Serum IL-10 Levels and Its Relationship with Parasitemia in Chronic Chagas Disease Patients

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Abstract. It is known that the immunoregulatory networks in human Chagas disease play a key role in parasitemia control during the acute phase. However, little is known regarding the control of parasitemia during the chronic phase. The aim of the study was to describe the serum cytokine profile of *Trypanosoma cruzi* chronically infected patients and to evaluate its relationship with the presence or absence of parasitemia in peripheral blood. This is a prospective observational study where adult Chagas disease patients were included. Patients previously treated for Chagas disease, pregnant women, and immunosuppressed patients were excluded. Demographic and clinical information was collected, and *T. cruzi* real-time polymerase chain reaction (RT-PCR) and serum cytokine profile were determined in peripheral blood. Forty-five patients were included. *Trypanosoma cruzi* RT-PCR in peripheral blood resulted positive in 19 (42.2%) patients. No differences in the serum cytokine profile were found depending on cardiac or digestive involvement. However, patients with positive *T. cruzi* RT-PCR had a higher median concentration of IL-10 and IL-1β and a lower median concentration of IL-8 than those with negative *T. cruzi* PCR. These results reinforce the key role that this anti-inflammatory cytokine (IL-10) plays in parasitemia control.

INTRODUCTION

Chagas disease, the parasitic disease caused by the hemoflagellated protozoan *Trypanosoma cruzi*, affects 6–7 million people worldwide.¹ During the last decades, the disease has spread from endemic areas in Latin America to other non-endemic regions such as the United States, Europe, Australia, and Japan.^{2,3} Although the main route of transmission is through blood-sucking triatomine insects (vectorial route), other routes of transmission include congenital, oral, transfusions, and organ transplantation.⁴

After the acute phase of the infection, which is asymptomatic in most of the cases and lasts in 4–8 weeks, a subsequent asymptomatic indeterminate form takes place during years. After 10–30 years, up to 30–40% of patients will develop the symptomatic chronic phase, with cardiac and/or digestive involvement.⁴ During the acute phase or in reactivations due to immunosuppression, patients have high parasitemia and diagnosis relies either on direct parasitological methods, such as the microhematocrit and the Strout method, or on molecular biology techniques such as the polymerase chain reaction (PCR). In the indeterminate/chronic phase, the parasitemia is low and intermittent, and the diagnosis is based on serological tests; however, PCR-based high-sensitive techniques can detect the parasite in peripheral blood and are presently used to monitor early detection of treatment failure.⁵

The different clinical evolution of Chagas disease patients depends on multiple factors, including both related to the parasite and the host. It is known that the immunoregulatory networks in human Chagas disease play a key role in the visceral involvement.⁶ Some studies have observed that in patients with Chagas disease myocardiopathy, there is a predominant Th1 immune response, with high levels of inflammatory cytokines, such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α.⁷ However, in patients in the indeterminate phase

of Chagas disease, a predominant production of anti-inflammatory cytokines (IL-10) is observed.⁸ In the acute phase, the Th1 immune response is crucial to control the parasitemia.⁹ However, scarce information regarding the control of the parasitemia during the indeterminate or chronic phase of the disease is available.

The aim of the present study was to describe the serum cytokine profile of *T. cruzi* chronically infected patients and to evaluate its relationship with the presence or absence of parasitemia through detection of *T. cruzi* DNA by PCR in peripheral blood.

METHODS

This is a retrospective analysis from a prospective observational study that was performed from March to July 2013 at Vall d'Hebron University Hospital (Barcelona, Spain). The initial study was designed to characterize the cutaneous toxicity of benznidazole in patients with Chagas disease and to define the immunological mechanisms involved.¹⁰ Adult patients (older than 18 years) with diagnosis of Chagas disease were included; those patients previously treated for Chagas disease, pregnant women, and immunosuppressed patients were excluded. For the present study, participants were only included in the analysis when all the relevant information was available: demographic information (gender, age, and country of origin), clinical data (cardiac or gastrointestinal involvement), and *T. cruzi* real-time polymerase chain reaction (RT-PCR), and serum cytokine profile was determined in peripheral blood before starting specific treatment.

The diagnosis of Chagas disease was performed through two positive different serological tests according to the WHO recommendations: an ELISA with recombinant antigen (Bioelisa Chagas; Biokit, Lliçà d'Amunt, Spain) and an ELISA with crude antigen (Ortho *T. cruzi* ELISA; Johnson & Johnson, High Wycombe, United Kingdom).¹¹ Visceral involvement was evaluated through physical examination, 12-lead electrocardiography, chest radiography, and barium enema. For cardiac involvement, patients were stratified following the Kuschner classification.¹² A RT-PCR to detect *T. cruzi* DNA in peripheral blood pretreated with guanidine ethylenediaminetetraacetic acid (EDTA) was

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TABLE 1

Clinical and epidemiological characteristics of patients with Chagas disease included in the study ($n = 45$)

Characteristic	Number of patients ($n = 45$)
Age (years)	36 (22–55)
Gender, female	34 (75.6%)
Country of origin	
Bolivia	43 (95.6%)
Argentina	1 (2.2%)
Honduras	1 (2.2%)
Cardiac involvement	
Kushnir 0	34 (75.6%)
Kushnir 1	8 (17.8%)
Kushnir 2	1 (2.2%)
Kushnir 3	2 (4.4%)
Gastrointestinal involvement	
None	36 (80%)
Dolichocolon	7 (15.6%)
Sigmoid dilatation	2 (4.4%)
Positive <i>Trypanosoma cruzi</i> polymerase chain reaction	19 (42.2%)

Data are reported as number (%) of patients or median (range).

performed in duplicate before starting treatment in all patients according to the method described by Piron et al.¹³

Serum samples were collected and remained frozen at -80°C until assay performance. Concentrations of the following cytokines (IFN- γ , IL-1 β , IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, and IL-17A) were measured in serum using cytometric bead array technology from BD™ Enhanced Sensitivity Flex Set (BD Biosciences, San Jose, CA). Fluorescence was measured using the FACSCanto II and FACSDiva V6.1.2 (BD Biosciences) software. Data were analyzed using FCAP Array V3.0 software based on five parameter logistic curve fits.

Categorical data were presented as absolute numbers and proportions, and continuous variables were expressed as medians and ranges. The χ^2 test was used to compare the distribution of categorical variables, and the Mann–Whitney U -test for continuous variables. Results were considered statistically significant if the two-tailed P -value was < 0.05 . SPSS software for Windows (version 19.0; SPSS Inc, Chicago, IL) was used for statistical analyses.

The strengthening the reporting of observational studies in epidemiology (STROBE) statement guidelines were used to improve the quality of the study. The study protocol was approved by the Ethical Review Board of the Vall d'Hebron University Hospital (Barcelona, Spain), and written informed consent was obtained from all patients. Procedures were performed in accordance with the ethical standards laid down in the Declaration of Helsinki as revised in 2013.

RESULTS

From the 54 patients of the initial study, 45 patients had enough information to be included in this retrospective analysis; nine patients were excluded because blood sample for serum cytokine analysis was not available. The median age was 36 (22–55) years, and 34 (75.6%) were female (see Table 1). Most of them were born in Bolivia (43 patients, 95.6%). Regarding visceral manifestations, nine (20%) patients had cardiac involvement, seven (15.5%) had digestive involvement, and two (4.4%) patients had both cardiac and digestive involvement. Cardiac involvement included eight patients in the stage 1 (four patients with right bundle branch block, two patients with anterior hemiblock, and one patient with posterior hemiblock), one patient in the stage 2, and two patients in the stage 3 of the Kushnir classification. Gastrointestinal involvement was observed in seven patients with dolichocolon and two patients with colonic dilatation (sigmoid diameter > 6 cm). *Trypanosoma cruzi* RT-PCR in peripheral blood resulted positive in 19 (42.2%) patients: 11 patients in the indeterminate phase of the disease, four patients with cardiac involvement, three patients with gastrointestinal involvement, and one patient with both cardiac and gastrointestinal involvement.

When analyzing the serum cytokine levels, patients with positive *T. cruzi* RT-PCR in peripheral blood had a higher median concentration of IL-10 (1,622.06 fg/mL versus 515.05 fg/mL, $P = 0.010$) and IL-1 β (1,195.22 fg/mL versus 682.21 fg/mL, $P = 0.022$) than those with negative *T. cruzi* PCR. Conversely, patients with negative *T. cruzi* PCR had a higher median concentration of IL-8 (1,960.96 fg/mL versus 622.70 fg/mL, $P < 0.001$) than those with positive *T. cruzi* PCR (see Table 2, Figure 1). When comparing serum cytokine concentrations depending on cardiac or digestive involvement, no statistically significant differences were observed (Tables 3 and 4). Similarly, we did not find any difference in the serum cytokine profile of patients with cardiac involvement compared with those with digestive involvement.

DISCUSSION

We studied the serum cytokine profile of 45 patients chronically infected by *T. cruzi* and residing in a non-endemic country. Interestingly, patients with detectable parasitemia measured through *T. cruzi* RT-PCR in peripheral blood had a higher concentration of the anti-inflammatory cytokine IL-10 than those with negative *T. cruzi* RT-PCR.

TABLE 2

Serum cytokine concentrations depending on *Trypanosoma cruzi* PCR result in Chagas disease patients

Cytokines (fg/mL)	Positive <i>T. cruzi</i> PCR ($n = 19$)	Negative <i>T. cruzi</i> PCR ($n = 26$)	P -value
IL-17A	1,225.50 (892.13–1,914.10)	1,185.58 (890.79–2,020.61)	0.783
IL-2	0 (0–0)	0 (0–6,807.51)	0.221
IL-5	391.02 (0–5,736.50)	0 (0–2,119.64)	0.206
IL-12p70	1,767.59 (0–3,709.10)	1,237.91 (0–5,966.8)	0.059
IFN- γ	514.68 (0–1,532.52)	242.40 (0–2,789.69)	0.070
IL-10	1,622.06 (197.16–4,961.17)	515.05 (0–3,096.84)	0.010
IL-1β	1,195.22 (0–2,230.71)	682.21 (0–7,275.95)	0.022
IL-4	0 (0–1,277.84)	0 (0–1,372.68)	0.412
IL-8	622.70 (126.50–6,647.20)	1,960.96 (193.60–35,384.60)	< 0.001

PCR = polymerase chain reaction. Data are reported as median value and range. Bold represent those values which showed statistically significance ($P < 0.05$).

FIGURE 1. Comparison of different serum cytokine concentrations depending on *Trypanosoma cruzi* polymerase chain reaction (PCR) result in Chagas disease patients. IL-10 and IL-1beta serum concentrations are higher in patients with positive *T. cruzi* PCR, whereas IL-8 serum concentrations are higher in those with negative *T. cruzi* PCR. This figure appears in color at www.ajtmh.org.

Anti-inflammatory cytokines play a key role in controlling inflammatory cytokine production; IL-10 (produced by immune cells such as dendritic and regulatory T cells) is an anti-inflammatory cytokine with pleiotropic effects in immunoregulation and inflammation. Whereas the pro-inflammatory Th1 immune response is necessary in the acute phase of Chagas disease to control the parasitemia, in the indeterminate phase, there is an increase in the IL-10 levels that controls the inflammation.⁹ It has been observed that this anti-inflammatory

control should be well balanced to avoid that production of IL-10 does not immunosuppress the cellular response necessary to control the parasitemia but has to be sufficient to control the establishment of inflammation during the chronic phase.⁸ This necessary and complicated cytokine balance in the chronic phase of the disease could explain the fact that, in our cohort of Chagas disease patients, those with detectable parasitemia had higher IL-10 serum concentrations. However, it is important to note that there are reports that indicate that

TABLE 3
Serum cytokine concentrations depending on cardiac involvement in Chagas disease patients

Cytokines (fg/mL)	With cardiac involvement (n = 11)	Without cardiac involvement (n = 34)	P-value
IL-17A	1,242.78 (892.13–2,020.61)	1,169.19 (890.79–1,914.10)	0.520
IL-2	0 (0–6,807.51)	0 (0–0)	0.416
IL-5	0 (0–5,736.50)	0 (0–1,840.79)	0.930
IL-12p70	1,292.13 (0–5,966.80)	1,767.59 (0–3,673.10)	0.298
IFN-gamma	446.07 (0–2,789.69)	455.90 (0–1,532.52)	0.706
IL-10	735.01 (0–4,961.17)	1,720.39 (6.16–2,462.95)	0.256
IL-1beta	1,019.30 (0–7,275.95)	1,078.61 (0–1,740.81)	0.979
IL-4	0 (0–1,372.68)	0 (0–1,277.84)	0.558
IL-8	1,123.98 (126.50–35,384.60)	1,312.21 (226.8–10,699.10)	0.958

Data are reported as median value and range.

TABLE 4
Serum cytokine concentrations depending on digestive involvement in Chagas disease patients

Cytokines (fg/mL)	With digestive involvement (n = 9)	Without digestive involvement (n = 36)	P-value
IL-17A	1,351.40 (1,040.92–1,477.76)	1,196.83 (890.79–2,020.61)	0.456
IL-2	0 (0–439.30)	0 (0–6,807.51)	0.302
IL-5	407.72 (0–1,334.22)	0 (0–5,736.50)	0.801
IL-12p70	1,141.11 (0–3,134.10)	1,613.30 (0–5,966.80)	0.422
IFN-gamma	425.58 (0–2,789.69)	455.86 (0–2,058.82)	0.909
IL-10	1,296.45 (53.12–2,145.36)	1,044.06 (0–4,961.17)	0.887
IL-1beta	839.78 (52.15–1,649.67)	1,072.60 (0–7,275.95)	0.865
IL-4	0 (0–718.93)	0 (0–1,372.68)	0.115
IL-8	1,153.02 (217–10,002.60)	1,176.77 (126.5–35,384.60)	0.977

Data are reported as median value and range.

both in the acute and chronic phases of Chagas disease, the parasitemia induces a serum increase of IL-10 as part of its immune-evasion mechanisms, making it difficult to know if this cytokine profile is the cause or the consequence of parasitemia levels.^{14,15}

There are some situations with a skewed immune response to Th2 (where IL-10 levels are also increased) in which is observed an increase in the probability to detect parasitemia in Chagas disease patients. During the pregnancy, there is a physiological bias to Th2 immune response to increase the fetal survival.¹⁶ Some studies have observed that pregnant women with Chagas disease have higher levels of parasitemia, hence being more probable to having a positive *T. cruzi* RT-PCR.¹⁷ Two studies performed by our group demonstrated that Chagas disease patients coinfecting with *Strongyloides stercoralis* (which produces a Th2 immune response and higher IL-10 production) had a higher proportion of patients with positive *T. cruzi* RT-PCR in peripheral blood than those without coinfection.^{18,19} These data support the hypothesis of the key role that plays IL-10 in parasitemia control in chronic Chagas disease patients.

As explained previously, the serum cytokine profile in Chagas disease patients is different depending on the form of the disease (acute phase, indeterminate form, and symptomatic chronic form).⁸ However, in our study, we did not observe differences neither between patients with or without cardiac involvement nor between patients with or without digestive involvement. It could be explained by the fact that patients with visceral involvement in our study presented very early or incipient stages of the disease: patients in the stage one of the Kushnir classification regarding cardiac involvement and patients with dolichocolon regarding digestive involvement. There were only a few patients with established cardiomyopathy (three patients) or intestinal dilatation (two patients).

Contradictory results have been observed in our study regarding cytokines involved in the innate immune response: serum IL-1beta concentrations were higher in patients with positive *T. cruzi* RT-PCR, whereas serum IL-8 concentrations were higher in patients with negative *T. cruzi* RT-PCR. IL-1beta and IL-8 are pro-inflammatory cytokines involved in the innate immune response, produced by different cells (including monocytes, neutrophils, fibroblasts, and endothelial cells), and act as chemotactic factors for neutrophils and lymphocytes. Although the study of the acute phase represents a challenge (it is asymptomatic most of the times), it seems that the innate immune response mediate the control of parasite replication in the early stages of infection.²⁰ The role of the innate immune response in the Chagas disease chronic phase is not well established; however, studies performed in African trypanosomiasis

show that the control of parasitemia is related to high serum levels of IL-10 and low levels of IL-8, similar to those results found in our study.²¹

This study has some limitations that should be mentioned. The low number of included patients makes it difficult to generalize the results, especially regarding the evaluation of the cytokine profile depending on visceral involvement, because most of the patients in the symptomatic chronic phase of the disease had a very incipient affectation. Moreover, most of the patients were women of Bolivian origin, which makes it difficult to extrapolate the results to a more general population. The determination of *T. cruzi* RT-PCR in peripheral blood was performed once (it is a cross-sectional study), and it could limit the result interpretation. However, this study gives relevant information about the serum cytokine profile and its relationship with the probability to detect the parasite in peripheral blood and performed in Chagas disease patients outside endemic areas, hence without risk of reinfections in the last years (that could modify the cytokine profile).

Summarizing, in our cohort of chronically infected Chagas disease patients, those patients with detectable parasitemia measured through *T. cruzi* RT-PCR in peripheral blood had a higher concentration of the anti-inflammatory cytokine IL-10 than those with negative *T. cruzi* RT-PCR. These results reinforce the key role that plays this anti-inflammatory cytokine in parasitemia control. Further studies are needed to confirm and deepen this immunoregulatory control.

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