CD45RO⁺ T Cells and T Cell Activation in the Long-Lasting Immunity after *Leishmania infantum* Infection

João F. Rodrigues-Neto,^{1,2} Gloria R. Monteiro,² Tatjana S. L. Keesen,¹ Henio G. Lacerda,^{2,3} Edgar M. Carvalho,^{4,5} and Selma M. B. Jeronimo^{1,2,4*}

¹Department of Biochemistry, Universidade Federal do Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil; ²Institute of Tropical Medicine of Rio Grande do Norte, Universidade Federal do Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil; ³Department of Infectious Diseases, Universidade Federal do Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil; ⁴National Institute of Science and Technology of Tropical Diseases (INCT-DT/CNPq), Federal University of Bahia, Salvador, Bahia, Brazil; ⁵Immunology Service, Federal University of Bahia, Salvador, Bahia, Brazil

Abstract. Manifestations of *Leishmania infantum* infection range from asymptomatic to symptomatic visceral leishmaniasis (VL). People with symptomatic VL (sVL) have suppressed immune responses against *Leishmania* antigens that are reversed after clinical cure. The intradermal leishmanin skin test (LST) is negative during sVL, but it becomes positive after treatment. The aim of this study was to compare T cell responses in individuals with sVL, recovered VL (RecVL), and endemic controls. Endemic controls were household contacts of a VL case and they were grouped by their LST results, either positive (LST+) or negative (LST−). Mononuclear cells were studied ex vivo or after stimulation with soluble *Leishmania* antigens (SLA); cell surface markers and cytokines were determined. T cells, ex vivo, from individuals with sVL and from LST+ individuals presented a higher activation for CD4⁺ and CD8⁺ cells expressing CD69. However, lymphocytes from sVL stimulated with SLA had lower percentages of CD4⁺ and CD8⁺ cells expressing CD69 and CD8⁺ cells expressing CD25, with no release of interferon- γ or tumor necrosis factor. sVL subjects had lower percentage of memory cells (CD4⁺ CD45RO⁺), ex vivo, without SLA stimulation than RecVL, LST+, or LST− ($P = 0.0022$). However, individuals with sVL had fewer regulatory cells after SLA stimulation (CD4⁺ CD25^{HIGH}, $P = 0.04$ and CD4⁺ FOXP3⁺, $P = 0.02$) than RecVL. The decrease in specific memory and activated CD4⁺ and CD8⁺ cells, as in response to *Leishmania* antigens, could explain, in part, the immune impairment during sVL. Finally, protective T cell responses are long lasting because both RecVL or LST+ individuals maintain a specific protective response to *Leishmania* years after the primary infection.

INTRODUCTION

Visceral leishmaniasis (VL) in Brazil, Europe, and northern Africa is caused by *Leishmania infantum* infections.^{1,2} The natural course of *L. infantum* infection depends on the host immune responses and environmental factors,^{3,4} and ranges from asymptomatic to symptomatic VL (sVL).^{5–8} VL can be fatal, even with treatment, in 5% to 10% of the cases.⁵ However, most of the people infected with *L. infantum* have self-resolution of the infection without presenting clinical symptoms,⁹ and usually they can be identified by a positive response to *Leishmania* antigens, in vitro, or by a positive leishmanin skin test (LST+). Both recovered VL (RecVL) individuals and people with self-resolving *Leishmania* infections tend to present long-term protection against disease development, if there is no immunosuppression.^{10,11}

Factors involved in susceptibility or resistance to *Leishmania* infection are due, in part, to the balance between pathogenic and protective immune responses.^{12,13} The latter depends on the genetic background of the host, strain of infecting *Leishmania*, sand fly factors, and comorbidity.^{14–16} sVL is characterized by impaired Th1 responses, whereas resistance to developing disease is characterized by activation of CD4⁺ T cells to a Th1 phenotype. However, the decreased ability of peripheral blood mononuclear cells (PBMCs) to proliferate and produce interferon (IFN)- γ upon *Leishmania* antigen stimulation^{17–19} contrasts with the detection of IFN- γ in sera of VL patients^{20–22} or its release and detection in whole blood assays.²³

Memory T cells stimulated by specific antigens aid the differentiation of T cells to effector T cells. Their responses via cytokines or chemokines enable CD4⁺ and CD8⁺ T cells to migrate to the site of infection and to secrete proinflammatory cytokines such as tumor necrosis factor (TNF) and IFN- γ .^{24,25} Heterogeneity in CD4⁺ T cells influences immune responses to *Leishmania* infection.^{26–28} Regulatory T (Treg) cells are capable of recognizing self- and non-self-antigens; they can downregulate both Th1 and Th2 immune responses,^{29,30} and they play a role in both experimental and human VL.^{29,31} The molecular mechanisms by which Treg cells suppress effector T cells are under investigation, but it is believed that Treg cells suppress the effector T cells by releasing suppressive cytokines (interleukin [IL]-10, transforming growth factor- β) or in a contact-dependent manner or both. Recently, it was observed that CD4⁺ T cells suppress T cell activation at the pathologic site of infection in human VL due to *Leishmania donovani*.³⁰ However, in another study, no increase in Foxp3 + Treg was observed in patients with sVL due to *L. donovani* infection.²⁹ In addition, Th17 cells seem to promote a proinflammatory environment by the release of cytokines and chemokines, which are key components in activating and attracting neutrophils and other cells to sites of inflammation.^{32,33}

The aim of this study was to assess activation in memory and Treg cells during sVL and after successful clinical recovery (RecVL), and in controls from the VL endemic area that present signs of *Leishmania* infection (LST+) or not (LST−). The overall goal was to assess whether the presence of long-term memory may explain the long-term immunity in RecVL individuals or among individuals who are LST+.

*Address correspondence to Selma M. B. Jeronimo, Institute of Tropical Medicine of Rio Grande do Norte, UFRN, Natal, RN 59078-970, Brazil. E-mail: smbj@cb.ufrn.br

METHODS

Study population. A total of 55 people were recruited from a cohort residing in an endemic area for VL in the state of Rio Grande Norte, northeast Brazil, as previously described.^{34,35} Table 1 shows the characteristics of the studied subjects. Majority of the population were adults (mean age 29.3 years). Patients with sVL had symptoms of disease plus parasitological confirmation (presence of *Leishmania* in the bone marrow) and/or positive anti-*Leishmania* antibodies.³⁶ VL cases were under treatment (sVL) or post-treatment (RecVL), whereas the controls had no history of VL or symptoms suggestive of VL. RecVL were individuals within 1 year or more than 10 years post-treatment. All VL subjects were treated with antimony and had no relapse of disease. The control subjects were household contacts of VL cases.

Blood was collected from all subjects and the LST was used as a measure of the delayed-type hypersensitivity response. Controls were examined for *Leishmania* infection (anti-*Leishmania* antibodies, by polymerase chain reaction and LST), and they were grouped in accordance to their LST, either positive (LST+) or negative (LST-). None of the controls had a history of VL. The clinical evaluations were performed on at least two occasions, with an interval of at least 1 year between assessments, to avoid boosting. In this way, the controls were grouped as either those who remained with a positive LST response over time (LST+/LST+), or those who were LST+ but lost this response over time (LST+/LST-) or remained LST negative (endemic control), (LST-/LST-). All study participants remained residing in the VL endemic area.

Assessment of *Leishmania* infection. Anti-*Leishmania* antibodies were determined by enzyme-linked immunosorbent assay using soluble *Leishmania* antigen (SLA) lysate from a local *Leishmania* isolate (IOC 3071) and rK39 (Infectious Disease Research Laboratory, Seattle, WA), as previously reported.³⁶ We assessed the delayed-type hypersensitivity responses using LST, which was performed by injecting 0.1 mL (25 µg) of the antigens at 3 cm from the cubital fold on the forearm of the study participants. The responses were read between 48 and 72 hours after injection of the antigens, by the ballpoint pen technique.^{37,38} An induration greater than 5 mm was considered positive. The antigens were kindly donated by Centro de Pesquisa e Produção de Imunobiológicos (Curitiba, Paraná, Brazil).

Isolation of cells, culture, and typing. The PBMCs were isolated by using a Ficoll-HyPaque gradient (GE Health Care, Freiburg, Germany). Cells were suspended in RPMI 1640

supplemented with glutamine and were incubated at a concentration of 10^7 cells/mL for 18 hours at 37°C, in 5% CO₂ atmosphere, either with or without stimulation by SLA at a concentration of 10 µg/mL. SLA was prepared from promastigotes grown in culture derived from a local *Leishmania* isolate that was typed by a reference laboratory (IOC 3071). The cells were stained ex vivo for CD25, CD69, CD45RO, CD4, and CD8 and after SLA stimulation for CD25, CD69, FOXP3, CD4, and CD8 markers. CD4 and CD8 memory cells were identified by the presence of the CD45RO marker, whereas Treg cells were identified by the presence of FOXP3 or CD25^{HIGH}. The cells were also stained with annexin/propidium iodide (PI) and programmed cell death 1 (PD-). For each sample, 30,000 events were analyzed using the FlowJo software (version 7.6.4; Treestar, Ashland, Oregon). Data acquisition was obtained by using FACSCanto II (BD Biosciences, San Jose, CA). Antibodies used in the experiments were acquired from eBiosciences or BD Pharmingen (San Diego, CA).

Cytokine analysis. Levels of cytokines in cell culture supernatants were determined by using a cytometric bead array (CBA; IL-17, IL-4, IL-2, IL-10, IL-6, TNF, and IFN-γ, Becton Dickinson [BD], San Jose, CA), following the instructions provided by the manufacturer. The supernatants were evaluated after 18 hours at 37°C, in 5% CO₂ atmosphere, either with or without stimulation by SLA at a concentration of 10 µg/mL. SLA was prepared from a local *L. infantum* isolate (IOC 3071). Cytokine concentrations were calculated based on fluorescence intensity measurements with the use of software designed for the CBA analysis (FCAP Array™ v3.0 Software; BD Biosciences).

Statistical analysis. Kruskal-Wallis or Wilcoxon tests were used for comparison between groups (sVL, RecVL, and controls) using data obtained from samples ex vivo or subjected to SLA stimulation. Analyses were performed using Graph-Pad Prism (Graph Pad Software, San Diego, CA) and a *P* value < 0.05 was considered significant.

Ethical considerations. This protocol and informed consent were reviewed and approved by the Federal University Ethical Committee. The certificate of ethical approval is CAAE 12584513.1.0000.5537. All participants or their legal guardians signed the informed consent.

RESULTS

T cell activation markers in *L. infantum* infection. Non-stimulated PBMCs from sVL and LST+/LST+, ex vivo, had higher expression of CD69 on CD4⁺ and CD8⁺ T cells when compared with RecVL < 1 year post-treatment, or

TABLE 1
Clinical and demographic data of the study groups

Parameters	Phenotypic groups					
	VL			Asymptomatic <i>Leishmania</i> infection		Potentially exposed
	sVL	RecVL* < 1 year	RecVL* > 10 years	LST+/LST+	LST+/LST-	LST-/LST-
<i>n</i>	11	11	9	9	6	9
Age (years)	32 ± 14.2	27 ± 14.9	17 ± 12.1	50 ± 17.6	33 ± 18.2	17 ± 3.5
Sex % (M/F)	66/34	80/20	44/56	55/45	16/84	29/71
Leukocytes† (mm ³)	2,525 ± 660.1	6,120 ± 286.3	7,814 ± 2,830.8	6,500 ± 1,831.6	9,766 ± 2,313.5	7,883 ± 2,885.6
Platelets (mm ³)	138,400 ± 79,682.5	261,000 ± 62,382.7	355,428 ± 74,631.8	277,000 ± 34,100.7	300,833 ± 64,436.2	340,333 ± 74,783.6
Treatment (days)	3 ± 2.6	193 ± 97.8	4,457 ± 728.1	N/A	N/A	N/A

N/A = not applicable; RecVL = recovered VL; sVL = symptomatic VL; VL = visceral leishmaniasis.

* RecVLs are not from the same donor.

† Leukocytes in sVL presented a statistical difference when compared with the other groups (*P* < 0.05). Values shown in absolute numbers or as mean ± SD.

FIGURE 1. Increased lymphocyte activation ex vivo in symptomatic VL (sVL) and LST+/LST+. Peripheral blood mononuclear cells from individuals with sVL, recovered VL (RecVL) (< 1 year or > 10 years), and endemic controls (LST+/LST+, LST+/LST–, or LST–/LST–) were stained for CD69 and CD25 in CD4⁺ and CD8⁺ T cells. The graphs represent the percentage of CD69 in CD4⁺ T cells (A), CD69 in CD8⁺ T cells (B), and CD25 in CD8⁺ T cells (C). Medians were compared using Mann–Whitney. A *P* value of < 0.05 was considered significant. (**P* < 0.05, ***P* < 0.01; LST = leishmanin skin test).

RecVL > 10 years or LST+/LST– (Figure 1A and B). CD25 expression was higher in CD8⁺ T cells from sVL subjects, when compared with RecVL subjects < 1 year (Figure 1C). However, T cells from sVL individuals stimulated with SLA had a lower percentage of CD69 or CD25 when compared with RecVL individuals (*P* = 0.008 for CD4⁺ CD69⁺, *P* = 0.0006 for CD8⁺ CD69⁺ and *P* = 0.01 for CD8⁺ CD25⁺), or LST+/+ (Figure 2A–C).

The LST+/LST+ individuals presented a median of 15% of CD69 in CD4 T cells, and the LST+/LST– individuals had a median of 8% of CD69 in CD4 T cells, whereas the LST–/LST– individuals had a median of 4% of CD69 in CD4⁺ T cells (Figure 2A). Similar findings were observed for CD8⁺ T cells expressing CD69, but individuals who were LST+/LST– had the highest percentages of CD8⁺ T cells expressing CD69 (about 15%). However, for CD8⁺ T cells, only RecVL patients < 1 year posttreatment presented a higher percentage of cells expressing CD25 (Figure 2C). RecVL subjects > 10 years posttreatment still responded to SLA stimulation, with increased CD4⁺ and CD8⁺ T cells expressing CD69 (*P* = 0.01 and *P* = 0.03, respectively; Supplemental Fig. 1). The percentage of cells expressing activation markers in the presence or absence of SLA, for all groups, is shown in Supplemental Fig. 1.

A representation of the dot plot graphs of cellular activation profiles in ex vivo condition or after SLA stimulation is shown in Supplemental Fig. 2. There was no increase in annexin/PI cell death in cells from sVL individuals after SLA stimulation, *P* > 0.05 (data not shown). CD4 and CD8 T cells from sVL individuals were stained for PD-1 ex vivo and after SLA stimulation. However, no difference was observed between the groups (*P* > 0.05) (Supplemental Fig. 3).

sVL has reduced levels of memory T cells. The percentage of memory CD4⁺ T cells in PBMCs without SLA stimulation was reduced in sVL subjects compared with that in the other groups (*P* < 0.05). Lymphocytes from sVL ex vivo presented 24% of CD45RO expression in CD4⁺ T cells, whereas RecVL presented 54% CD45RO expression in this population (Figure 3A). There was a difference in the percentage of CD4⁺ T cells expressing CD45RO between sVL and RecVL individuals 1 year post-treatment (*P* = 0.002) and between sVL individuals and the other groups (*P* < 0.05). There was no difference in CD8⁺ CD45RO⁺ in sVL or RecVL individuals (Figure 3B), respectively, 22% and 29%. There was also no difference in CD45RO expression in both CD4 and CD8 T cells between RecVL groups and endemic controls, (*P* > 0.05),

FIGURE 2. T lymphocyte activation after *Leishmania* antigen stimulation. Peripheral blood mononuclear cells from symptomatic VL (sVL), recovered VL (RecVL) (< 1 year or > 10 years) and endemic controls (LST+/LST+, LST+/LST–, or LST–/LST–) were cultured in the presence of soluble *Leishmania* antigens. The graphs represent the percentage of CD69 in CD4⁺ T cells (A), CD69 in CD8⁺ T cells (B), and CD25 in CD8⁺ T cells (C). Medians were compared using Mann–Whitney. A *P* value of < 0.05 was considered significant. (**P* < 0.05, ***P* < 0.01; LST = leishmanin skin test).

FIGURE 3. CD45RO expression in CD4 and CD8 T, in ex vivo condition. Peripheral blood mononuclear cells from symptomatic VL (sVL), recovered VL (RecVL) (< 1 year or > 10 years) and endemic controls (LST+/LST+, LST+/LST-, LST-/LST-) were stained for CD45RO in CD4 and CD8 T cells. The graphs represent the percentage of CD45RO in CD4⁺ T cells (A) and CD45RO in CD8⁺ T cells (B). (** $P < 0.01$; LST = leishmanin skin test).

either LST+/LST+, LST+/LST-, or LST-/LST-. There was no difference in CD45RO expression in either CD4 and CD8 T cells after SLA stimulation (data not shown).

Decreased Treg cells during sVL. The percentage of T cells expressing CD25^{High} and FOXP3 is shown in Figure 4. There was no difference in the percentage of CD4⁺ CD25^{High} between the sVL group (median of 0.6%) and the RecVL < 1 year post-treatment (median of 0.8%), ex vivo, but there was a difference when compared with cells from RecVL > 10 years post-treatment (median of 1.5%, $P = 0.009$) (Figure 4A). Cells from sVL subjects had a median of 0.7% for CD4⁺ CD25^{High} when compared with RecVL < 1 year (1.3%) ($P = 0.04$), or > 10 years (2.8%) after SLA stimulation (Figure 4B). Moreover, the group that was LST+/LST- had a mean of 2.2% of CD4⁺ CD25^{High} (Figure 4B). In addition, the expression of FOXP3 in the CD4⁺ T cells was increased in RecVL but not in sVL (median of 1.8% in sVL and 3.7% in RecVL < 1 year, $P = 0.02$) after SLA stimulation (Figure 4C). CD4⁺ T cells expressing FOXP3 were increased in subjects who were LST+/LST+ (median of 6.0%). The LST+/LST- group had a median of 1.3% of FOXP3 expression in CD4⁺ T cells, which was similar to the sVL group (median of 1.9%) (Figure 4C).

Increased IFN- γ , TNF, and IL-6 release after VL clinical recovery. There was no difference in IL-17, IL-4, IL-2, IL-10, IL-6, TNF, and IFN- γ in the supernatants of cells isolated from individuals with sVL after SLA stimulation ($P > 0.05$) when compared with that in cells stimulated with media, but an increase in IFN- γ , TNF, and IL-6 was observed for RecVL < 1 year ($P = 0.01$) (Figure 5A–C). Likewise, an increase in the ratio of IFN- γ /IL-10 after SLA stimulation in RecVL < 1 year was detected, showing a predominance of IFN- γ production after clinical cure (Figure 5D). There was no production of IL-17, IL-10, IL-4, and IL-2 in cells isolated from individuals with sVL or RecVL < 1 year after SLA stimulation (data not shown).

DISCUSSION

VL is a severe disease that is usually associated with decreased ability of the host to kill *Leishmania*, and there is about 5% to 10% mortality, even with treatment.^{39,40} This disease is the result of an interplay between the host defense and the parasite survival strategies.¹² Studies have demonstrated the importance of cytokines, such as TNF and IFN- γ , and T lymphocytes in *Leishmania* infection.^{41–43} The activation of

FIGURE 4. Profile of Regulatory T (Treg) cells in *Leishmania* infection. Peripheral blood mononuclear cells from symptomatic VL (sVL), recovered VL (RecVL) (< 1 year or > 10 years), and controls (LST+/LST+, LST+/LST- or LST-/LST-) were evaluated ex vivo and cultured in the presence or absence of *Leishmania* antigen. The graphs represent the ex vivo percentage of CD4⁺ CD25^{High} T cells (A), the percentage after antigen stimulation of CD4⁺ CD25^{High} T cells (B), and FOXP3 in CD4⁺ T cells (C). Medians were compared using Mann–Whitney with a P value of < 0.05 considered significant. (* $P < 0.05$, ** $P < 0.01$; LST = leishmanin skin test).

FIGURE 5. Cytokine evaluation in symptomatic VL (sVL) and recovered VL (RecVL) < 1 year after soluble *Leishmania* antigen (SLA) stimulation. Plots show the quantity of interferon (IFN)- γ (A), tumor necrosis factor (B), and interleukin (IL)-6 (C), and the ratio of IFN- γ /IL-10 (D) in sVL and RecVL < 1 year. Data are represented in pg/mL, * $P < 0.05$.

CD4 and CD8 T cells is known to have an important role in controlling infection by intracellular pathogens, by eliciting protective immunity through the proinflammatory cytokines. In this study, we observed a decreased frequency in activated T cells from sVL patients after SLA stimulation, which was accompanied by a lack of IFN- γ and TNF production after specific antigen stimulation and decreased memory T cells. The increase in the ratio of IFN- γ /IL-10 after the clinical cure was in accordance with the restoration of the specific immune response. Moreover, as no increased frequency in Treg cells was found in sVL, the impairment in the immune response might be due to the lack of memory T cells, rather than an increase in Treg cells, as seen in other diseases.^{44,45}

sVL patients are anergic to *Leishmania* antigen stimulation,^{17,46} which is reversed after clinical cure. This can be estimated by a positive LST response, by the proliferation of T lymphocytes, or by the amount of IFN- γ released by PBMCs in response to parasite antigen stimulation. However, mRNA expression for IFN- γ has been documented in T cells,⁴⁷

and IFN- γ is detected in the sera and whole blood cell culture of sVL patients.²¹ In this study, we found that cells from sVL individuals displayed, ex vivo, a greater activation pattern, but T cell activation was absent in response to SLA stimulation. Whereas cytokines such as IFN- γ are found in sera from sVL patients, PBMCs from these patients do not proliferate in vitro and do not produce IFN- γ after SLA stimulation. However, this reduction in activated T cells (in vitro culture) in sVL could be due to cell exhaustion, because of the overstimulation during chronic *Leishmania* infection leading to the inability of T cells to respond to an additional load of antigen stimulation. However, as annexin and PD-1 expression was not enhanced, it is likely that the increase in costimulatory molecules, such as cytotoxic T-lymphocyte-associated protein-4, and regulatory cytokines, such as IL-10, could be involved in promoting a negative regulation. Studies of tegumentary leishmaniasis showed that subjects with diffuse cutaneous leishmaniasis have an anergic response to *Leishmania* antigens similar to VL.⁴⁸ In a murine model of schistosomiasis, decreased T cell

function due to general T cell overstimulation or "T cell exhaustion" was also shown.⁴⁹ In addition, the role of antibodies in the pathogenesis of VL is not entirely understood, but an effective T cell response is usually observed after a decrease in anti-Leishmania antibodies in the blood when there is a development of a positive LST response.⁵⁰

Interestingly, the sVL and LST+/LST+ groups showed an increase in CD4⁺ and CD8⁺ T cell activation markers ex vivo. However, a decrease in cells expressing CD69 in the sVL was observed after SLA stimulation, whereas an increase in activated cells was seen in LST+/LST+. RecVL individuals had an increase in the percent of CD4⁺ and CD8⁺ T cells expressing CD69 and CD8⁺ T cells expressing CD25, indicating long-lasting immunity to Leishmania. These findings may explain the resistance to relapse once a protective T cell response is mounted. Moreover, one potential explanation for the higher activation observed for both sVL and LST+/LST+ groups could be the presence of Leishmania. It could be that individuals who had recovered from VL either within 1 year or more than 10 years ago had successfully responded to the specific Leishmania therapy and might have better controlled parasite replication. Persistence of Leishmania parasites has been shown to occur in leishmaniasis.⁵¹ The sVL group had reduced levels of circulating memory T cells, similar to those observed for sVL because of *L. donovani*.⁵² This could reflect the migration to and retention of cells in infected organs, such as bone marrow.⁵³ The level of CD45RO ex vivo has been used to predict the intensity of T cell response in cutaneous leishmaniasis.⁵⁴ In our study, RecVL individuals, either < 1 year or > 10 years, had an increase in the percent of cells expressing activation markers after antigen stimulation and an increase in memory cells (CD45RO+) in ex vivo conditions when compared with individuals with sVL. Although peripheral memory cells were low in sVL patients, we cannot exclude the possibility that these individuals may have memory cells in tissues (central memory cells) and that, after cure, memory cells are found in the periphery. In addition, expression of memory cells is time dependent, and cells were only stimulated for 18 hours. In an experimental model of Leishmania major infection, two types of CD4⁺ memory T cells were shown, one that was dependent on the presence of Leishmania antigen and a second that was parasite independent and long lasting.⁵⁵ All subjects studied herein continued to live in the endemic area and potentially could have been re-exposed to Leishmania or could have failed to clear the initial infection entirely. Development of sVL years after people have moved from the endemic area supports the likelihood that there is persistence of *L. infantum*.¹⁰ In that report, the sVL development was associated with immunosuppression.

Treg cells, which constitutively express the alpha subunit receptor IL-2 (CD25), have a central and prominent role in maintaining immune balance.^{23,56,57} We found that Treg cells during sVL were decreased, but increased levels were observed after recovery. Both CD4⁺ CD25^{high} and CD4⁺ FOXP3⁺ were detected in low percentages in sVL patients. An increase in the relative amount of Treg cells in VL patients from India was observed, followed by a decline after healing.³⁰ These differences might reflect some antigen variation between *Leishmania* species. Although in an experimental model of leishmaniasis emphasis has been given to the participation of Treg cells in the progression of Leishmania infection,³¹ our

data do not support an increase in the percent of Treg cells in sVL.

Finally, in this study, we documented that memory to Leishmania antigens is long lasting, both in RecVL people and in people with asymptomatic Leishmania infection. Whether long-term memory is due to new infection or due to parasite persistence is not known.⁵⁷ Finally, a decrease in activated T cells after restimulation in vitro with SLA and a reduction in the percentage of memory T cells may explain the impairment of T cell responses to Leishmania antigens and the inability of patients who develop VL to control parasite growth.

Received September 12, 2016. Accepted for publication August 21, 2017.

Published online December 26, 2017.

Note: Supplemental figures appear at www.ajtmh.org.

Acknowledgments: We thank the staff of Hospital Giselda Trigueiro (Secretaria de Saúde do Estado do Rio Grande do Norte) and Hospital Infantil Varella Santiago, in Natal, Brazil, for aiding the recruitment of individuals with symptomatic visceral leishmaniasis; John Donelson, (University of Iowa) for helping the revision of this manuscript and Manoel Gomes for his help with the field studies.

Financial support: This work was supported by the National Institutes of Health (AI-30639). J. F. R-N. received a fellowship from CAPES.

Authors' addresses: João F. Rodrigues-Neto and Tatjana S. L. Keesen, Department of Biochemistry, Universidade Federal do Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil, E-mails: joao_meto@yahoo.com.br and tat.keesen@gmail.com. Gloria R. Monteiro, Instituto de Medicina Tropical do Rio Grande do Norte, Universidade Federal do Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil, E-mail: gloriag74@hotmail.com. Henio G. Lacerda, Department of Infectious Diseases, Universidade Federal do Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil, E-mail: heniolacerda@ufrnet.br. Edgar M. Carvalho, Serviço de Imunologia, Universidade Federal da Bahia, Salvador, Bahia, Brazil, E-mail: edgar@ufba.br. Selma M. B. Jeronimo, Department of Biochemistry, Universidade Federal do Rio do Norte, Natal, Rio Grande do Norte, Brazil and Universidade Federal do Rio Grand do Norte, Natal, Rio Grande do Norte, Brazil, E-mail: smbj@cb.ufm.br.

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