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MAJOR ARTICLE

Circulating Cytokine and Chemokine Profiles of *Trypanosoma cruzi*-Infected Women During Pregnancy and Its Association With Congenital Transmission

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Background. *Trypanosoma cruzi*, the causative agent of Chagas disease, can be transmitted to the offspring of infected women, which constitutes an epidemiologically significant parasite transmission route in nonendemic areas. It is relevant to evaluate differentially expressed factors in *T. cruzi*-infected pregnant women as potential markers of Chagas congenital transmission.

Methods. Circulating levels of 12 cytokines and chemokines were measured by enzyme-linked immunosorbent assay or cytometric bead array in *T. cruzi*-infected and uninfected pregnant women in their second trimester of pregnancy and control groups of *T. cruzi*-infected and uninfected nonpregnant women.

Results. *Trypanosoma cruzi*-infected women showed a proinflammatory Th1-biased profile, with increased levels of tumor necrosis factor (TNF)-α, interleukin (IL)-12p70, IL-15, and monokine induced by interferon-gamma (MIG). Uninfected pregnant women presented a biased response towards Th2/Th17/Treg profiles, with increased plasma levels of IL-5, IL-6, IL-1β, IL-17A, and IL-10. Finally, we identified that high parasitemia together with low levels of TNF-α, IL-15, and IL-17, low TNF-α/IL-10 ratio, and high IL-12p70 levels are factors associated with an increased probability of Chagas congenital transmission.

Conclusions. *Trypanosoma cruzi*-infected pregnant women who did not transmit the infection to their babies exhibited a distinct proinflammatory cytokine profile that might serve as a potential predictive marker of congenital transmission.

Keywords. congenital transmission; cytokines; immune response; pregnancy; *Trypanosoma cruzi*.

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*. It currently affects 6–7 million people, mostly in Latin America. Due to migrations, *T. cruzi* vertical transmission has also become a health problem in nonendemic countries [1].

Maternal-foetal *T. cruzi* transmission has been reported to occur in a large percentage of pregnancies, with an average rate of 5% [2]. There is frequent underdiagnosis of *T. cruzi* infection in women during prenatal care and in congenitally infected children [3].

Seropositive pregnant women are usually in the chronic phase of *T. cruzi* infection, and their parasitemia is frequently low, but it has been observed that mothers of infected children showed higher parasitemia compared to the mothers of uninfected offspring [4–8]. In the acute phase of the infection or reactivated Chagas disease, a 100% rate of *T. cruzi* congenital transmission has been observed [9, 10], with all women treated with trypanocidal drugs before pregnancy giving birth to uninfected children, thus supporting the hypothesis that the parasite load in pregnant women is a key factor for mother-to-child *T. cruzi* transmission [11–13], reviewed in [14]. It has been suggested that the cytokine pattern for a successful pregnancy varied in healthy women from a proinflammatory stage, associated with implantation and placentation, to a predominantly Th2 profile in the anti-inflammatory stage, associated with foetal growth [15, 16], followed by a proinflammatory stage, which is responsible for the initiation of parturition [17]. In pregnant women with chronic *T. cruzi* infection, higher levels of tumor necrosis factor (TNF)-α and interferon (IFN)-γ were observed in women who did not transmit the infection to their offspring, suggesting a shift towards a Th1 profile [18–22].

In the group of *T. cruzi*-infected pregnant women who gave birth to uninfected children, those with detectable parasitemia showed increased levels of IFN-γ and TNF-α in peripheral, placental, and cord blood, compared with pregnant women with undetectable parasitemia, suggesting that higher parasite loads balanced by a robust proinflammatory response could prevent congenital transmission [21, 23]. In addition, a decreased *T. cruzi*-specific production of IFN-γ was observed in peripheral blood mononuclear cells

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from mothers who transmitted the congenital infection [8]. TNF-α and IFN-γ are key mediators to control parasitemia in *T. cruzi* infection, a strong innate and adaptive immune response involving chemokines and cytokines would likewise control parasite load in pregnant women, thus preventing transmission [12].

The purpose of this study was to evaluate a set of circulating immune mediators to understand the relationship between the mother's immune response, parasite load, and congenital transmission by measuring the plasma levels of IFN-γ, IL12p70, IL-4, IL-5, IL-17A, IL-6, IL-10, IL-15, IL-1β, TNF-α, monocyte chemotactic protein (MCP)-1, and monokine induced by IFN-γ (MIG), as well as the parasite burden in pregnant women who transmitted *T. cruzi* infection versus those who did not. Our data suggest differential chemokine and cytokine profiles in seropositive pregnant women, which could potentially act as risk factors for congenital Chagas transmission.

METHODS

Study Location and Participants

Our research was conducted at the Instituto Nacional de Parasitología (INP) "Dr Mario Fatala Chaben." Groups of *T. cruzi*-infected (P+, n = 70) and uninfected (P−, n = 37) pregnant women, as well as control groups of infected (NP+, n = 30) and uninfected (NP−, n = 30) women without clinically recognized pregnancies, were included in our study, all from nonendemic areas. After diagnosis of the babies born to *T. cruzi*-infected mothers, P+ were reclassified as mothers of congenitally infected babies (P+B+, n = 35) and mothers who delivered uninfected children (P+B−, n = 35) (Table 1). *Trypanosoma cruzi*-infected women were healthy, in the asymptomatic phase of chronic infection, and did not received previous trypanocidal treatment. P+ and P− were at least 16 weeks pregnant. The pregnant women chosen for enrollment in the study were in the second trimester of pregnancy because of the predominant Th2 profile that rules the anti-inflammatory stage associated with a stable gestation before the inflammatory environment leading to delivery. The average age was not significantly different among the 5 groups of women studied, and the gestation time during which the blood sample was drawn did not differ among the 3 groups of pregnant women (Table 1). Venous blood was drawn with and without anticoagulant for the collection of plasma and serum, respectively. Sera were used for serological assays, and plasma was kept at −70°C until use.

Diagnosis of *Trypanosoma cruzi* Infection

The presence of specific antibodies in serum samples was determined by means of indirect hemagglutination, indirect immunofluorescence, and enzyme-linked immunosorbent assays (ELISAs) as previously described [5]. Women were considered infected when at least 2 of the serological tests were reactive [24]. Babies born to *T. cruzi*-infected subjects were monitored at our institution to assess congenital *T. cruzi* infection diagnosis. The presence of *T. cruzi* in the blood of children was determined by the INP Micromethod at 1 and 6 months of age [25]. The serological assays described above were also carried out to evaluate the presence of *T. cruzi*-specific antibodies, and when detected after 10 months of age the babies were considered congenitally infected and referred for trypanocidal treatment. Seropositive women (P+) were then grouped into mothers of *T. cruzi*-infected (P+B+) or uninfected (P+B−) babies.

Trypanosoma cruzi Deoxyribonucleic Acid Quantification

The blood samples collected from infected women (5 mL) were mixed with 1 volume of buffer containing equal volumes of 6 M guanidine hydrochloride (Sigma Chemical Co., St. Louis, MO)

Table 1. Characteristics of the Groups of Women Studied

	Nonpregnant		Pregnant		
	Noninfected (NP−)	Infected (NP+)	Noninfected (P−)	Infected Mothers of Noninfected Babies (P+B−)	Infected Mothers of Infected Babies (P+B+)
n	30	30	37	35	35
Age (years)[a]	30 ± 8	34 ± 11	31 ± 7	25 ± 7	29 ± 7
Month of gestation (months)[b]	-	-	5.0 ± 2.0	6.8 ± 1.4	5.2 ± 1.4
Serological Diagnosis					
IHA[c]	<5	6.9 ± 1.1	<5	6.9 ± 0.9	6.8 ± 0.9
IIF[c]	<5	7.0 ± 0.8	<5	7.0 ± 0.9	7.0 ± 1.0
ELISA[d]	49 ± 34	345 ± 69	65 ± 38	346 ± 75	340 ± 69
Babies					
Birth weight (grams)[e]	-	-	-	3530 ± 95.5	3486 ± 96.6
Preterm birth[f]	-	-	-	0/31	4/32

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IHA, immunohemagglutination; IIF, indirect immunofluorescence.

[a,b]Arithmetic mean ± standard error of the mean values are shown. The groups did not differ significantly with respect to age and months of gestation ($P > .05$ analysis of variance).

[c]Results of IHA and IIF are expressed as the $\log_2$ of the inverse of the titer and considered reactive when >5.

[d]ELISA is expressed as the optical density at 490 nm × 1000 and considered reactive when >200.

[e,f]Groups of infected or uninfected babies born from born to infected mothers did not differ significantly with respect to weight at birth or preterm birth.

and EDTA (0.1 M, pH 8). Deoxyribonucleic acid was isolated and quantified by real-time polymerase chain reaction as previously described [5].

Determination of Plasma Cytokine Production by Enzyme-Linked Immunosorbent Assay

The levels of IL-4, IL-5, IL-10, IL12p70, IFN-γ, TNF-α, and IL-15 were determined by 2-site ELISA (OptEIA Kit; BD PharMingen, San Jose, CA), as previously described [19]. The assay sensitivity was 4 pg/mL for IL-4, 5 pg/mL for IL-5, 4 pg/mL for IL-10, 12 pg/mL for IL-12p70, 2 pg/mL for IFN-γ, 2 pg/mL for TNF-α, and 8 pg/mL for IL-15.

Determination of Plasma Cytokine Production by Cytometric Bead Array

The levels of IL-1β, IL-6, IL-17A, MIG/CXCL9, and MCP-1/CCL2 were determined by cytometric bead array, as previously described [26], using commercial kits (BD Biosciences-US). Data were acquired on a FACSAria flow cytometer (BD Biosciences) and analyzed with FCAP Array software (BD Biosciences). The sensitivity of each assay was as follows: 2.3 pg/mL for IL-1β, 1.6 pg/mL for IL-6, 0.3 pg/mL for IL-17A, 1.1 pg/mL for MIG, and 1.3 pg/mL for MCP-1.

Statistical Analysis

Data normality was evaluated using the Shapiro-Wilk test. Cytokine levels were presented as medians with interquartile ranges. Comparison of median values between 2 groups were performed using the Mann-Whitney test, and among 3 or more groups using the Kruskal-Wallis test, followed by Dunn's posttest to compare pairs. Cytokine and chemokine profiles in the different groups were also compared using principal component analysis (PCA).

Finally, a Spearman's correlation test was used to analyze the association between cytokine concentrations and parasitemia. The result matrix was built with a 2-dimensional visualization technique called "heat map" analysis. Statistical analysis and graphs were performed using GraphPad Prism 8.00 software and Statistix 10.0. A $P < .05$ was considered statistically significant.

Ethical Approval

The study was approved by Administración Nacional de Laboratorios e Institutos de Salud "Carlos G. Malbrán" Ethics Committee and carried out according to the declaration of Helsinki. Women eligible for this study signed a written informed consent before enrollment.

RESULTS

Differential Circulating Levels of Cytokines Associated With *Trypanosoma cruzi* Infection and Pregnancy

We studied a set of Th1, Th2, Th17, regulatory, and proinflammatory cytokines, cell differentiation-induced cytokines, and chemokines in the plasma of the women enrolled.

We first analyzed the changes in cytokine and chemokine sets induced during chronic *T. cruzi* infection. A skew towards a proinflammatory profile balanced by regulatory cytokines was observed in NP+ with increased levels of TNF-α, IL-12p70, IL-15, IL-10, and MIG (Figures 1A and C and 2E, C, and F) compared with NP−. Among uninfected women, pregnancy induced a Th2/Th17/T regulatory profile, with increased levels of IL-5, IL-6, IL17-A, IL-1β, and IL-10 (Figures 2A, D and B and 1D and 2C) compared with NP−.

Differential Cytokine and Chemokine Expression in *Trypanosoma cruzi*-Infected Pregnant Women

When comparing the groups of seropositive women, increased levels of IL-5 but decreased levels of IL-10 were found in the P+ group (ie, P+B− and P+B+ regardless of congenital transmission compared with NP+) (Figure 2A and C). P+B+ had significantly lower levels of TNF-α and increased levels of IFN-γ compared with NP+ (Figure 1A and B), whereas P+B− showed higher levels of IL-15, IL-6, and IL-1β than the NP+ group (Figures 2E and D and 1D).

When the effects of *T. cruzi* infection in pregnant women were evaluated, we found that regardless of the congenital transmission, P+ had higher levels of IFN-γ and MIG but lower IL-10 compared with P− (Figures 1B and 2F and C, respectively). P+B− showed increased levels of TNF-α and IL-15, whereas only P+B− showed increased IL-12p70 compared with P− (Figures 1A, 2E, and C, respectively). Overall, a mixed Th1/Th2 profile was observed in P+ with a more robust proinflammatory profile in mothers who delivered uninfected children compared with those who delivered *T. cruzi*-infected children. The circulating levels of IL-4 and MCP-1 remained unaltered among the 5 groups of women studied.

Differential Circulating Levels of Cytokines and Chemokines and Parasite Load Associated With *Trypanosoma cruzi* Congenital Transmission

To evaluate the immune mediators associated with congenital transmission, the levels of cytokines and chemokines were compared between P+B+ and P+B− through a univariate analysis. Higher parasite loads and IL-12p70 levels, along with lower levels of IL-15, TNF-α, IL-17, and lower TNF-α/IL-10 ratio, were associated with congenital transmission (Table 2). Regarding parasitemia, a variable percentage of women with chronic *T. cruzi* infection had detectable parasite loads: 55% in the NP+ group and 87% in the P+ group (P+B− and P+B+). NP− and P− showed, as expected, undetectable parasitemia.

Principal Component Analysis

Because cytokines and chemokines do not necessarily function independent from one another, PCA was conducted to determine whether any specific groups of cytokines could be associated with congenital transmission. Two principal components with eigenvalues greater than 1 were extracted: these components cumulatively explained 57.6%, 47.9%, and 63%

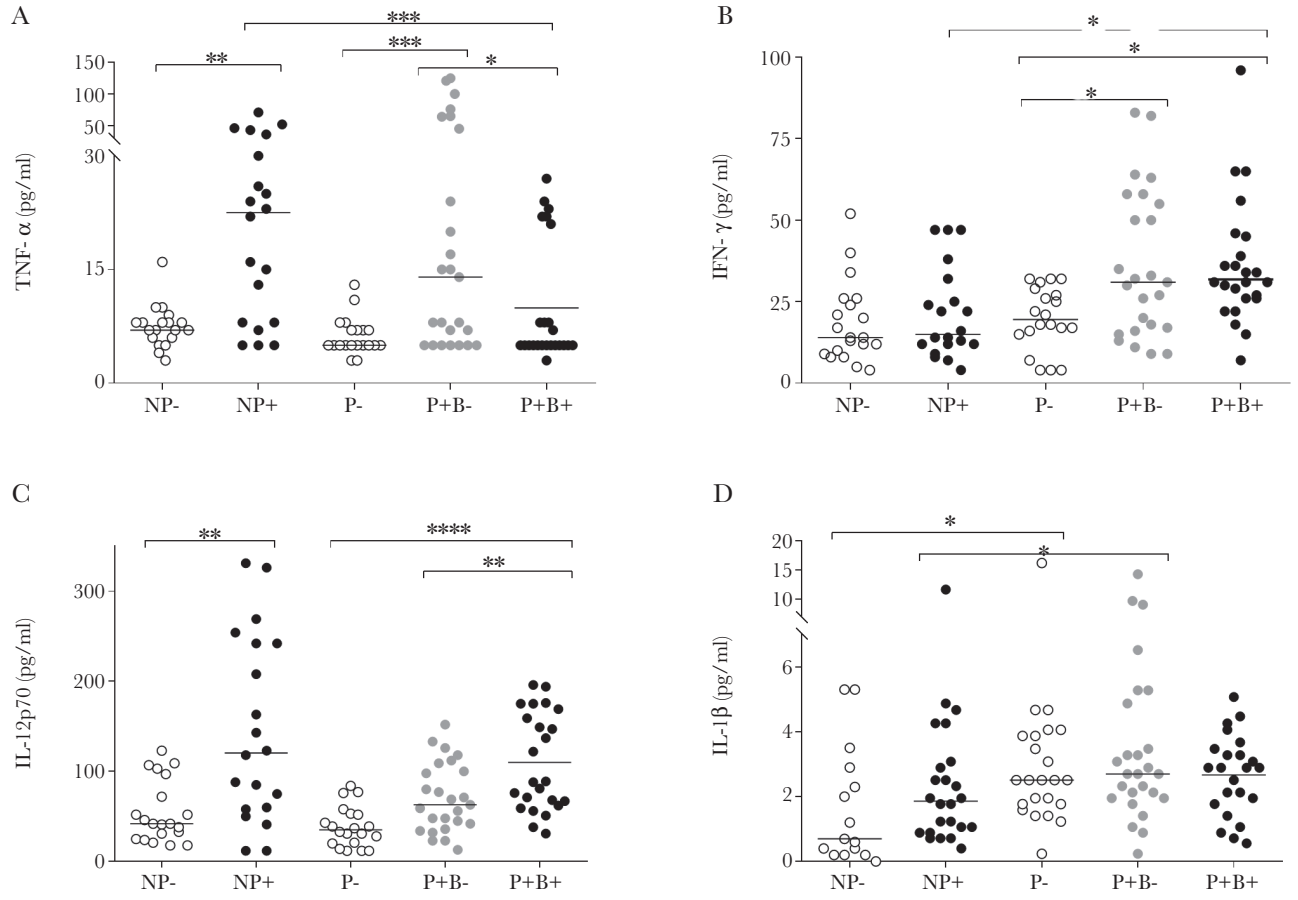

Figure 1. Circulating plasma levels of Th1 and proinflammatory cytokines in *Trypanosoma cruzi*-infected and uninfected pregnant and nonpregnant women. Tumor necrosis factor (TNF)-α (A), interferon (IFN)-γ (B), interleukin (IL)-12p70 (C), and IL-1β (D), expressed as pg/mL in *T. cruzi*-infected (P+) and uninfected (P–) pregnant women, and control groups of *T. cruzi*-infected (NP+) and uninfected (NP–) nonpregnant women were measured by enzyme-linked immunosorbent assay or cytometric bead array. Horizontal lines represent median values for each group. Differences were tested using Kruskal-Wallis, followed by Dunn's test for post hoc comparisons. *, $P < .05$; **, $P < .01$; and ***, $P < .001$.

of the variance in cytokine and chemokine levels in P+B–, P+B+, and P–, respectively. When the subject groups were plotted based on PC1 and PC2, 3 distinct clusters were observed (Figure 3A). P+B– was clearly segregated from P–, whereas P+B+ was in an intermediate position between P+B– and P–.

The component loadings for each variable showed that PC1 scores in P+B– were inversely correlated with IL-12p70, whereas in P+B+, they were positively correlated with IL-4 and IL-5 and inversely correlated with IL-15 (Figure 3B). The variance in PC2 was inversely correlated (1) with IL-5 and IL-17 in P+B– and (2) with IFN-α and TNF-α in P+B+. P– showed a more limited repertoire of mediators influencing cytokine and chemokine responses, with PC1 and PC2 scores inversely correlating to IL-1β and IL-6, respectively (Figure 3B).

Correlation Between Cytokine/Chemokine Responses and Parasitemia in Chagas Congenital Transmission

The circulating levels of cytokines and chemokines were correlated with parasite loads, as shown in Figure 4. In NP+, IL-12p70, IL-15, IFN-γ, and TNF-α levels were positively correlated with parasitemia, whereas IL-10 was not (Figure 4A).

In P+B–, parasitemia showed a positive correlation with the levels of IL-12p70, IL-15, IFN-γ, and TNF-α and a negative correlation with IL-6 (Figure 4B). In P+B+, parasitemia was only positively correlated with IL-15 but inversely correlated with IL-1β (Figure 4C).

DISCUSSION

In this study, we measured the circulating levels of 12 cytokines and chemokines in pregnant *T. cruzi*-infected women to evaluate whether congenital transmission could be predicted based on a specific profile of these mediators during pregnancy. The cytokine and chemokine profiles differed depending on the situation related to *T. cruzi* infection, pregnancy, pregnancy in the context of parasite infection, and Chagas congenital transmission.

Normal pregnancy has long been described as a Th1/Th2/Treg phenomenon, where cytokines play a dual role,

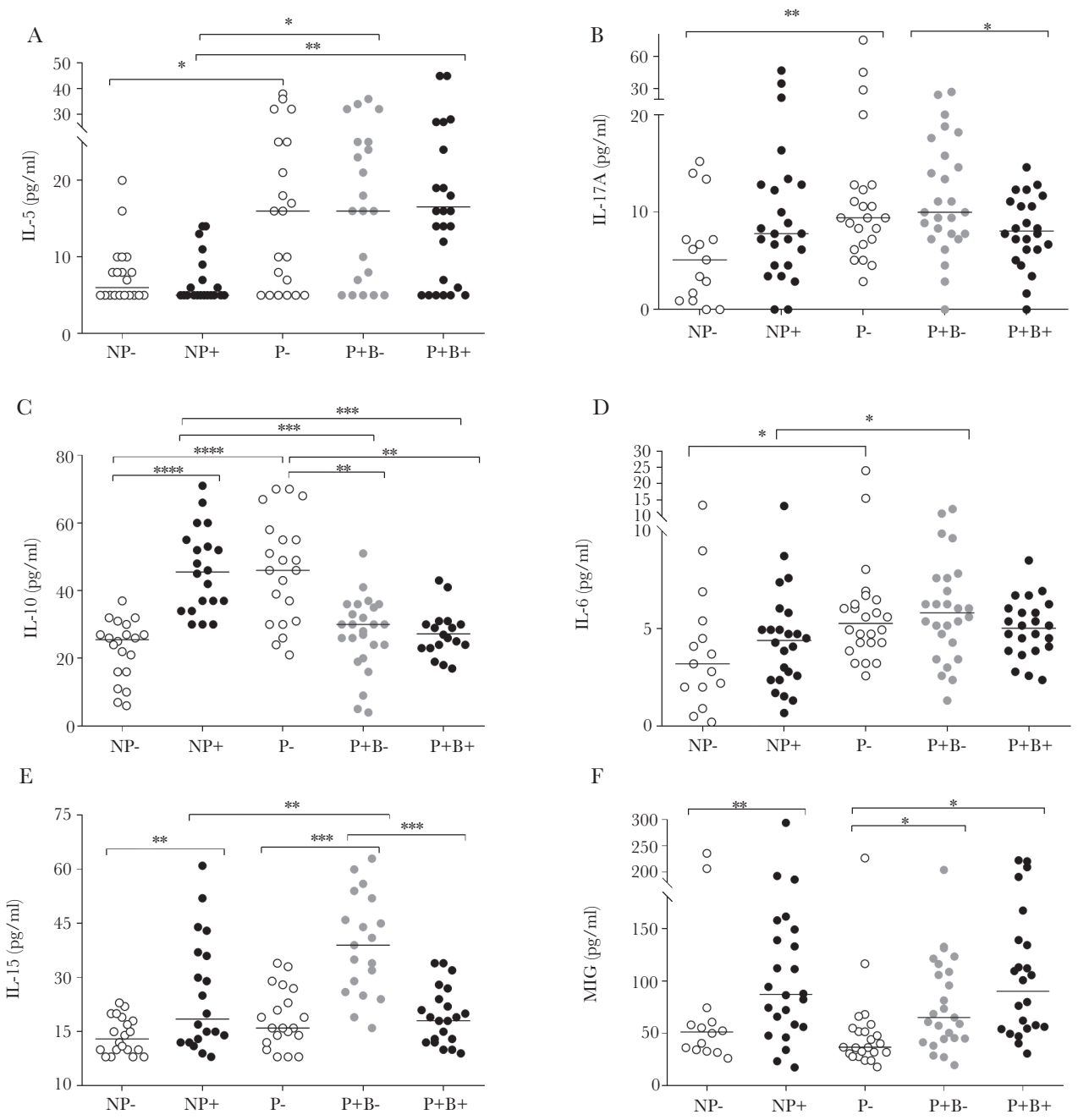

Figure 2. Circulating plasma levels of Th2, Th17, Treg, inmunomodulatory, cell differentiation-induced cytokines, and chemokines in *Trypanosoma cruzi*-infected and uninfected pregnant and nonpregnant women. Interleukin (IL)-5 (A), IL-17 (B), IL-10 (C), IL-6 (D), IL15 (E), and monokine induced by interferon-gamma (MIG) (F) expressed as pg/mL in *T. cruzi*-infected (P+) and uninfected (P–) pregnant women, and control groups of *T. cruzi*-infected (NP+) and uninfected (NP–) nonpregnant women were measured by enzyme-linked immunosorbent assay or cytometric bead array. Horizontal lines represent median values for each group. Differences were tested using Kruskal-Wallis, followed by Dunn's test for post hoc comparisons. *, $P < .05$; **, $P < .01$; ***, $P < .001$.

contributing to the generation of tolerance to avoid rejection of the semi-allogeneic foetus while protecting the foetus against pathogens [15, 27]. The pregnant women chosen for enrollment were women in the second trimester of their pregnancy, because parasite infection is associated with a proinflammatory stage, and there is a higher possibility of detecting a congenital infection in the mid-trimester when the Th2 profile rules the anti-inflammatory stage associated with a stable gestation. Therefore, the shift towards a proinflammatory stage might be attributed to *T. cruzi*-infection. In addition, by this time, the main immune cell populations have been developed [11, 12].

The cytokine profile in P– in our study presented a bias toward a Th2/Th17/Treg phenotype, with increased levels of IL-5, IL-6, IL-1β, IL-17A, and IL-10 in their plasma. Several studies

Table 2. Univariate Analysis of Factors Associated With *Trypanosoma cruzi* Mother-to-Child Transmission

Variable (Unit)[a]	P+B− (n = 35)	P+B+ (n = 35)	*P* Value[b]
T. cruzi Infection			
Parasite load (mE/mL)	*1.16 (0.22–2.22)*	*7 (1.5–14)*	*0.0001*
ELISA (OD 450 nm)	0.336 (0.310–0.403)	0.319 (0.284–0.382)	.5498
IHA (titer)	1/128 (1/64–1/128)	1/128 (1/128–1/128)	.6080
Log IHA	7 (6–7)	7 (7–7)	.6080
IIF (titer)	1/128 (1/64–1/256)	1/128 (1/128–1/256)	.7318
Log IIF	7 (6–8)	7 (7–8)	.7318
Cytokines (pg/mL)			
IFN-γ	31 (16.5–53)	31 (26–43.5)	.7038
IL-12p70	*63 (39–104.5)*	*88.5 (63.25–166.5)*	*.0105*
IL-4	16 (7–25)	8.5 (7.75–18.25)	.3136
IL-5	16 (6–25)	15 (5.25–22.75)	.6055
IL-17A	*9.99 (7.64–16.2)*	*7.78 (6.15–11.11)*	*.0398*
IL-6	5.82 (4.07–7.6)	5.16 (3.86–6.04)	.1273
IL-10	30 (22–35.5)	26.5 (23–31)	.8411
IL-15	*37 (26.7–50.5)*	*18.5 (12.25–23.5)*	*<.0001*
IL-1β	2.70 (1.95–4.97)	2.89 (1.77–3.47)	.5540
TNF-α	*14 (5–54.5)*	*5 (5–17.75)*	*.0213*
Chemokines (pg/mL)			
MCP-1	92.12 (49.31–128.26)	81.35 (41.33–120.66)	.8374
MIG	66.85 (44.96–117.48)	100.8 (54.59–139.09)	.1963
Ratios			
IFN-γ/IL-4	1.9 (1.1–5.1)	3 (1.22–6.02)	.3372
IFN-γ/IL-10	1.3 (0.6–2.5)	1.05 (0.8–2.15)	.5087
TNF-α/IL-10	*0.5 (0.2–1.85)*	*0.2 (0.2–0.65)*	*.0121*

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IFN, interferon; IHA, immunohemagglutination; IIF, indirect immunofluorescence; IL, interleukin; MIG, monokine induced by interferon-gamma; MCP, monocyte chemotactic protein; TNF, tumor necrosis factor.

[a]Data for continue variables are shown as median and interquartile ranges.

[b]Italic bold values indicate $P < .05$ analyzed by the 2-sample *t* test or the Wilcoxon rank-sum test as appropriate. *T. cruzi*-infected pregnant women, mothers of noninfected (P+B−) or congenitally infected babies (P+B+).

have shown the presence of IL-5 in peripheral blood in P− during the different pregnancy trimesters [28, 29], suggesting a possible role of IL-5 beyond endometrial tissue remodeling [30, 31]. The increased levels of IL-1β and IL-6 in P−, which appear to play a crucial role during embryo implantation and placental development [32], are in line with previous observations of elevated IL-1β and IL-6 cytokine plasma levels in normal pregnancies [33].

In line with our results, IL-17 concentration and an increased number of Th17 cells throughout pregnancy have been reported [34, 35]. In contrast, Santner-Nanan et al [36] described a decreased prevalence of Th17 cells in the peripheral blood of healthy pregnant women, whereas other studies found no differences between nonpregnant and pregnant women [37]. *Trypanosoma cruzi*-infected women showed a proinflammatory Th1-biased profile, regardless of whether they were pregnant or not, with increased levels of TNF-α, IL-12p70, and MIG, whereas IFN-γ levels were only increased in P+. IL-12p70 is known as an immune-modulatory cytokine that mediates IFN-γ production through the induction of Th1 cells and activation of NK cells [31]. Interferon-γ activates macrophage-mediated cytotoxicity through nitric oxide production, limiting parasite proliferation [38]. All infected women also showed increased levels of MIG, which is induced by IFN-γ and is important in Th1 polarization, functioning by attracting Th1 cells and inhibiting Th2 migration. A strong MIG-mediated Th1 response has been found to be a key factor in protection against *T. cruzi* [39].

The inflammatory mediator IL-15, a pleiotropic interleukin involved in the development and maintenance of effector cells that share many biological activities with IL-2 [40], showed increased levels in the plasma of *T. cruzi*-infected women regardless of pregnancy. Along with IL-12, IL-15 is considered an important costimulator of IFN-γ and TNF-α production, both of which are essential cytokines in the immune control of intracellular pathogens [41]. Indeed, elevated IL-15 levels have been found in several infections caused by intracellular pathogens such as dengue virus [42] and *Leishmania infantum* [43]. It is likely that one of the most important roles of IL-15 during *T. cruzi* infection is to amplify the levels of IFN-γ and TNF-α produced by NK cells to control parasitemia. The behavior of IL-10 was very particular, because it increases during *T. cruzi* infection and pregnancy, but in *T. cruzi*-infected pregnant women and regardless of congenital transmission, the levels were comparable to those found in uninfected nonpregnant women. It is

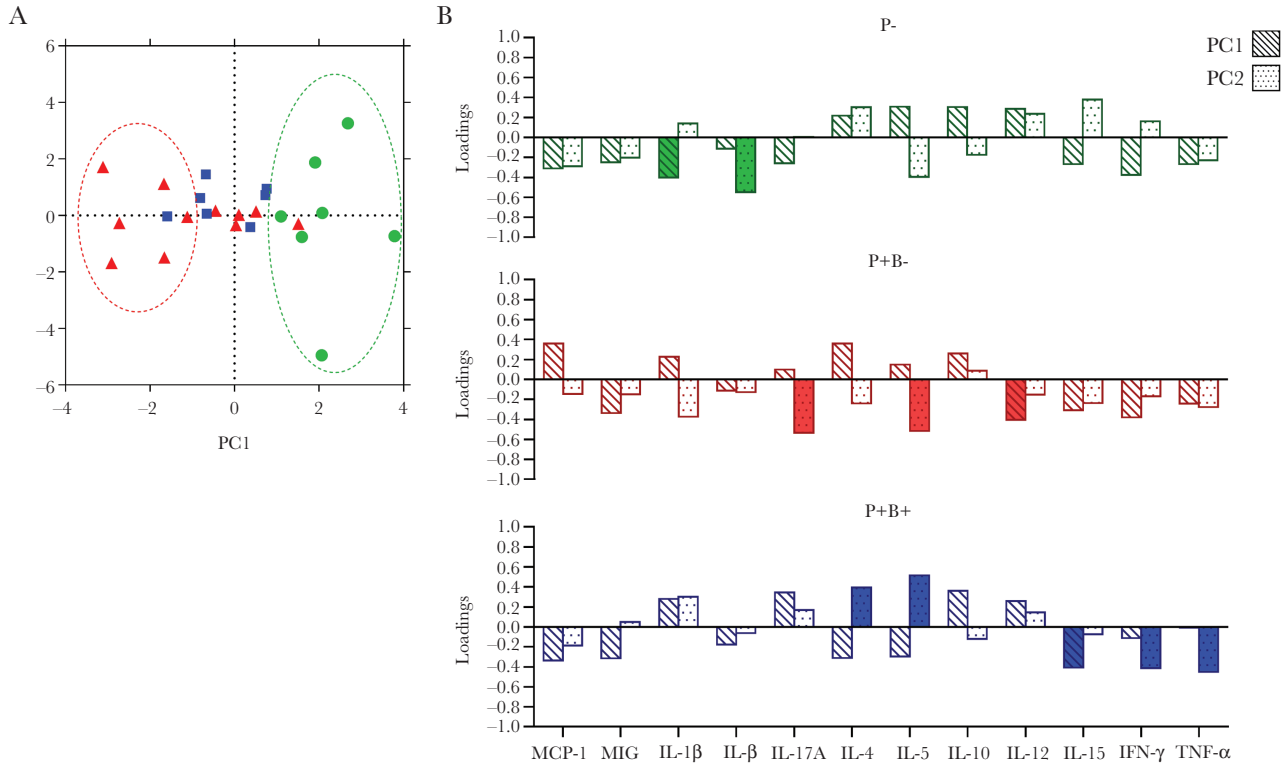

Figure 3. Principal component analysis of cytokine and chemokine production. Circulating immune-mediators were measured in uninfected pregnant women (P–, n = 37), and *Trypanosoma cruzi*-infected pregnant women, mothers of uninfected babies (P+B–, n = 35) or congenitally infected babies (P+B+, n = 35). After log transformation, the principal components were extracted with eigenvalues higher than 1.0. Loading plots depict the relationship between the first 2 principal components PC1 and PC2 and explained the 63%, 57.6%, and 47.9% of the variance, respectively. P– (green), P+B– (red), and P+B+ (blue) women plotted based on the first 2 extracted principal components, PC1 and PC2 (A). Monocyte chemotactic protein 1, monokine induced by interferon-gamma, interleukin (IL)-1β, IL-6, IL-17A, IL-4, IL-5, IL-10, IL-12, IL-15, interferon-γ, and tumor necrosis factor-α loading values of PC1 and PC2 are represented by the bars for each group (B). Loadings of >0.4 or < –0.4 were considered as influential mediators (filled bars).

possible that higher levels of IFN-γ, IL-5, and IL-6 observed in *T. cruzi*-infected pregnant women work as an inhibiting factor in the Treg pathway.

We found lower expression of TNF-α during pregnancy in mothers of *T. cruzi*-infected children, which is in line with previous observations of TNF-α response downregulation, as well as lower circulating levels of the soluble receptor TNFR1 in this group of women [19, 20]. We also showed similar IFN-γ and IL-10 plasma levels in both seropositive pregnant women subgroups (P+B+ and P+B–), as previously observed [19]. However, *T. cruzi*-stimulated peripheral blood mononuclear cells from P+B– evidenced a higher production of IFN-γ than PBMC from P+B+. Because IFN-γ is a key cytokine for parasite control, its increased production observed in T cells from P+B– might contribute to reduced parasite load, thus decreasing mother-to-child parasite transmission [8].

Principal component analysis revealed the relative importance of certain cytokines to explain variability within the

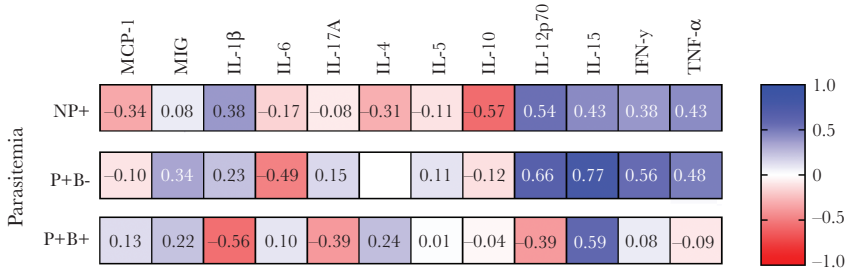

Figure 4. Heat maps of the correlations between plasma cytokine/chemokine concentrations and parasitemia. Immune mediators and parasite load were measured in *Trypanosoma cruzi*-infected nonpregnant women (NP+), mothers of noninfected children (P+B–), and mothers of *T. cruzi* congenitally infected babies (P+B+). The Spearman's rank correlation coefficient ranges from –1.0 to 1.0 corresponding to a strongly negative to a strongly positive correlation ($P < .05$).

pregnant women groups. Cytokine profiles in P+B− formed a cluster that was clearly different from P−, comprising more inflammatory mediators than the cytokine profiles of P+B+. Because IL-4 and IL-5 provided the largest contribution to variance in P+B+, a Th2 profile could be associated with congenital parasite transmission.

As previously described by our laboratory and other researchers [4–8], parasite load in P+B+ was significantly higher than in the P+B− group, and this could be explained by the fact that these pregnant women do not exhibit the appropriate immune responses to control parasite load, thus facilitating parasite transmission through the placenta. In line with this finding, a positive correlation was found among IFN-γ, TNF-α, IL-12p70, and IL-15 levels and parasitemia in pregnant *T. cruzi*-infected women who did not transmit the infection to their babies, supporting a more balanced response in these women. We are aware that circulating levels might not directly reflect the cell capacity to produce these cytokines/chemokines, which is an issue that needs to be addressed in future studies.

CONCLUSIONS

In summary, our findings support and contribute to previous observations in which a strong and protective immune response of seropositive pregnant women is associated with lower congenital parasite transmission. In *T. cruzi*-infected mothers who did not transmit the infection to their babies, we identified a set of cytokines with a bias towards Th1/Th17 over Treg and Th2 profiles.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases online*. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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References

1. World Health Organization. Chagas disease (American trypanosomiasis). Available at: https://www.who.int/health-topics/chagas-disease. Accessed 13 December 2019.
2. Howard EJ, Xiong X, Carlier Y, Sosa-Estani S, Buekens P. Frequency of the congenital transmission of *Trypanosoma cruzi*: a systematic review and meta-analysis. BJOG **2014**; 121:22–33.
3. Picado A, Cruz I, Redard-Jacot M, et al. The burden of congenital Chagas disease and implementation of molecular diagnostic tools in Latin America. BMJ Glob Health **2018**; 3:e001069.
4. Bern C, Verastegui M, Gilman RH, et al. Congenital *Trypanosoma cruzi* transmission in Santa Cruz, Bolivia. Clin Infect Dis **2009**; 49:1667–74.
5. Bua J, Volta BJ, Velazquez EB, Ruiz AM, Rissio AM, Cardoni RL. Vertical transmission of *Trypanosoma cruzi* infection: quantification of parasite burden in mothers and their children by parasite DNA amplification. Trans R Soc Trop Med Hyg **2012**; 106:623–8.
6. Rendell VR, Gilman RH, Valencia E, et al. *Trypanosoma cruzi*-infected pregnant women without vector exposure have higher parasitemia levels: implications for congenital transmission risk. PLoS One **2015**; 10:e0119527.
7. Murcia L, Carrilero B, Munoz-Davila MJ, Thomas MC, López MC, Segovia M. Risk factors and primary prevention of congenital Chagas disease in a nonendemic country. Clin Infect Dis **2013**; 56:496–502.
8. Hermann E, Truyens C, Alonso-Vega C, et al. Congenital transmission of *Trypanosoma cruzi* is associated with maternal enhanced parasitemia and decreased production of interferon-gamma in response to parasite antigens. J Infect Dis **2004**; 189:1274–81.
9. Scapellato PG, Bottaro EG, Rodríguez-Brieschke MT. Mother-child transmission of Chagas disease: could coinfection with human immunodeficiency virus increase the risk? Rev Soc Bras Med Trop **2009**; 42:107–9.
10. Agosti MR, Ercoli P, Dolcini G, Andreani G, Martínez Peralta L, González Ayala S. Two cases of mother-to-child transmission of HIV and *Trypanosoma cruzi* in Argentina. Braz J Infect Dis **2012**; 16:398–9.
11. Carlier Y, Truyens C. Congenital Chagas disease as an ecological model of interactions between *Trypanosoma cruzi* parasites, pregnant women, placenta and fetuses. Acta Trop **2015**; 151:103–15.

12. Carlier Y, Truyens C. Maternal-fetal transmission of *Trypanosoma cruzi*. In: Telleria J, Tibayrenc M, eds. American Trypanosomiasis-Chagas Disease. One Hundred Years of Research. 2nd ed. UK, USA: Elsevier; **2017**: pp 517–59.
13. Carlier Y, Schijman AG, Kemmerling U. Placenta, *Trypanosoma cruzi*, and congenital chagas disease. Curr Trop Med Rep **2020**; 7:172–82.
14. Bustos PL, Milduberger N, Volta BJ, Perrone AE, Laucella SA, Bua J. *Trypanosoma cruzi* infection at the maternal-fetal interface: implications of parasite load in the congenital transmission and challenges in the diagnosis of infected newborns. Front Microbiol **2019**; 10:1250.
15. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today **1993**; 14:353–6.
16. Raghupathy R. Th1-type immunity is incompatible with successful pregnancy. Immunol Today **1997**; 18:478–82.
17. Mor G, Aldo P, Alvero AB. The unique immunological and microbial aspects of pregnancy. Nat Rev Immunol **2017**; 17:469–82.
18. Vekemans J, Truyens C, Torrico F, et al. Maternal *Trypanosoma cruzi* infection upregulates capacity of uninfected neonate cells to produce pro- and anti-inflammatory cytokines. Infect Immun **2000**; 68:5430–4.
19. Cardoni RL, García MM, De Rissio AM. Proinflammatory and anti-inflammatory cytokines in pregnant women chronically infected with *Trypanosoma cruzi*. Acta Trop **2004**; 90:65–72.
20. García MM, De Rissio AM, Villalonga X, Mengoni E, Cardoni RL. Soluble tumor necrosis factor (TNF) receptors (sTNF-R1 and -R2) in pregnant women chronically infected with *Trypanosoma cruzi* and their children. Am J Trop Med Hyg **2008**; 78:499–503.
21. Cuna WR, Choque AG, Passera R, Rodriguez C. Pro-inflammatory cytokine production in chagasic mothers and their uninfected newborns. J Parasitol **2009**; 95:891–4.
22. Carlier Y, Altcheh J, Angheben A, et al. Congenital Chagas disease: updated recommendations for prevention, diagnosis, treatment, and follow-up of newborns and siblings, girls, women of childbearing age, and pregnant women. PLoS Negl Trop Dis **2019**; 13:e0007694.
23. Kemmerling U, Osuna A, Schijman AG, Truyens C. Congenital transmission of *Trypanosoma cruzi*: a review about the interactions between the parasite, the placenta, the maternal and the fetal/neonatal immune responses. Front Microbiol **2019**; 10:1854.
24. Ministerio de Salud de la Nación Argentina. [*Chagas: Atención del Paciente Infectado con Trypanosoma cruzi*]. 3ª ed. Ciudad Autónoma de Buenos Aires: Ministerio de Salud de la Nación; **2018**.
25. De Rissio AM, Riarte AR, García MM, Esteva MI, Quaglino M, Ruiz AM. Congenital *Trypanosoma cruzi* infection. Efficacy of its monitoring in an urban reference health center in a non-endemic area of Argentina. Am J Trop Med Hyg **2010**; 82:838–45.
26. Albareda MC, Natale MA, De Rissio AM, et al. Distinct treatment outcomes of antiparasitic therapy in *Trypanosoma cruzi*-infected children is associated with early changes in cytokines, chemokines, and T-cell phenotypes. Front Immunol **2018**; 9:1958.
27. Tagoma A, Haller-Kikkatalo K, Roos K, et al. Interleukin-7, T helper 1, and regulatory T-cell activity-related cytokines are increased during the second trimester of healthy pregnancy compared to non-pregnant women. Am J Reprod Immunol **2019**; 82:e13188.
28. Goines PE, Croen LA, Braunschweig D, et al. Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: a case-control study. Mol Autism **2011**; 2:13.
29. van der Giessen J, Binyamin D, Belogolovski A, et al. Modulation of cytokine patterns and microbiome during pregnancy in IBD. Gut **2020**; 69:473–86.
30. Robertson SA, Mau VJ, Young IG, Matthaei KI. Uterine eosinophils and reproductive performance in interleukin 5-deficient mice. J Reprod Fertil **2000**; 120:423–32.
31. Kayama H, Takeda K. The innate immune response to *Trypanosoma cruzi* infection. Microbes Infect **2010**; 12:511–7.
32. Prins JR, Gomez-Lopez N, Robertson SA. Interleukin-6 in pregnancy and gestational disorders. J Reprod Immunol **2012**; 95:1–14.
33. Montagnana M, Lippi G, Albiero A, Salvagno GL, Franchi M, Guidi GC. Serum pro-inflammatory cytokines in physiological and pre-eclamptic pregnancies. Gynecol Endocrinol **2008**; 24:113–6.
34. Martínez-García EA, Chávez-Robles B, Sánchez-Hernández PE, et al. IL-17 increased in the third trimester in healthy women with term labor. Am J Reprod Immunol **2011**; 65:99–103.
35. Wu HX, Jin LP, Xu B, Liang SS, Li DJ. Decidual stromal cells recruit Th17 cells into decidua to promote proliferation and invasion of human trophoblast cells by secreting IL-17. Cell Mol Immunol **2014**; 11:253–62.
36. Santner-Nanan B, Peek MJ, Khanam R, et al. Systemic increase in the ratio between Foxp3+ and IL-17-producing CD4+ T cells in healthy pregnancy but not in preeclampsia. J Immunol **2009**; 183:7023–30.
37. Poordast T, Najib FS, Baharlou R, Bijani A, Alamdarloo SM, Poordast A. Assessment of T helper 17-associated cytokines in third trimester of pregnancy. Iran J Immunol **2017**; 14:172–9.

38. Vespa GN, Cunha FQ, Silva JS. Nitric oxide is involved in control of *Trypanosoma cruzi*-induced parasitemia and directly kills the parasite in vitro. Infect Immun **1994**; 62:5177–82.
39. Hardison JL, Wrightsman RA, Carpenter PM, Lane TE, Manning JE. The chemokines CXCL9 and CXCL10 promote a protective immune response but do not contribute to cardiac inflammation following infection with *Trypanosoma cruzi*. Infect Immun **2006**; 74:125–34.
40. Carson WE, Giri JG, Lindemann MJ, et al. Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. J Exp Med **1994**; 180:1395–403.
41. Fehniger TA, Shah MH, Turner MJ, et al. Differential cytokine and chemokine gene expression by human NK cells following activation with IL-18 or IL-15 in combination with IL-12: implications for the innate immune response. J Immunol **1999**; 162:4511–20.
42. Azeredo EL, De Oliveira-Pinto LM, Zagne SM, Cerqueira DI, Nogueira RM, Kubelka CF. NK cells, displaying early activation, cytotoxicity and adhesion molecules, are associated with mild dengue disease. Clin Exp Immunol **2006**; 143:345–56.
43. Milano S, Di Bella G, D'Agostino P, et al. IL-15 in human visceral leishmaniasis caused by *Leishmania infantum*. Clin Exp Immunol **2002**; 127:360–5.