MAJOR ARTICLE

Helminthic Infection Down-Regulates Type 1 Immune Responses in Human T Cell Lymphotropic Virus Type 1 (HTLV-1) Carriers and Is More Prevalent in HTLV-1 Carriers than in Patients with HTLV-1–Associated Myelopathy/Tropical Spastic Paraparesis

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Human T cell lymphotropic virus type 1 (HTLV-1) infection is associated with an exacerbated type 1 immune response and secretion of high levels of proinflammatory cytokines. In contrast, helminthic infection induces a type 2 immune response. In the present study, the cytokine profile in HTLV-1 carriers coinfected with helminths (*Strongyloides stercoralis* and/or *Schistosoma mansoni*) was compared with that in HTLV-1 carriers not coinfected with helminths. Levels of interferon (IFN)–γ were higher in HTLV-1 carriers not coinfected with helminths than in HTLV-1 carriers coinfected with helminths ($P < .05$). The overall frequency of IFN-γ–expressing $CD8^+$ and $CD4^+$ cells was decreased in HTLV-1 carriers coinfected with helminths ($P < .05$). The percentage of interleukin (IL)–5– and IL-10–expressing T cells in HTLV-1 carriers coinfected with helminths was higher than that in HTLV-1 carriers not coinfected with helminths ($P < .05$). Moreover, we found that the prevalence of helminthic infection was 7-fold higher in HTLV-1 carriers than in patients with HTLV-1–associated myelopathy/tropical spastic paraparesis ($P < .05$). These data show that helminthic infection decreases activation of type 1 cells, which may influence the clinical outcome of HTLV-1 infection.

Human T cell lymphotropic virus type 1 (HTLV-1) infection is associated with spontaneous activation of T cells, uncontrolled proliferation of lymphocytes, and an exacerbated type 1 immune response including secretion of high levels of proinflammatory cytokines [1–3]. The great majority of individuals infected with HTLV-1 display an asymptomatic form of the infection and are referred to as "HTLV-1 carriers." HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T cell leukemia/lymphoma (ATLL) are the main clinical manifestations associated with HTLV-1 infection. HAM/TSP is characterized by hyperreflexia, muscle weakness, and spasticity in the lower extremities. Evidence that the immunological response participates in the pathogenesis of HAM/TSP includes the following: (1) cytotoxic activity against viral Tax protein is present in patients with HAM/TSP [4, 5]; (2) an increase in proinflammatory cytokines—such as tumor necrosis factor (TNF)–α, interleukin (IL)–1, and IL-6—has been observed in the cerebral spinal fluid (CSF)

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of patients with HAM/TSP [6–8]; and (3) spinal cord lesions are associated with infiltration of $CD4^+$ and $CD8^+$ T cells, presence of macrophages, proliferation of astrocytes, and fibrillary gliosis [9]. Although proinflammatory cytokines are more prominent in patients with HAM/TSP, HTLV-1 carriers also have high production of proinflammatory cytokines such as interferon (IFN)–γ and TNF-α [2].

In contrast to HTLV-1 infection, helminthic infection is associated with a type 2 immune response and with high levels of IL-4, IL-5, and IL-10 and low levels of IFN-γ [10, 11]. It has been shown that, as a regulatory mechanism of the immune response, cytokines secreted by type 2 cells may down-regulate type 1 immune responses, and vice versa. For instance, IL-4 and IL-10 may down-regulate the IFN-γ response [12], and IFN-γ decreases the secretion of type 2 cytokines [13]. We and others have previously shown a high frequency of strongyloidiasis [14–17] and an increased susceptibility to develop disseminated *Strongyloides stercoralis* infection in HTLV-1 carriers [18–20]. It is also well known that helminthic infection—in particular, schistosomiasis—down-regulates type 1 immune responses and decreases the severity of autoimmune disease in experimental animals [21, 22]. To evaluate whether helminthic infection influences the immunological response in patients infected with HTLV-1, the cytokine profile and HTLV-1 proviral DNA load were determined in both HTLV-1 carriers coinfected with helminths (*S. stercoralis* and/or *Schistosoma mansoni)* and HTLV-1 carriers not coinfected with helminths. Additionally, the prevalence of helminthic infection in patients with HAM/TSP and that in HTLV-1 carriers were compared.

PATIENTS, MATERIALS, AND METHODS

Patients. The present study included 310 HTLV-1 carriers and 32 patients with HAM/TSP from the HTLV-1 multidisciplinary clinic located at Hospital Universitário Prof. Edgard Santos in Salvador, Bahia, Brazil. A clinical history was obtained from and a physical examination was performed on all patients. For all patients, HTLV-1 infection was confirmed by Western-blot analysis, and 3 examinations of stool specimens were performed (Hoffman and Baermann techniques). Twenty-five percent of the patients infected with *S. stercoralis* had complained of diarrhea. Patients with schistosomiasis were asymptomatic and had <25 eggs/g of stool. Immunological evaluation was performed for 35 HTLV-1 carriers coinfected with helminths (*S. stercoralis* and/or *S. mansoni*) and for a control group of 35 HTLV-1 carriers matched by age and sex but without evidence of helminthic infection. Immunological studies were also performed for 18 patients with HAM/TSP, including the 1 with HAM/TSP and helminthic infection. Immunological evaluation consisted of measurement of cytokines (IFN-γ and IL-5) in supernatants of unstimulated peripheral blood mononuclear cell (PBMC) cultures by ELISA and measurement of intracellular cytokines (IFN-γ, IL-10, and IL-5) and phenotypic immunological markers by flow-cytometric analysis. Moreover, HTLV-1 proviral DNA load was determined. The mean $\pm$ SD ages of HTLV-1 carriers coinfected with helminths and of HTLV-1 carriers not coinfected with helminths were 45 ± 17 and 46 ± 12 years, respectively, and the male:female ratios were 6:1 and 5:1, respectively. This was the naturally occurring bias found in the sample population. The criterion for a diagnosis of strongyloidiasis or schistosomiasis was a positive identification of either *S. stercoralis* larvae (Baermann technique) or *S. mansoni* eggs (Hoffman technique) in a stool specimen. By the examination of stool specimens, 13 patients were found to have *S. stercoralis* infection alone, 15 patients were found to have *S. mansoni* infection alone, and 7 patients were found to have both *S. stercoralis* and *S. mansoni* infection. After collection of blood, all patients infected with *S. stercoralis* were treated with cambendazol (5 mg/kg of weight), and those infected with *S. mansoni* were treated with praziquantel (50 mg/kg of weight, divided into 2 doses) or oxaminiquine (20 mg/kg of weight, in a single dose).

For evaluation of the prevalence of helminthic infection, 342 patients attending the HTLV-1 clinic were included in the present study. These patients had been evaluated by 2 neurologists and were divided into 2 groups according to the Osames' Motor Disability Score [23] and the Expanded Disability Status Scale [24]: (1) patients with HAM/TSP and (2) HTLV-1 carriers who did not fulfill the World Health Organization criteria for HAM/TSP. Informed consent was obtained from all participants, and the human-experimentation guidelines of the Hospital Universitário Prof. Edgard Santos were followed in the conduct of this clinical research.

Immunological Studies

Determination of levels of cytokines. Levels of cytokines (IFN-γ and IL-5) in supernatants of unstimulated PBMCs were measured by ELISA. Briefly, PBMCs were obtained by density-gradient centrifugation by use of lymphocyte separation media (Organon Teknika). After being washed in saline, the cells were adjusted to 3×10^6 cells/mL in RPMI 1640 medium (Gibco) supplemented with 10% AB^+ serum containing 100 U of penicillin/g and 10 μg/mL streptomycin. All cultures were incubated without stimulus, for 72 h at 37°C in 5% CO_2. Supernatants were collected and stored at −20°C. Levels of IFN-γ (Genzyme) and IL-5 (PharMingen) were measured by the ELISA sandwich technique, and the results were expressed in picograms per milliliter on the basis of a standard curve generated by use of recombinant cytokines.

Single-cell cytoplasmic cytokine staining. Briefly, 2×10^5 PBMCs were cultured in RPMI 1640 medium supplemented with 5% AB Rh^+ serum, in 96-well plates. On the basis of preliminary results, all the cytokine staining was performed

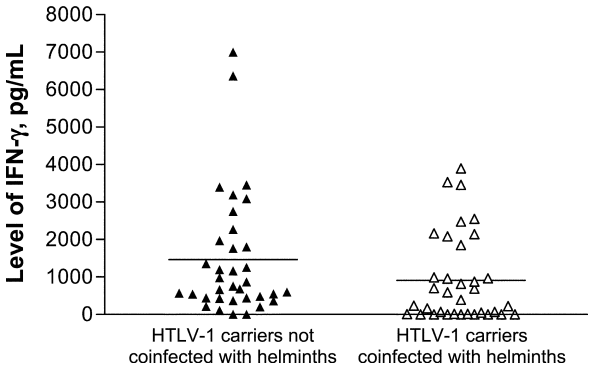

Figure 1. Levels of interferon (IFN)–γ in human T cell lymphotropic virus type 1 (HTLV-1) carriers coinfected or not coinfected with helminths *(Strongyloides stercoralis* and/or *Schistosoma mansoni).* Levels of IFN-γ were determined in unstimulated 72-h culture supernatants of peripheral blood mononuclear cells. The horizontal lines represent the means of the populations.

after 20 h of incubation with or without αCD3/CD28 stimulus. During the last 4 h of incubation, brefeldin A (1 μg/mL) was added to the culture. The cells were then washed and centrifuged by use of ice-cold PBS plus sodium-azide, were stained for surface markers, and were fixed by use of 2% formaldehyde. The fixed cells were then permeabilized with a solution of saponin and stained for 30 min at 4°C by use of anticytokine monoclonal antibodies directly conjugated with phosphatidylethanolamine (IFN-γ, IL-5, and IL-10) (Pharmingen). Preparations were then washed, fixed, and analyzed by use of a FACSCalibur flow cytometer (Becton Dickinson). In all cases, the cells were double-stained for cytokines and for cell-surface markers. In all cases, because of the low frequency of positive events being analyzed, 30,000 gated events were acquired for later analysis.

Proviral Load

Patients and cells. The sample population consisted of 12 HTLV-1 carriers not coinfected with helminths and 17 HTLV-1 carriers coinfected with helminths from whom frozen PBMCs were available; determination of HTLV-1 proviral DNA load was performed only in a subgroup of patients, because of the limited number of available samples.

Real-time polymerase chain reaction analysis of DNA. The HTLV-1 proviral DNA load in PBMCs was measured by use of an ABI PRISM 7700 Sequence Detector (Applied Biosystems), as described elsewhere [25]. DNA was extracted from 10^6 cells by use of a Puregene DNA Isolation Kit (Gentra), in accordance with the manufacturer's instructions, and 100 ng of sample DNA solution/well was analyzed. The HTLV-1 proviral DNA load was calculated by use of the following formula: copy number of HTLV-I (*pX*) per 100 cells = (copy number of *pX*)/(copy number of β-actin/2) × 100.

Serological Testing for HTLV-1

All serum samples were screened for HTLV-1 and HTLV-2 antibodies by ELISA (Cambridge Biotech). Repeatedly reactive samples were subjected to Western-blot analysis, to distinguish between HTLV-1 and HTLV-2, by use of HTLV blot 2.4 (Genelabs), in accordance with the manufacturer's instructions.

Statistical Analysis

The Wilcoxon rank sum test was used to compare means. Fisher's exact test was used to compare proportions. The χ^2 test was used to compare the prevalence of helminthic infection.

RESULTS

The levels of IFN-γ in supernatants of lymphocyte cultures from HTLV-1 carriers coinfected with helminths and those not coinfected with helminths are shown in figure 1. The levels (mean ± SD) of IFN-γ were higher in 35 HTLV-1 carriers not coinfected with helminths (1465 ± 1648 pg/mL) than in 35 HTLV-1 carriers coinfected with helminths (913 ± 1163 pg/mL) ($P < .05$). Both *S. mansoni* and *S. stercoralis* infections contribute to the decreasing levels of IFN-γ, but the down-regulation of IFN-γ was mainly observed in HTLV-1 carriers coinfected with *S. mansoni* (474 ± 838 pg/mL). Although the levels (mean ± SD) of IL-5 did not differ between the 2 groups, there was a tendency for higher levels of IL-5 in HTLV-1 carriers coinfected with helminths (199 ± 476 pg/mL), compared with those in HTLV-1 carriers not coinfected with helminths (132 ± 258 pg/mL) ($P > .05$) (data not shown). As we have reported elsewhere [2], levels of IFN-γ in HTLV-1 carriers were quite variable, and patients could be divided into low-level (<400 pg/mL; range, 0–370 pg/mL) and high-level (>400 pg/mL; range, 430–6995 pg/mL) producers of IFN-γ. The frequencies of low-level producers of IFN-γ were 52% in the HTLV-1 carriers not coinfected with helminths and 80% in the HTLV-1 carriers coinfected with helminths ($P < .05$) (table 1). The mean production of IFN-γ in patients with HAM/TSP was 4246 ± 2924 pg/mL. Because only 1 patient with HAM/TSP was in-

Table 1. Frequency of high-level and low-level producers of interferon-γ among human T cell lymphotropic virus type 1 (HTLV-1) carriers coinfected or not coinfected with helminths *(Strongyloides stercoralis* and/or *Schistosoma mansoni).*

	Infection, no. (%) of patients	
Level	HTLV-1 alone (n = 35)	HTLV-1 plus helminths (n = 35)
High (>400 pg/mL)	17 (48)	7 (20)
Low (<400 pg/mL)	18 (52)	28 (80)[a]

[a] $P < .05$, Fisher's exact test.

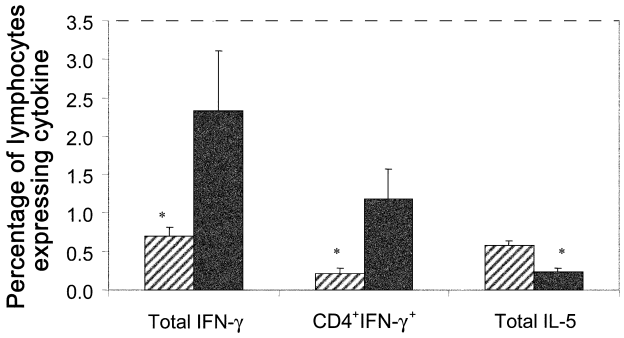

Figure 2. Lower frequency of CD4+ interferon (IFN)–γ^+ cells and higher frequency of cells secreting interleukin (IL)–5 in human T cell lymphotropic virus type 1 (HTLV-1) carriers coinfected with helminths *(striped bars)* than in HTLV-1 carriers not coinfected with helminths *(black bars)*. The columns and error bars represent the mean ± SD, respectively. $n = 3$ patients/group. $^*P < .05$

fected with helminths, no comparison could be performed between patients with HAM/TSP infected with helminths and patients with HAM/TSP not infected with helminths.

The frequencies of cytokine-producing cells in HTLV-1 carriers not coinfected with helminths and HTLV-1 carriers coinfected with helminths, after stimulation with αCD3/CD28, were determined by fluorescence-activated cell sorter analysis. Figure 2 shows that, although the frequency of total cells secreting IFN-γ was 2.33% in 3 HTLV-1 carriers not coinfected with helminths, in 3 HTLV-1 carriers coinfected with helminths, only 0.70% of cells secreted IFN-γ ($P < .05$). In contrast, the frequency of cells secreting IL-5 was 2-fold higher in HTLV-1 carriers coinfected with helminths (0.58%) than in HTLV-1 carriers not coinfected with helminths (0.24%) ($P < .05$). Most of the IFN-γ was secreted by CD4+ T cells, and the frequency of IFN-γ–expressing CD4+ T cells (1.18%) was higher in HTLV-1 carriers not coinfected with helminths than in HTLV-1 carriers coinfected with helminths (0.22%) ($P < .05$) (figure 2).

We have previously shown that most of the IFN-γ–producing cells in HTLV-1 carriers were CD4+ T cells, although both CD4+ and CD8+ T cells are responsible for the high levels of IFN-γ observed in HTLV-1 carriers [26]. Figure 3 shows the frequency of CD8+ T cells secreting IFN-γ or IL-10 and the frequency of total cells secreting IL-10 in unstimulated cultures. Coinfection with HTLV-1 and helminths significantly decreases the frequency of CD8+ T cells secreting IFN-γ ($P < .05$). In contrast, the frequency of total cells secreting IL-10 and the frequency of CD8+ T cells secreting IL-10 were higher in 4 HTLV-1 carriers coinfected with helminths (0.58%) than in 7 HTLV-1 carriers not coinfected with helminths (0.21%) ($P < .05$). Moreover, the frequency of total CD8+ T cells was higher in HTLV-1 carriers coinfected with helminths (data not shown).

Figure 4 shows the HTLV-1 proviral DNA load in a subset of patients from the 2 groups of patients from whom frozen PBMCs were available. Although the number of copies were quite variable in both groups, the HTLV-1 proviral DNA load was significantly lower in the 17 HTLV-1 carriers coinfected with helminths (2.2 ± 1.5 copies/100 cells) than in the 12 HTLV-1 carriers not coinfected with helminths (3.7 ± 1.2 copies/100 cells) ($P < .05$).

The frequency of infection with the intestinal helminths *S. stercoralis* and *S. mansoni* is higher in patients infected with HTLV-1 than in seronegative individuals [27]. By comparing the prevalence of these helminths in HTLV-1 carriers with that in patients with HAM/TSP, we found that HTLV-1 carriers had a 7-fold higher prevalence of infection with intestinal helminths than did patients with HAM/TSP (table 2).

DISCUSSION

The present study has shown that helminthic infection decreases both production of IFN-γ and the overall frequency of IFN-γ–expressing CD8+ and CD4+ cells in HTLV-1 carriers. In contrast, the percentage of IL-10–expressing cells in HTLV-1 carriers coinfected with helminths was higher than that in HTLV-1 carriers not coinfected with helminths. Moreover, the prevalence of helminthic infection was significantly lower in patients with HAM/TSP than in HTLV-1 carriers.

Coinfection with HTLV-1 and helminths has clinical and immunological implications. It is known that the prevalence of strongyloidiasis and schistosomiasis is higher in HTLV-1 carriers than in seronegative control subjects [14–17, 27] and that coinfection with HTLV-1 and *S. stercoralis* is associated with dissemination of parasites and development of severe forms of strongyloidiasis [18–20]. We have previously shown that HTLV-1 infection decreases the type 2 immune response in patients with strongyloidiasis and schistosomiasis [27–29].

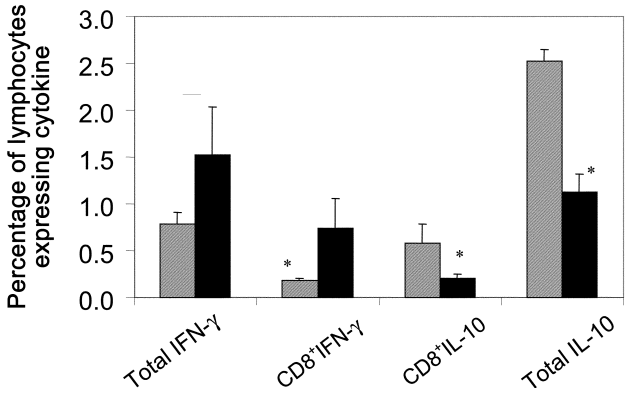

Figure 3. Lower frequency of CD8+ T cells secreting interferon (IFN)–γ and higher frequency of cells secreting interleukin (IL)–10 in human T cell lymphotropic virus type 1 (HTLV-1) carriers coinfected with helminths ($n = 4$) *(gray bars)* than in HTLV-1 carriers not coinfected with helminths ($n = 7$) *(black bars)*. The columns and error bars represent the mean ± SD, respectively. $^*P < .05$.

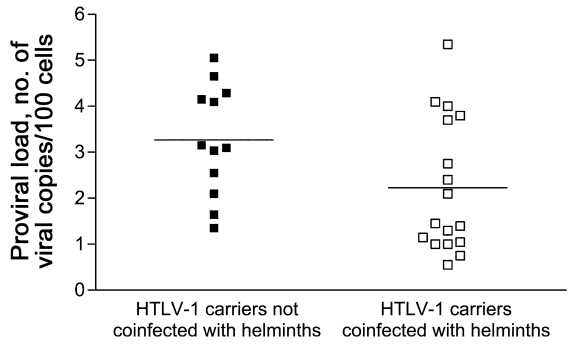

Figure 4. Proviral load in human T cell lymphotropic virus type 1 (HTLV-1) carriers coinfected or not coinfected with helminths (*Strongyloides stercoralis* and/or *Schistosoma mansoni).* The horizontal lines represent the means of the populations.

Here, we have shown that helminthic infection can down-regulate the exaggerated inflammatory response observed in HTLV-1 carriers. Additionally, coinfection with HTLV-1 and helminths was associated with a decreased HTLV-1 proviral DNA load and a decreased frequency of myelopathy.

HTLV-1 infects predominantly T cells, leading to spontaneous proliferation of lymphocytes and increased secretion of cytokines. Although levels of both type 1 and type 2 cytokines are increased in unstimulated lymphocyte cultures from HTLV-1 carriers, compared with those from control subjects, the most striking finding in this regard is the high levels of IFN-γ secreted by both CD4 and $CD8^+$ T cells [26]. Because helminthic infection is associated with increasing levels of IL-4, IL-5, and IL-10 [10–11], the immunological consequences of the association between HTLV-1 infection and helminthic infection was evaluated. That levels of IFN-γ and numbers of $CD4^+$ and $CD8^+$ T cells were decreased in HTLV-1 carriers coinfected with helminths indicates that helminthic infection may down-regulate production of IFN-γ in HTLV-1 carriers.

We have previously shown that exogenous IL-10 can decrease production of IFN-γ in lymphocyte cultures from HTLV-1 carriers [26]. That HTLV-1 carriers coinfected with helminths have an increased frequency of cells secreting IL-10, compared with HTLV-1 carriers not coinfected with helminths, indicates that, in HTLV-1 infection, helminths may down-regulate production of IFN-γ through the induction of IL-10.

Although little is known about defense mechanisms against HTLV-1, killing of infected T cells by $CD8^+$ T cells participates in this phenomenon [5]. Given that helminthic infection down-regulates the type 1 immune response, it is plausible that helminthic infection increases the HTLV-1 proviral DNA load. In fact, a previous study [30] showed that coinfection with *S. stercoralis* increases the HTLV-1 proviral DNA load. Here, we have shown that the proviral load in HTLV-1 carriers coinfected with helminths is lower than that in HTLV-1 carriers not coinfected with helminths, suggesting that helminths may inhibit HTLV-1 transcription. Since the spread of the virus is accelerated by activation of T cells [31], it is possible that the low proviral load in HTLV-1 carriers coinfected with helminths may be due to down-regulation of the immune system. Interestingly, a study of patients with T cell non-Hodgkin lymphoma and patients with ATLL showed that there was a better response to treatment and longer survival in HTLV-1 carriers coinfected with *S. stercoralis* than in HTLV-1 carriers not coinfected with *S. stercoralis* [32].

HAM/TSP is one of the most important consequences of HTLV-1 infection and is characterized by weakness, hyperreflexia, urinary manifestations, and spastic paraparesis. Several studies have emphasized the role of the immune response in the pathogenesis of HAM/TSP, with the following observations: (1) infiltration of the spinal cord by T cells with an increasing number of $CD8^+$ T cells expressing *tax* [9]; (2) increasing levels of proinflammatory cytokines in lymphocyte cultures and CSF [6–8]; and (3) occurrence of fibrosis of the neurological tissue associated with inflammation [9]. We have previously shown that the frequencies of *S. stercoralis* and *S. mansoni* infections were higher in HTLV-1 carriers than in HTLV-1–seronegative blood donors [17, 27]. In the present study, we found that the frequencies of *S. mansoni* and *S. stercoralis* infections were much lower in patients with HAM/TSP than in HTLV-1 carriers. Although it can be argued that patients with HAM/TSP are potentially less frequently exposed to *S. stercoralis* and *S. mansoni,* because of their physical limitations, the group of HTLV-1 carriers coinfected with helminths reported here had no recent exposure to these helminths. In fact, all of the HTLV-1–infected patients in the present study now live in urban areas, where *S. mansoni* transmission is not documented and contamination of the adult population with *S. stercoralis* is less likely. These observations, together with the data suggesting that HTLV-1 infection increases the failure rate of antihelminthic drugs [33, 34], suggest that most of the HTLV-1 carriers coinfected with *S. stercoralis* and/or *S. mansoni* acquired the helminthic infection during childhood. In such cases, the low frequency of helminthic infection in patients with HAM/TSP may suggest that helminths,

Table 2. Frequency of helminths *(Strongyloides stercoralis* and/or *Schistosoma mansoni)* in human T cell lymphotropic virus type 1 (HTLV-1) carriers and patients with HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP).

Clinical form of HTLV-1 infection	No. of patients with helminths/ no. of patients tested (%)
HTLV-1	71/310 (23)[a]
HAM/TSP	1/32 (3)

[a] $P < .05$, χ^2 test.

by decreasing the production of IFN-γ and the HTLV-1 proviral DNA load, protect HTLV-1 carriers from development of myelopathy. Interestingly, the majority of the studies of coinfection with HTLV-1 and *S. stercoralis* in Japan have been performed in Okinawa, and there are no data in the literature on the prevalence of HAM/TSP in this area of Japan [34].

The present study has clearly demonstrated that HTLV-1 carriers coinfected with helminths display an immune phenotype consistent with suppression of the type 1 response, resulting in decreased proviral load. These findings, together with the finding of a lower prevalence of helminthic infection in the patients with more-severe cases of HAM/TSP, aid in understanding the events that lead to the development of this more severe clinical outcome of HTLV-1 infection. Lastly, they highlight an important interaction, between viral and parasitic pathogens, within the infected host.

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