

Schistosoma Antigens Downmodulate the in vitro Inflammatory Response in Individuals Infected with Human T Cell Lymphotropic Virus Type 1

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Key Words

Human T cell lymphotropic virus type 1 · *Schistosoma* spp. antigens · Interferon- γ · Interleukin-10

Abstract

Human T cell lymphotropic virus type 1 (HTLV-1) is the causal agent of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). While the immune response to HTLV-1 infection is polarized to the Th1-type, chronic helminth infections drive the Th2- and T regulatory-type, and are able to downregulate the inflammatory response in some autoimmune diseases. **Objective:** To evaluate whether *Schistosoma* spp. antigens alter the in vitro cytokine response in HTLV-1 infection. **Methods:** The recombinant *Schistosoma* antigens Sm29 and ShTSP2 (tetraspanin) and PIII, a fraction of the *Schistosoma mansoni* adult worm antigen were added to peripheral blood mononuclear cell (PBMC) cultures of HTLV-1-infected individuals and the levels of interferon (IFN)- γ and interleukin (IL)-10 in the supernatants were measured using the ELISA sandwich technique. **Results:** Compared to the levels of cytokine in nonstimulated cultures, the levels of IFN- γ were reduced in 50, 47 and

50% of patients by the presence of Sm29, ShTsp2 and PIII, respectively. The downregulation of IFN- γ production in the presence of Sm29 antigen was observed mainly in subjects who had lower basal levels of this cytokine. The levels of IL-10, however, increased by the addition of the three antigens in the cultures in 74, 62 and 44% of individuals, respectively. In addition, there was a decrease in the ratio of IFN- γ /IL-10 levels in cultures stimulated with Sm29 and ShTSP2 when compared to nonstimulated ones. **Conclusions:** The *Schistosoma* spp. antigens used in this study were able to downmodulate IFN- γ production in vitro in HTLV-1 infection. This may be associated with the increased levels of IL-10 induced by the antigens.

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Introduction

The human T cell lymphotropic virus type 1 (HTLV-1) is the causal agent of the adult T cell leukemia/lymphoma and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [1]. About 10–20 million individuals worldwide are infected, and Salvador, the capital

of the state of Bahia in Brazil, has the highest prevalence of HTLV-1 infection in the country, with a frequency of 1.76% in the general population [2, 3].

HAM/TSP is clinically characterized by the insidious onset of spastic paraparesis that is progressive and can eventually lead to wheelchair dependency [1, 4]. Other manifestations associated with HTLV-1 infection include Sjögren syndrome, polyarthritis, uveitis, urologic manifestations and erectile dysfunction [5, 6]. Patients with HAM/TSP have a higher proviral load and higher production of interferon (IFN)- γ compared to HTLV-1 carriers [7, 8]. While HTLV-1 virus induces a Th1-polarized immune response, in chronic *Schistosoma mansoni* infection the immune response is polarized towards the Th2 and T regulatory type. In experimental models, it has been shown that infection with *S. mansoni* or injection of their products prevents Th1-inflammatory diseases, such as type I diabetes, psoriasis and colitis [9, 10]. Our hypothesis is that *Schistosoma* spp. antigens are able to downmodulate the inflammatory immune response in HTLV-1-infected individuals. The aim of this study was to evaluate the in vitro ability of *Schistosoma* spp. antigens in modifying the profile of cytokine production by peripheral blood mononuclear cells (PBMC) of HTLV-1-infected individuals.

Methodology

Study Population

A total of 26 HTLV-1-infected subjects from the Multidisciplinary HTLV-1 Clinic of the Immunology Service, Federal University of Bahia, Salvador, Bahia, Brazil were included in this study.

All patients donated blood for PBMC isolation and subsequent cell cultures in the presence or absence of *Schistosoma* spp. antigens. This study was approved by the ethical committee of the Maternidade Climério de Oliveira/Federal University of Bahia, and informed consent was obtained from all study participants or their legal guardians.

Laboratorial Evaluation

Anti-HTLV-1 antibody titers were measured using ELISA technique (Cambridge Biotech Corp, Worcester, Mass., USA). Positive sample results were confirmed using Western blot (HTLV blot 2.4, Genelabs, Science Park Drive, Singapore).

Schistosoma spp. Antigens

Antigens used in this study included a *S. mansoni* recombinant protein Sm29, a membrane-bound glycoprotein located on the tegument of the adult worm and lung stage schistosomula [11], ShTSP-2, a recombinant protein (tetraspanin) from *S. haematobium* tegument [12] and PIII, which is a fraction of *S. mansoni* soluble adult worm antigen obtained by ionic chromatography [13].

The Sm29 and ShTSP2 recombinant proteins were cloned in *Escherichia coli* and levels of lipopolysaccharide contamination tested by a commercially available chromogenic LAL end-point assay kit

(Cambrex, Charles City, Iowa, USA), were <0.25 ng/ml. In order to neutralize the potential effects of lipopolysaccharide found in low levels in *Schistosoma*-recombinant antigens, polymyxin B was added to cell cultures every 12 h according to an established protocol [14].

PBMC Cultures and Cytokine Determination

Sm29 (5 μ g/ml), ShTSP2 (5 μ g/ml) and PIII (10 μ g/ml) were added to PBMC (3×10^6 cells/ml) of HTLV-1-infected individuals, cultured with RPMI 1640 (Gibco, Grand Island, N.Y., USA) plus 10% of heat-inactivated human AB Rh+ serum (Sigma Chemical Co., St. Louis, Mo., USA), antibiotics and glutamine. Cells were incubated at 37°C in 5% CO₂ atmosphere in a 24-well plates for 72 h. Supernatants were collected to cytokine measurement.

Levels of IFN- γ and interleukin (IL)-10 were measured by an ELISA sandwich technique using commercially available kits (OptEIA; BD Bioscience, San Jose, Calif., USA). The results were expressed as picograms per milliliter (pg/ml), on the basis of a standard curve.

Statistical Analyses

A nonparametric Wilcoxon signed-rank test for matched pairs was used to analyze the effect of the addition of *Schistosoma* spp. antigens in the production of IFN- γ and IL-10. The Fisher exact test was used to compare proportions. An alpha (α) error of 5% ($p < 0.05$) was considered as statistically significant. The SPSS 9.0 software (IBM Software, New York, N.Y., USA) was used for statistical analysis.

Results

From 26 HTLV-1-infected individuals included in this study, 17 were asymptomatic (65%) and were considered as HTLV-1 carriers, while 9 patients (35%) had the HAM/TSP form of the disease. The mean age did not differ between carriers and symptomatic patients (47 ± 10 years and 50 ± 6 years, respectively; $p > 0.05$). In the HAM/TSP group, there was a significantly higher prevalence of males (56% vs. 29%; $p < 0.05$).

Levels of IFN- γ were higher in nonstimulated PBMC cultures ($3,334 \pm 3,822$ pg/ml) when compared to the cultures stimulated with ShTSP-2 ($2,188 \pm 2,560$ pg/ml, 34% of reduction; $p = 0.004$; table 1). No significant difference was found in the mean levels of IFN- γ between nonstimulated and Sm29- or PIII-stimulated cultures (table 1). On the other hand, mean levels of IL-10 were lower in nonstimulated culture (123 ± 157 pg/ml) compared to the levels of this cytokine in cultures stimulated with Sm29 (223 ± 391 pg/ml, 81% of increase; $p = 0.008$) or with ShTSP-2 (556 ± 502 pg/ml, 352% of increase; $p = 0.0001$, table 1).

The frequency of individuals who presented a significant reduction in the levels of IFN- γ when the antigens Sm29, ShTSP2 and PIII were added to the cultures was 50, 69, and 50%, respectively (table 2). Among those who dis-

Table 1. Effect of the addition of *Schistosoma* spp. antigens on cytokine production by PBMC of HTLV-1-infected individuals

	IFN- γ levels (mean \pm SD) pg/ml	Reduction %	p value	IL-10 levels (mean \pm SD) pg/ml	Increase %	p value*
Without antigen	3,334 \pm 3,822	–	–	123 \pm 157	–	–
rSm29	2,802 \pm 3,769	16	0.104	223 \pm 391	81	0.008
rShTSP-2	2,188 \pm 2,560	34	0.004	556 \pm 502	352	0.0001
PIII	2,812 \pm 2,741	17	0.534	125 \pm 185	1.6	0.07

* Cytokine levels in nonstimulated cultures vs. cultures stimulated with *Schistosoma* spp. antigens; Wilcoxon signed-rank test.

Table 2. Frequency of individuals who presented significant changes in the levels of IFN- γ and IL-10 in the presence of *Schistosoma* spp. antigens in the cultures (n = 26)

<i>Schistosoma</i> spp. antigens	Reduction in IFN- γ levels	Increase in IL-10 levels
	patients, n (%)/ percentage of reduction	patients, n (%)/ percentage of increase
rSm29	13 (50)/59%	20 (77)/327%
rShTSP-2	18 (69)/47%	22 (85)/573%
PIII	13 (50)/35%	12 (46)/35%

played a decrease in the levels of IFN- γ , the reduction was 59% when the cultures were stimulated with Sm29, 47% after stimulation with ShTSP-2 and 35% in the presence of PIII (table 2).

Regarding IL-10 production, the frequency of individuals who displayed a significant increase in the levels of this cytokine after Sm29, ShTSP2 and PIII stimulation was 74, 62, and 44%, respectively (table 2). Among those who displayed an increase in the levels of IL-10, the augment reached 327, 573 and 35% in response to rSm29, rShTSP-2 and PIII, respectively (table 2).

We then compared the main clinical and laboratorial features of individuals who displayed a significant reduction in the levels of IFN- γ with those who did not have a reduction in the levels of this cytokine according to whether *Schistosoma* spp. antigens were present (table 3).

The median age and gender distribution did not differ between the 2 groups of subjects for any antigen tested (table 3). In addition, the frequency of patients with HAM/TSP among those individuals who experienced a reduction in the levels of IFN- γ in the presence of Sm29, ShTSP2 and PIII was similar to those who did not present inhibition of IFN- γ production (table 3).

We observed, however, that the basal levels of IFN- γ were lower in the group of individuals who had a decrease

in the levels of this cytokine in the presence of Sm29 in the cultures [median (minimum and maximum values) = 469 pg/ml (63–3,277 pg/ml)] when compared to the group of patients who did not have a reduction in the cytokine production [3,133 pg/ml (479–17,297 pg/ml); $p < 0.01$, table 3]. The basal levels of IL-10 in individuals who showed decreased levels of IFN- γ when the *Schistosoma* spp. antigens were added to the cultures did not differ significantly from those who did not experience this reduction (table 3). There was also no significant difference in the HTLV-1 proviral load in individuals who experienced a reduction in IFN- γ production (table 3).

In order to analyze the balance between Th1- and T regulatory-type cytokines, we evaluated the ratio of IFN- γ and IL-10 levels produced by nonstimulated and *Schistosoma* spp. antigen-stimulated cultures (fig. 1). We observed that the ratio of IFN- γ /IL-10 levels in the totality of HTLV-1-infected individuals was higher in the PBMC cultures without *Schistosoma* spp. antigens (IFN- γ /IL-10 ratio, mean \pm SD = 116 \pm 207.1) than in cultures stimulated with the antigens Sm29 and ShTSP-2 (72.2 \pm 207.7 and 14.3 \pm 25.1, respectively; $p < 0.001$; fig. 1a). There was no significant difference in the ratio of IFN- γ /IL-10 levels when PIII was added to the cultures (75.5 \pm 121.9; fig. 1a). In HTLV-1 carriers, we observed that there was also a higher ratio of IFN- γ /IL-10 levels in nonstimulated cultures (79.8 \pm 85.6) compared to those stimulated with Sm29 and ShTSP-2 (0.17 \pm 0.49 and 15.1 \pm 20.5, respectively; $p < 0.001$; fig. 1b). The addition of PIII to the cultures did not alter the ratio of IFN- γ /IL-10 (69.4 \pm 96.7) in relation to the nonstimulated cultures. Furthermore, in HAM/TSP individuals, the ratio of IFN- γ /IL-10 was higher in PBMC cultures stimulated with Sm29 (0.44 \pm 0.48) than in PBMC cultures without the antigen (148.3 \pm 311; $p = 0.01$; fig. 1c). There was no significant difference in the ratio of IFN- γ /IL-10 levels in the presence of ShTSP-2 (12.7 \pm 32) and PIII (87.2 \pm 158.1) compared to nonstimulated cultures (148.3 \pm 311; $p > 0.05$; fig. 1c).

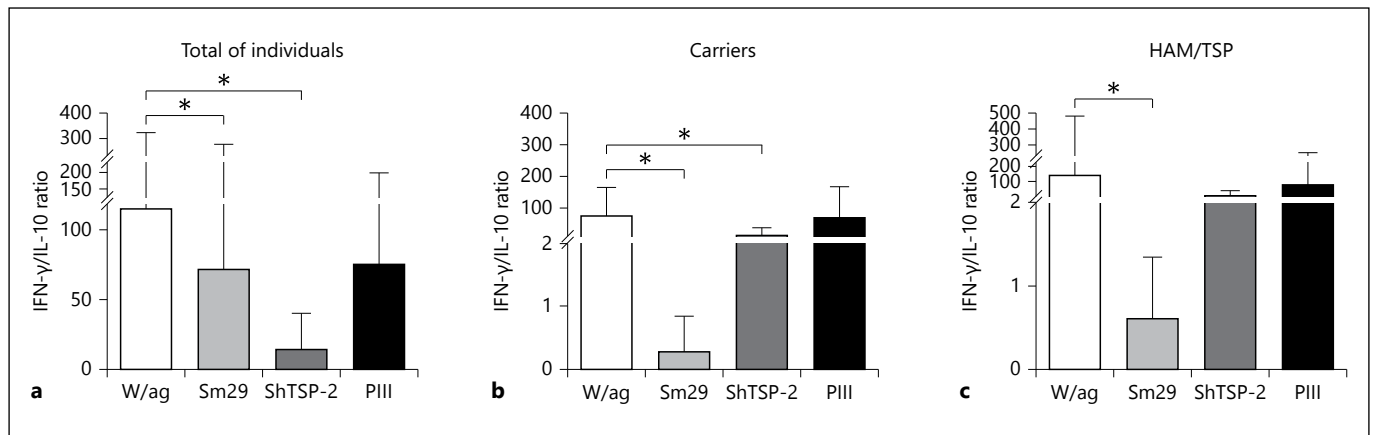


Fig. 1. The ratio of IFN- γ /IL-10 levels in the supernatants of PBMC cultures without stimulation (W/ag) and cultures stimulated with *Schistosoma* spp. antigens was evaluated in the totality of HTLV-1-infected individuals (a), in HTLV-1 carriers (b) and in individuals with HAM/TSP (c). Statistical differences are indicated by asterisks ($p < 0.05$; Wilcoxon signed-ranks test).

Table 3. Characteristics of individuals who displayed inhibition or no inhibition of IFN- γ production by the addition of *Schistosoma* spp. antigens to the cultures

	Individuals who displayed inhibition of IFN- γ production			Individuals who did not display inhibition of IFN- γ production		
	Sm29 n = 13/26	ShTSP-2 n = 18/26	PIII n = 13/26	Sm29 n = 13/26	ShTSP-2 n = 8/26	PIII n = 13/26
Age, years median (range)	48 (23–58)	48 (23–58)	48 (23–58)	49 (23–59)	53 (35–59)	50 (43–59)
Male gender, n (%)	5 (38)	7 (39)	3 (23)	5 (38)	3 (38)	7 (54)
HAM/TSP, n (%)	6 (46)	7 (39)	4 (31)	3 (23)	2 (25)	5 (39)
IFN- γ , pg/ml median (range)	469 (63–3,277) ^a	667 (54–6,566)	656 (41–7,898)	3,133 (479–17,297) ^a	959 (348–9,991)	1,783 (690–8,650)
Increase in IL-10, n (%)	10 (77)	13 (72)	4 (31)	10 (77)	8 (100)	8 (62)
Proviral load, median (range)	4.94 (4.33–5.84)	4.63 (3.62–5.59)	4.6 (3.62–5.84)	4.54 (1.54–5.53)	4.73 (1.54–5.53)	5.02 (1.54–5.59)

^a Individuals who presented inhibition of IFN- γ production after the addition of Sm29 to the PBMC cultures vs. individuals who did not present inhibition of IFN- γ production ($p < 0.01$; Mann-Whitney U test).

Discussion

In this study, we evaluated whether *Schistosoma* spp. antigens are able to change the cytokine response produced by PBMC of HTLV-1-infected individuals.

The host immune response to HTLV-1 infection explains why some HTLV-1 individuals develop serious illness while most of them remain asymptomatic during their whole lives. Studies have shown that in HTLV-1-infected individuals, there is a high level of IFN- γ and TNF production without any additional in vitro stimulation, when compared to the cells of HTLV-1-seronegative blood donors [8, 15].

There is still no satisfactory treatment or a way to prevent the development of disease in infected individuals.

As HTLV-1 is able to induce an exacerbated immune response and this is the basis of the pathology [8], strategies capable of reducing the Th1-inflammatory response are desirable. A previous study conducted by the Immunology Service of the Federal University of Bahia showed that in HTLV-1-infected individuals coinfecting with *S. mansoni*, the levels of IFN- γ in the supernatants of nonstimulated PBMC cultures were higher when compared to HTLV-1 non-coinfecting individuals [16].

In addition, we showed previously that the antigens Sm29 and PIII are able to induce the IL-10 production by PMBC of *S. mansoni*-infected individuals [17, 18] and that they suppress the Th2-inflammatory response in an experimental model of allergic asthma [19]. The antigens Sm29, ShTSP2 and PIII were tested in this study with re-

gard to their ability to induce IL-10 production and suppress the Th1-response in vitro in the cells of HTLV-1-infected individuals.

We observed that rShTSP-2 was able to significantly reduce the mean levels of IFN- γ . The 3 antigens, however, were capable of diminishing this cytokine production in a significant number of individuals. Moreover, the downmodulation of IFN- γ production by rSm29 and rShTSP-2 antigens was followed by an increase in IL-10 production in the majority of individuals, and this augment in IL-10 production reached more than 300%. Reduction of IFN- γ production and an increase in the levels of IL-10 by the use of rSm29 and PIII antigens in PBMC cultures was also seen by our research team in patients with cutaneous leishmaniasis, the pathology of which is also linked to a Th1-immune response [20].

Evaluating possible individual features associated with the ability of the antigens to downmodulate IFN- γ production, we found that subjects displaying inhibition of production of this cytokine by the addition of rSm29 to the cultures had lower basal levels when compared to those who did not display inhibition of IFN- γ production. Unexpectedly, we did not observe any other difference between the group of individuals who had reduction in the levels of IFN- γ and those who did not. In patients with cutaneous leishmaniasis [20], the addition of Sm29 and PIII to cell cultures stimulated with the *Leishmania*-soluble antigen resulted in a reduction of IFN- γ production, also with no correlation to the clinical features of the disease.

The relationship between coinfection with HTLV-1 and *S. mansoni* is not well studied and the existing data are controversial. For example, the frequency of *S. mansoni* infection in HTLV-1 individuals from a blood bank was higher than in HTLV-1-negative individuals from the same blood bank. However, interestingly, the frequency of HAM/TSP was lower in *S. mansoni*-infected individuals than in noninfected ones [16].

We showed that the antigens we used were able to induce in vitro IL-10 production, which is in agreement with other studies showing that Sm29 and PIII induce high IL-10 production via the PBMC of individuals infected with *S. mansoni* or even in noninfected individuals [17].

It has been proposed that IL-10 plays an important role in the maintenance of the asymptomatic carrier status in HTLV-1-infected subjects, counterbalancing the proinflammatory cytokine IFN- γ [21]. Recently, the inability of the recombinant human IL-10 in downmodulating IFN- γ production in vitro in HAM/TSP individuals was described [22]. However, the low ratio of IFN- γ /IL-10 induced by some *Schistosoma* spp. antigens in our study support the hypothesis that these antigens could be beneficial in preventing pathology in HTLV-1-infected individuals. Nevertheless, we cannot rule out the possibility of other existing molecules induced by *Schistosoma* spp. antigens being involved in the downmodulation of the Th1-immune response in HTLV-1 infection. Indeed, in a model of ovalbumin-induced asthma, *S. mansoni* antigens induce an expansion of CD4+CD25+Foxp3+ T cells [23, 19].

Taken together, our results show that the *Schistosoma* spp. antigens used in this study are able to downmodulate the in vitro exacerbated type 1 immune response in a high percentage of HTLV-1-infected individuals. The downmodulation is generally followed by an increase in IL-10 production. These findings may contribute to the development of new strategies to prevent the inflammatory process induced by HTLV-1 infection and the progression to severe forms of the disease, such as HAM/TSP.

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