from individuals with low and high load, the expression of CD25, FoxP3, and IL-10 molecules in these cells was evaluated. Initially, $CD4^+$ T cells were classified into subpopulations that presented absence, low, and high expression of CD25 ($CD25^{neg}$, $CD25^{low}$, and $CD25^{high}$, respectively), and the $TCD8^+$ cells, in turn, were classified through the expression of CD25 in $TCD8^+$CD25neg and subpopulations $TCD8^+CD25^+$.

The frequency of $TCD4^+$CD25 high and $TCD4^+$CD25 low cell's subpopulations was similar between the studied groups ($p > 0.05$). As for the $TCD8^+CD25^{neg}$ subpopulation, no significant differences were observed in the frequency of these cells between the studied groups ($p > 0.05$).

The expression of regulatory molecules FoxP3 and IL-10 in $TCD4^+CD25^{neg}$, $TCD4^+CD25^{low}$, $TCD4^+CD25^{high}$, $TCD8^+CD25^{neg}$, $TCD8^+CD25^+$, $CD19^+CD5^{neg}$, and $CD19^+CD5^+$ B lymphocytes in stimulated cultures is shown in Table 4. It was observed that, for all subpopulations evaluated, there was greater expression of FoxP3 and IL-10 in cultures of individuals with low parasite load, when compared to the high load group (Table 4).

In this study, the expression of CD5 by $CD19^+$ lymphocytes in PBMC cultures from individuals with different parasite loads was also evaluated. No significant differences were observed in the frequency of $CD19^+CD5^+$ and $CD19^+CD5^{neg}$ B cells between groups. As for the frequency of FoxP3 in these subpopulations, no significant differences were observed between the groups evaluated, regardless of the stimulus used ($p > 0.05$).

Regarding the expression of IL-10 by $CD19^+CD5^+$ B cells, a higher expression of this cytokine was observed in individuals with low load compared to that found in the high load group ($p < 0.05$). As for the expression of IL-10 by B $CD19^+CD5^{neg}$ lymphocytes, this was also higher in the group of individuals with low load compared to values found in individuals with high load ($p < 0.05$).

Discussion

Natural resistance to *Schistosoma mansoni* infection has been associated with a mixed Th1 and Th2 response profile (Oliveira et al. 2012). Resistance to reinfection, in turn, has been associated with a predominantly Th2 response with the presence of eosinophilia (Hagan et al. 1985), parasite-specific IgE, and production of IL-4 and IL-5 by blood mononuclear cells peripherally stimulated in vitro (Dunne et al. 1992). Previous studies in Conde, Bahia, showed that about 5% of individuals had natural resistance to infection by *Schistosoma* (Oliveira et al. 2012). However, the immune response of infected individuals with low parasite loads, regardless of the level of exposure to contaminated water, still needs to be studied. The present study evaluated the phenotypic and functional profile of T and B cells in individuals residing in an endemic area for *S. mansoni* who presented, over 10 years, low parasite loads. Furthermore, the association between the phenotype and function of these cells and their possible participation in the mechanisms involved in resistance to infection by *S. mansoni* was evaluated.

In this study, no significant differences were observed when evaluating the sex and age of the low and high load groups. The risk of infection by *S. mansoni* is directly associated with contact with contaminated water. No significant differences were observed between the degree of exposure to contaminated water. This data reinforces the idea that resistance or susceptibility to infection is not conditioned to the degree of exposure to contaminated water sources. Furthermore, our data suggests that resistance to infection by *S. mansoni* confers resistance to infections by other helminths. Corroborating this hypothesis, Oliveira et al. (2012) demonstrated that resistance to infection by *Schistosoma mansoni* would not be associated with behavioral and socioeconomic characteristics (Oliveira et al. 2012).

Because it is a disease with a chronic course, in which the more severe forms are associated (among other factors), with high parasite loads, and that the different clinical forms of the disease can present different immune responses (Andrade 2009; Caldas et al. 2008); it was necessary in this study to evaluate the hematological and biochemical aspects of individuals with different parasite loads, since the immune response in individuals with decompensated forms of the disease could interfere in the interpretation of the results obtained in this work. In chronic schistosomiasis, laboratory tests do not reveal major changes, but in hepatosplenic forms with hypersplenism, anemia and thrombocytopenia can be observed (Mccormick and Murphy 2000) and in severe nephropathy, decreased renal function can be observed, due to the deposit of immune complexes to renal glomeruli, which

Table 1 Demographic characteristics of studied population

	Low burden ($n=20$)	High burden ($n=16$)	Healthy control ($n=5$)	p
Age (median/age group in years)*				
	21 (13–58)	16 (10–65)	28 (21–39)	> 0.05
Gender (%)**				
Male	09 (45.0)	09 (56.2)	02 (40.0)	> 0.05
Female	11 (55.0)	07 (43.8)	03 (60.0)	
Schistosoma mansoni parasite load in median (min–max) of eggs per gram of feces (epg)*				
	00 (00–72)	930 (228–2376)	-	< 0.0001
Contact with contaminated water, *n* (%)**				
Low contact	04 (20)	02 (12)	-	> 0.05
Medium contact	06 (30)	03 (19)		
High contact	10 (50)	11 (69)		
Praziquantel treatment history, *n* (%)**				
No treatment record	04 (20)	04 (25)	-	> 0.05
One treatment cycle	16 (80)	12 (75)		

*Kruskal–Wallis test; **chi-square test

Table 2 Laboratory evaluation of individuals with different parasitic loads for *Schistosoma mansoni*

Categories	Low burden ($n=20$)	High burden ($n=16$)	Healthy control ($n=5$)	p
Renal function; median (range)*				
Urea (mg/dL)	19 (9–38)	24 (13–42)	31 (25–34)	> 0.05
Creatinin (mg/dL)	0.80 (0.60–1.20)	0.61 (0.42–1.02)	0.70 (0.60–0.93)	> 0.05
Hepatic function; median (range)*				
AST (U/L)	25 (13–67)	25 (16–45)	20 (19–29)	> 0.05
ALT (U/L)	24 (9–54)	18 (10–46)	17 (13–48)	> 0.05
GGT (U/L)	33 (10–166)	27 (10–68)	28 (15–52)	> 0.05
ALP (U/L)	109 (65–1002)	284 (139–740)	178 (83–283)	< 0.05[a,b]
Albumin (g/dL)	4.3 (3.0–4.8)	4.4 (4.1–4.7)	4.4 (4.3–4.5)	> 0.05
INR	1.10 (1.00–1.38)	1.07 (1.00–1.27)	1.15 (1.10–1.19)	> 0.05
Hematological parameters; median (range)*				
RBC (10^6/µL)	4.69 (3.98–5.66)	4.49 (4.24–5.11)	4.73 (4.29–5.76)	> 0.05
HBG (g/dL)	13.2 (11.2–15.4)	13.0 (11.3–14.8)	13.2 (13.0–15.8)	> 0.05
HCT (%)	39.9 (34.6–45.7)	38.7 (34.8–42.9)	40.5 (37.4–44.7)	> 0.05
WBC (10^3/µL)	6240 (3890–11,020)	6060 (3720–9010)	6300 (5130–7570)	> 0.05
NEUT (10^3/µL)	3461 (696–6191)	3254 (1339–5198)	3749 (1919–4732)	> 0.05
EOS (10^3/µL)	417 (81–2442)	697 (348–1848)	208 (47–292)	< 0.05[b]
BASO (10^3/µL)	21.3 (0.0–56.0)	0.0 (0.0–53.3)	37.9 (6.7–69.7)	> 0.05
LYMPH (10^3/µL)	2148 (1101–4026)	1817 (1320–3136)	1998 (1758–2378)	> 0.05
MONO (10^3/µL)	408 (219–844)	316 (112–558)	549 (407–689)	< 0.05[b]
PLT (10^3/µL)	243 (166–369)	224 (144–359)	294 (221–356)	> 0.05

*Kruskall-Wallis test ([a]low burden vs high burden; [b]high burden vs healthy controls)

cause the development of glomerulopathies (Elsheikh et al. 1989; Gryschek and Chieffi 2008). No laboratory evidence of severe forms of schistosomiasis disease was observed in this study. There were no significant differences in the parameters that assessed the renal profile of the individuals evaluated in this study.

In the evaluation of the hepatic profile, however, it was observed that individuals with high parasite loads presented ALP levels higher than those observed in the group with low

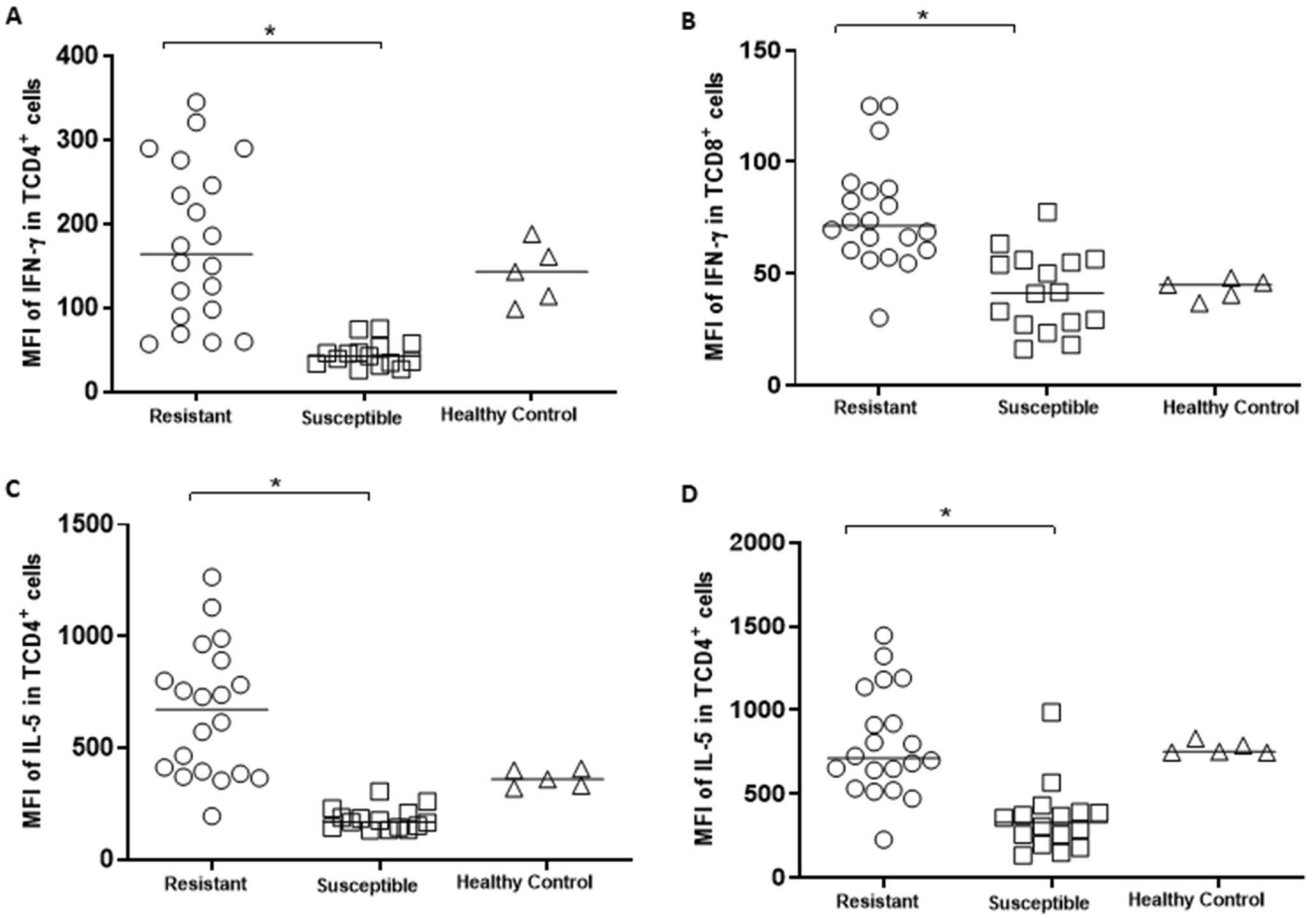


Fig. 3 Evaluation of IFN-γ and IL-5 production by $TCD4^+$ and $TCD8^+$ lymphocytes in CMSP cultures of individuals with low and high parasitic loads of S. *mansoni*, stimulated in vitro with swap antigen. Expression of IFN-γ by $TCD4^+$ (A) and $TCD8^+$ (B) lymphocytes. Expression of IL-5 by $TCD4^+$ (C) and $TCD8^+$ (D) lymphocytes. The results obtained represent the mean of fluorescence intensity (MFI) and were expressed as median, minimum, and maximum values. *$p < 0.05$, Kruskal–Wallis test

Table 3 Expression of CD28 and CTLA-4 in $TCD4^+$ and $TCD8^+$ lymphocytes in PBMC cultures from individuals with low and high parasite loads of *S. mansoni*

T lymphocytes	Groups		
	Resistant individuals	Susceptible individuals	*p*
$CD4^+CD28^+$	3024 (1305–6682)	1211 (598–2229)	$p < 0.05$
$CD4^+CTLA\text{-}4^+$	663 (147–1653)	158 (141–590)	$p < 0.05$
$CD8^+CD28^+$	1909 (767–4140)	622 (534–1666)	$p < 0.05$
$CD8^+CTLA\text{-}4^+$	1843 (230–3035)	216 (195–262)	$p < 0.05$

Unpaired *t* test. Values are expressed as mean fluorescence intensity (MFI)

parasite load and healthy individuals. One possible explanation is that most of the high-load individuals who had increased serum ALP levels were adolescents. And, due to the osteoclastic process observed at this stage of life, the bone isoenzyme is increased, producing higher ALP values than in adults (Ridefelt et al. 2014). Therefore, this finding would not represent a significant change observed in severe forms of *SchistosomiasiS. mansoni*.

Among the hematological evaluation data, eosinophilia was the only parameter that differed between groups. The increase in the absolute number of eosinophils observed in the group with a high parasite load is associated with the pattern of Th2 response to helminthic infection, with production of the cytokines IL-4, IL-5, and IL-13 (Rumbley et al. 1999). It should be noted that IL-5 is considered the most important cell growth factor for eosinophils, which participates in the process of maturation and increased survival of eosinophils. These cells can migrate to inflammatory sites and are decisive in the granulomatous response, the phase that involves the participation of different cell types such as T and B cells (Swartz et al. 2006).

The granulomatous response observed in schistosomiasis is mediated by several cell types, among which the specific

Table 4 Expression of IL-10 and FoxP3 in TCD4+, TCD8+, and B lymphocytes in PBMC cultures from individuals with low and high parasite loads *of S. mansoni*, stimulated in vitro with the SWAP antigen

Subpopulations	Low parasite load ($n=20$)	High parasite load ($n=16$)	Healthy controls ($n=5$)	*p*
	IL-10 expression			
$TCD4^+ CD25^{neg}$	736 (56–1303)	45 (36–78)	188 (165–214)	$p<0.05$
$TCD4^+ CD25^{low}$	776 (49–2254)	46 (32–802)	179 (153–205)	$p<0.05$
$TCD4^+ CD25^{high}$	806 (48–3194)	44 (30–834)	158 (148–194)	$p<0.05$
$TCD8^+ CD25^{neg}$	927 (33–1922)	48 (22–589)	64 (46–78)	$p<0.05$
$TCD8^+ CD25^+$	1151 (50–1920)	47 (19–866)	70 (41–80)	$p<0.05$
$BCD19^+ CD5^{neg}$	966 (43–1688)	47 (26–758)	95 (20–108)	$p<0.05$
$BCD19^+ CD5^+$	1284 (51–9772)	66 (38–1016)	104 (31–140)	$p<0.05$
	FOXP3 expression			
$TCD4^+ CD25^{neg}$	436 (185–702)	154 (122–493)	346 (332–362)	$p<0.05$
$TCD4^+ CD25^{low}$	516 (186–852)	168 (34–618)	360 (350–365)	$p<0.05$
$TCD4^+ CD25^{high}$	708 (205–1541)	201 (145–989)	378 (372–401)	$p<0.05$
$TCD8^+ CD25^{neg}$	571 (209–1182)	277 (136–850)	730 (550–837)	$p<0.05$
$TCD8^+ CD25^+$	552 (271–1403)	330 (180–484)	778 (646–923)	$p<0.05$
$BCD19^+ CD5^{neg}$	408 (226–614)	210 (154–507)	436 (245–478)	$p<0.05^a$
$BCD19^+ CD5^+$	757 (246–1595)	235 (142–597)	500 (366–640)	$p<0.05^a$

*Kruskall-Wallis test ([a]low burden vs high burden, [b]low burden vs helathy controls, [c]high burden vs healthy controls)

$CD4^+$ T cells for the parasitic antigens stand out, which are important sources of Th2 and regulatory cytokines associated with the protective immune response in *Schistosoma mansoni* infection. $CD8^+$ T cells, in turn, are important sources of cytokines and are associated with modulation of the granulomatous response (Chensue et al. 1981). Furthermore, some works demonstrate the ability of B cells to produce several cytokines associated with the Th1, Th2, and regulatory response pattern. However, its role in schistosomiasis in humans is still poorly explored (Harris et al. 2000).

In this study, the frequency of $TCD4^+$ and $TCD8^+$ cells, the $TCD4^+/TCD8^+$ ratio, and the frequency of B cells ($CD19^+$) did not differ between the group of individuals with low load and high parasite load of *S. mansoni*. This data reinforces the idea that, despite the similarity in the proportion of these cells in the different groups evaluated, the cell phenotype should influence the pattern of responses and that the phenotypic differences found here would not be a consequence of the increased proportion of one or another cell compartment. Work developed by Oliveira-Prado and collaborators (Oliveira-Prado et al. 2009), in an endemic area for *S. mansoni*, showed lower $TCD4^+$ cell frequencies and $TCD4^+/TCD8^+$ ratio in infected individuals when compared to non-infected individuals (Oliveira-Prado et al. 2009). However, differences in the selection strategies of the patients studied in this study differ from the selection criteria of the previous study, which evaluated individuals with low parasite loads over 10 years.

Several authors have shown that variations in the cellular immune response can influence both the development of immunopathological events and resistance to infection. However, these studies only evaluated the production of cytokines in PBMC supernatants stimulated by several antigens. These authors demonstrated that PBMC from endemic normal individuals produced high levels of IFN-γ, an important Th1 cytokine (Bahia-Oliveira et al. 1992; Viana et al. 1994; Brito et al. 2000; Oliveira et al. 2012). Allied to this, some works show the fact that resistance is associated with a mixed Th1 and Th2 response pattern (Reviewed by Corrêa-Oliveira et al. 2000). Reinforcing this hypothesis, studies carried out by our group in Conde-Ba demonstrated that PBMC from natural resistant individuals stimulated by *S. mansoni* antigens produced significant levels of IL-5, IL-13, and IFN-γ (Oliveira et al. 2012). In contrast, individuals who exhibit drug-induced resistance have a predominant Th2 profile (Couissinier-Paris and Dessein 1995).

In this study, it was possible to evaluate the possible sources of IFN-γ and IL-5, and it was observed that both $TCD4^+$ and $TCD8^+$ lymphocytes are important sources of these cytokines. Individuals with low loads showed a higher expression of IFN-γ and IL-5 when compared to individuals with high parasite load. These data suggest that the mixed Th1 and Th2 response pattern may be associated with the control of the parasite load in these individuals, and that the susceptibility is associated with an inappetence of $TCD4^+$ and $TCD8^+$ cells to produce these cytokines. What reinforces this hypothesis is that individuals with high parasite loads showed lower expression of costimulatory molecules CD28 and CTLA-4 crucial for the expansion and optimization processes in the protective response (Boesteanu and Katsikis 2009). Additionally, the increase in CTLA-4 expression demonstrated in

these individuals would act to prevent damage caused by the Th1 and Th2 responses observed in individuals with low parasite load (Walsh et al. 2007). In this work, it was not possible to establish the participation of B cells in the production of IFN-γ and IL-5 cytokines.

Regulatory cells play key roles in the modulation process of the immune response, preventing possible harmful effects and IL-10 is the key cytokine in the regulatory process of the immunopathogenesis of schistosomiasis (Burke et al. 2009).

The hypothesis of this work was that susceptibility to infection would be associated with a predominantly regulatory response. Surprisingly, it was found in this study that individuals with low parasite loads presented a regulatory profile in both $TCD4^+CD25^{neg}$, $TCD4^+CD25^{low}$, and $TCD4^+CD25^{high}$ classical regulatory cells, as these subpopulations showed high expression of the regulatory molecules FoxP3 and IL-10 when compared to individuals with high loads and even healthy controls. A possible explanation for this finding would be the attempt to control the Th1 and Th2 responses presented by individuals with low parasite loads and control the cellular activation process, since these same individuals showed higher expression of CD28 both for $TCD4^+$ and $TCD8^+$ cells. Parallel to this, the concomitant increase in CTLA-4 expression by T lymphocytes reinforces the idea of the participation of regulatory cells in this context (Walsh et al. 2007) that carry out their potent suppression activity through cell-to-cell contact (Baecher-Allan et al. 2001) or through the synthesis of regulatory cytokines, such as IL-10 and TGF-β (Saito et al. 2005).

Similar to the results observed in the evaluation of $TCD4^+$ regulatory cells, the expression of FoxP3 and IL-10 in $TCD8^+$ cells was higher in individuals with low parasite load when compared to that observed in individuals with high load. Little is known about the role of $TCD8^+$ regulatory cells, which have functional similarities with $CD4^+$ Treg cells (Churlaud et al. 2015).

Recently, the participation of B cells in the modulation of effector, regulatory, and memory T cells has been demonstrated by mechanisms independent of antibodies (Lund and Randall 2010). The concept of B cells as important sources of Th1, Th2, and regulatory cytokines has been arousing considerable interest. B lymphocytes are subdivided into two lineages: B1 and B2. B1 cells can be subdivided into B1a cells ($CD11^+CD5^+$) and B1b cells ($CD11^+CD5^{neg}$) and, together with B2 cells, are able to respond rapidly to antigenic stimuli. However, IL-10 production is not exclusive to $CD5^+$ cells (Takanaski et al. 1994; Tung et al. 2006). In this study, the regulatory profile of B cells was evaluated through the expression of FoxP3 and IL-10 and, like the results observed in the evaluation of $TCD4^+$ and $TCD8^+$ lymphocytes, individuals with low parasite load showed greater expression of molecules associated with the regulatory profile. For the first time, an association between B lymphocytes and resistance to helminth infection and, in particular, schistosomiasis was demonstrated.

Preliminary analyzes showed that IL-2 levels determined in PBMC culture supernatants from these low load individuals are higher than those found in the high parasite load group (unpublished data). This data reinforces the idea of probable energy in cells or the existence of inhibitory mechanisms in individuals with a high parasite load, a characteristic that would be associated with a decrease in the production of IL-2, the main factor in the development of regulatory cells (Bensinger et al. 2004) and participate in the inhibition of cell polarization (Wohlfert et al. 2011; Wang et al. 2011). However, the mechanisms involved in this controversial data need further investigation.

Several authors have been evaluating the participation of *Schistosoma mansoni* antigens in the development process of resistance to schistosomiasis. The immune response against *S. mansoni* antigens of endemic normal individuals differs significantly from the response of chronically infected individuals or those who present resistance to reinfection after drug treatment (Correa-Oliveira et al. 1989; Bahia-Oliveira et al. 1992; Viana et al. 1994, 1995). In this work, the participation of *Schistosoma mansoni* antigens considered as possible vaccine candidates was evaluated.

SWAP is considered the main target of the IgE response in experimental and human models of *S. mansoni* infection. Some studies have shown that endemic normal individuals have considerably higher levels of anti-schistosomulus tegument IgE when compared to chronic infected individuals (Viana et al. 1995), whereas after specific chemotherapy, the levels of these antibodies rise only in those individuals who have acquired resistance to reinfection (Caldas et al. 2000). We did not observe differences between unstimulated and SWAP-stimulated cultures for most of the evaluated markers (not shown). One explanation for this would be that, since they are lymphocytes, the antigen-specific response to SWAP in vitro by memory cells would take longer than that used in cultures, which was 48 h of stimulation. In the study published by our group in 2019 (Souza et al. 2019), we also stimulated the cells with *Schistosoma mansoni* adult worm antigen (SWAP) for 48 h and it did not modify the frequency of memory cells.

Our findings show that resistance to infection by *Schistosoma mansoni*, demonstrated by the low parasite load, is associated with a mixed response between the Th1, Th2, and regulatory profiles represented by $CD4^+$ and $CD8^+$ T lymphocytes and by B cells, with the objective of determining which mechanisms are involved in the reduction of the protective response in individuals with high parasite loads.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were

performed by all authors. The first draft of the manuscript was written by Robson da Paixão de Souza and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of the Gonçalo Moniz Research Center – CPqGM/Fiocruz/Ba (Opinion/Resolution n° 385.806 – 05/09/2013).

Consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare no competing interests.

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