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Ex vivo infection of human placental chorionic villi explants with *Trypanosoma cruzi* and *Toxoplasma gondii* induces different Toll-like receptor expression and cytokine/chemokine profiles

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Problem: *Trypanosoma cruzi* and *Toxoplasma gondii* present, respectively, low and high congenital transmission rates. The placenta as an immune regulatory organ expresses TLRs, leading to the secretion of cytokines. Both parasites are recognized by TLR-2, TLR-4, and TLR-9. Here, we studied if the parasites induce differences in TLR protein expression, cytokine profiles, and whether receptor inhibition is related to parasite infection.

Method of study: Placental tissue explants were infected *ex vivo* with each parasite, TLRs protein expression, cytokine profile and parasite infection were determined by Western blotting, ELISA and qPCR.

Results: *Trypanosoma cruzi* and *Toxoplasma gondii* infection is related to TLR-2 and TLR-4/TLR-9, respectively. *Trypanosoma cruzi* elicits an increase in TNF-α, IL-1β, IL-6, IL-8 and IL-10 cytokine secretion whereas *T. gondii* only increases the secretion of IL-8.

Conclusion: The susceptibility of the placenta to each parasite is mediated partially by the innate immune response.

KEYWORDS
Cytokines, placental infection, Toll-like receptors, *Toxoplasma gondii*, *Trypanosoma cruzi*

1 | INTRODUCTION

Chagas disease (American Trypanosomiasis) is caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*) and constitutes a devastating but neglected health problem in Latin America. Due to the extensive global migration of asymptomatic people, Chagas disease has also become an emerging disease in non-endemic countries. Congenital transmission, in spite of its low transmission rates, is partially responsible for the progressive globalization of the disease.[1,2]

Toxoplasmosis is caused by another protozoan parasite, *Toxoplasma gondii* (*T. gondii*), one of the most successful parasites on earth. This parasite is estimated to infect over one billion people worldwide.[3] Although the majority of infected healthy individuals have no symptoms, in immunocompromised or congenitally infected individuals, the parasite can cause severe disease or even death and is often associated with damage to the brain, eyes or other organs.[4]

The congenital transmission of pathogens is the consequence of complex interactions among the parasite, maternal and fetal/newborn immune responses, and placental factors; the placenta is the least-studied component of this "trilogy".[5] Interestingly, the congenital transmission

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rate for *T. cruzi* is much lower (≈1%-12%)[2,6] than the transmission rate for *T. gondii* (≈ 22%-72%).[7] To reach the fetus, both parasites have to cross the placental barrier.[7] Therefore, the placenta might play an important role in avoiding or allowing parasite infection. The placental barrier in the human villous placenta is formed by the trophoblast, which contacts the maternal blood beneath the trophoblast, a basal lamina and an underlying villous stroma (VS), or connective tissue, are present, the latter includes vascular endothelium, fibroblastic cells and macrophages.[8,9]

Importantly, the placenta is considered to be an immune regulatory organ, since it acts as a modulator of fetal and maternal immune responses.[10] The innate immune response against pathogens is initiated by pathogen pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), which recognize and bind highly conserved sequences known as pathogen-associated molecular patterns (PAMPs). The human trophoblast expresses all 10 of the known functional TLRs.[11] *T. cruzi* and *T. gondii* are both recognized by TLR-2, TLR-4, TLR-7, and TLR-9.[12–14] Surface TLRs (TLR-2 and TLR-4) recognize glycosylphosphatidylinositol (GPI)-anchored lipids from both parasites and a profilin-like protein from *T. gondii*.[15,16] Intracellular receptors TLR-7 and TLR-9 recognize single-stranded RNA and CpG-rich DNA, respectively. Each TLR initiates downstream signaling that culminates in the activation of signaling pathways which regulate the expression of cytokines, chemokines, and interferons (IFNs).[17]

Here, we studied ex vivo-infected human chorionic villi explants (HPCVE) to determine whether *T. cruzi* and *T. gondii* induce differences in TLR protein expression and differential cytokine/chemokine profiles, and whether receptor inhibition is related to parasite infection. Our results show that *T. cruzi* infection is related to TLR-2 expression and activation while *T. gondii* infection is mediated by TLR-4 and TLR-9. Additionally, *T. cruzi* elicits increases in TNF-α, IL-1β, IL-6, IL-8, and IL-10 cytokine expression and secretion, whereas *T. gondii* increases the expression of TNF-α and IL-8 but only increases the secretion of IL-8.

2 | MATERIALS AND METHODS

2.1 | Cell cultures

VERO cells (ATCC® CCL-81) were grown in RPMI medium supplemented with 5% fetal bovine serum (FBS) and antibiotics (penicillin-streptomycin).[18] Human foreskin fibroblasts (HFF) (ATCC® SCRC-1041) were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS and antibiotics. Both cell cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2 and with replacement of the culture medium every 24-48 hours.[19]

2.2 | Parasite culture and harvesting

T. cruzi: Semi-confluent VERO cells were incubated with a culture of Ypsilon strain epimastigotes of *T. cruzi* in the late stationary phase. Trypomastigotes also present in that culture invade VERO cells and replicate intracellularly as amastigotes. After 72 hours, amastigotes transform back into trypomastigotes which lyse the host cells. The parasites were recovered by low-speed centrifugation (500×*g*), which produces trypomastigotes in the supernatant and amastigotes in the sediment.[18]

T. gondii: Semi-confluent HFF were infected with RH tachyzoites (kindly gifted by Dr. Sergio Angel, IIB-INTECH, CONICET-UNSAM, Chascomús, Buenos Aires, Argentina) at a multiplicity of infection of 3-5 parasites per cell. After 40 hours of infection, infected cells were washed, and monolayers were scraped from the flasks and passed through 20-, 23-, and 25-gauge needles. Tachyzoites were purified from host cell debris with a 3.0-μm Isopore filter (Merck Millipore Cork, Ireland).[19]

2.2.1 | Human placental chorionic villi (HPCVE) culture and parasite infection

Human term placentas were obtained from uncomplicated pregnancies from vaginal or caesarean delivery. Informed consent for the experimental use of the placenta was given by each patient as stipulated by the Code of Ethics of the Faculty of Medicine, University of Chile. Exclusion criteria were the following: major fetal abnormalities, placental tumor, intrauterine infection, obstetric pathology, positive serology for Chagas disease, and any other maternal disease. Donor patients were negative for anti-*T. gondii* IgG/IgM antibodies. The organs were collected in cold, sterile, saline-buffered solution (PBS) and were processed no more than 30 minutes after delivery. Maternal and fetal surfaces of each placenta were discarded, and the villous tissue was obtained from the central part of the cotyledons. The isolated chorionic villi were washed with PBS to remove blood, cut in approximately 0.5 cm^3 pieces, and co-cultured with *T. cruzi* trypomastigotes or *T. gondii* tachyzoites (1×10^5/mL) for 2 hours. For TLR inhibition, HPCVE were incubated in the presence and absence of blocking antibodies (TLR-2, -4; [Pab-hTLR2 and Pab-hTLR4 InvivoGen®]) or suppressive oligonucleotides (TLR-9; [ODN TTAGGG, InvivoGen®]) for 2 hours prior to and during parasite challenge. LPS and CpG-DNA were used as positive controls (LPS-PG and ODN 2395, InvivoGen®). All the experiments were performed in triplicate in at least three different placentas.

2.2.2 | Western blotting

HPCVE were homogenized in a lysis buffer (Tris 10 mM pH 8.0; SDS 1% w/v and protease inhibitor cocktail [Complete Mini (Roche®)]) at 4°C using a Potter-Elvehjem homogenizer, and the homogenate was centrifuged at 15 000×*g* for 15 minutes at 4°C to remove debris. Protein concentration was measured by the Bradford assay using Sigma bovine serum albumin as a protein standard. Fifty micrograms of protein was separated in a 10% sodium dodecyl sulfate–polyacrylamide gel, blotted onto a nitrocellulose membrane, and probed with monoclonal antibodies against TLR-2 (ab24192 Abcam®; 1:500, v/v), TLR-4 (ab13556 Abcam®; 1:500, v/v) or TLR-9 (ab134368 Abcam®; 1:1000, v/v). To correct for loading, membranes were stripped and re-probed with anti-human GAPDH (sc-51905 Santa Cruz Biotechnology®; 1:5000 v/v) antibody. Immunoreactive proteins were detected using enhanced chemiluminescence reagents according to the manufacturer's instructions (SuperSignal West Pico, Thermo Fisher). The films were scanned and the NIH-Image software program V 1.6 (NIH, Bethesda, MD) was employed for densitometric analysis of the bands.

2.2.3 | Immunohistochemistry

Standard immunoperoxidase methods[9] were used to show TLR-2, TLR-4, and TLR-9 in HPCVE. The primary antibodies were applied individually to each section at 4°C overnight (ab24192, ab13556 and ab134368 Abcam®; dilution 1:1000 v/v each). Immunostaining was performed using a horseradish peroxidase-labelled streptavidin biotin kit (RTU-Vectastain kit) following the manufacturer's directions and with diaminobenzidine as the chromogen. Sections were counterstained with Mayer's haematoxylin (Scytek) and mounted with Entellan (Merck®). Immunohistochemical controls performed by replacing the primary antibodies with phosphate-buffered saline were negative. All sections were examined by light microscopy (Leitz Orthoplan). Then, ten fields were selected randomly, the localization of the immunoreactivity was analyzed, and the signal intensity was scored as follows: ±, patchy; +, weak; ++, moderate; +++, high [25]. Images were captured with a Canon 1256 camera.

2.2.4 | DNA amplification by real-time PCR

Genomic DNA was extracted from the placental tissue with the Wizard Genomic DNA Purification Kit (Promega®) according to the manufacturer's instructions and quantified by QUBIT Fluorometric System (Invitrogen®). For amplification of human and parasite DNA, two specific pairs of primers were used. A 100-bp human GAPDH sequence was amplified using the primers hGDH-F (5′-TGATGCGTGTACAAGCGTTTT-3′) and hGDH-R (5′-ACATGGT ATTCACCACCCCACTAT-3′), which were designed using the Primer Express software (version 3.0; Applied Biosystems®). For *T. cruzi* DNA detection, a 182-bp sequence of satellite DNA was amplified by using TCZ primers: TCZ-F 5′-GCTCTTGCCCACAMGGGTGC-3′ and TCZ-R 5′-CAAGCAGCGGATAGTTCAGG-3′.[20] For *T. gondii* DNA detection, a 98-bp sequence of the *T. gondii* B1 gene was amplified by using TOXO-F: 5′-AGCGTTCGTCCTCAACTATCGATTG-3′ and TOXO-R:5′-TCCCCTCTGCTCGCGAAAAGT-3 primers.[21] Each reaction mix contained 200 nM of each primer (forward and reverse), 1 ng of DNA from samples, 12.5 μL of SensiMix® SYBR Green Master Mix (Bioline®) and H_20 for a total of 25 μL. Amplification was performed in an ABI Prism 7300 sequence detector (Applied Biosystems®). The cycling programs were as follows: *T. cruzi*: A first step at 20°C for 2 minutes, a denaturation step at 95°C for 10 minutes and 40 amplification cycles of: 95°C (15 seconds), 60°C (15 seconds) and 72°C (30 seconds). Finally, a dissociation stage was added ranging from 60 to 95°C.[22] *T. gondii:* a first step at 50°C for 2 minutes, a denaturation step at 95°C for 10 minutes, and 40 amplification cycles of 90°C (15 seconds), 60°C (15 seconds), and 72°C (30 seconds) following a dissociation stage ranging from 60 to 95°C.[21] Relative quantification analysis of the results was expressed as RQ values by the comparative Control (ΔΔCt) method.[22,23]

2.2.5 | ELISA assays

The concentrations of TNF-α (KHC3011, Thermo Fisher Scientific®), IL-1β (KHC00111, Thermo Fisher Scientific®), IL-6 (KHC0062, Thermo Fisher Scientific®), IL-8 (KHC0082, Thermo Fisher Scientific®), IL-10 (KHC0102, Thermo Fisher Scientific®), and IFNγ (KAC1231, Thermo Fisher Scientific®) in the HPCVE culture supernatants were determined using commercial ELISA kits according to the manufacturer's instructions. Data were normalized with respect to the values obtained in control conditions. Intra- and inter-assay coefficients of variation were less than 10%.

2.2.6 | Statistics

The results are expressed as the mean±SD. The significance of differences was evaluated using Student's *t* tests for paired data or ANOVA followed by Dunnett's posttest as indicated.

3 | RESULTS

3.1 | *T. cruzi* and *T. gondii* induce different protein expression of TLR-2, TLR-4, and TLR-9 in HPCVE

HPCVE were co-incubated with 10^5 *T. cruzi* trypomastigotes or *T. gondii* tachyzoites for 2 hours; LPS and CpG-DNA were used as positive controls. *T. cruzi* trypomastigotes induced statistically significant increases in TLR-2 (284.01±8.95%; $P \leq .0001$, Figure 1A) and TLR-4 (146±5.16%; $P \leq .0001$, Figure 1B) protein expression compared to non-infected or *T. gondii*-infected explants. However, *T. gondii* tachyzoites induced a significant increase of TLR-9 compared to *T. cruzi* challenged or non-infected samples (32.99±1.27%; $P \leq .05$, Figure 1C). The increase in TLR expression in HPCVE after the incubation with the each of the parasites was confirmed by immunohistochemistry. *T. cruzi* induced strong (+++), moderate (++), and weak (+) immunoreactivity in the trophoblast and villous stroma for TLR-2, TLR-4, and TLR-9, respectively. In the same tissue compartments, *T. gondii* induced weak (+), patchy (±), and strong (+++) immunoreactivity for TLR-2, TLR-4, and TLR-9, respectively. Control non-infected HPCVE presented patchy (±) immunoreactivity for each of the studied TLRs (Figure 2).

3.2 | TLR-2 inhibition increases *T. cruzi* DNA load in HPCVE

HPCVE were co-incubated with 10^5 *T. cruzi* trypomastigotes in the presence and absence of blocking antibodies (TLR-2, TLR-4) or suppressive oligonucleotides (TLR-9) for 24 hours. Only the inhibition of TLR-2 significantly increased the amount of parasite DNA in the HPCVE (267.07±2.10%; $P \leq .001$, Figure 3A); the inhibition of TLR-4 and TLR-9 did not change the parasite DNA load in the placental tissue.

3.3 | TLR-4 and TLR-9 inhibition increases *T. gondii* DNA load in HPCVE

HPCVE were co-incubated with 10^5 *T. gondii* tachyzoites in the presence and absence of blocking antibodies (TLR-2, TLR-4) or suppressive oligonucleotides (TLR-9) for 24 hours. Contrary to the results with *T. cruzi*, the inhibition of both TLR-4 (460±6.50%, $P \leq .001$) and

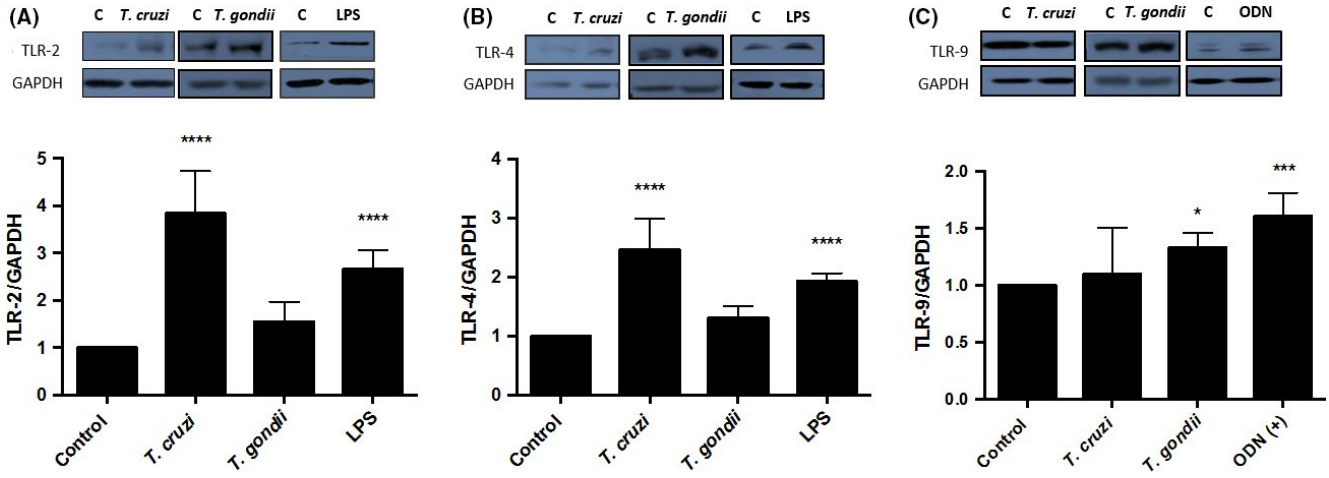

FIGURE 1 *Trypanosoma cruzi* and *Toxoplasma gondii* induce different protein expression of TLR-2, TLR-4, and TLR-9 in HPCVE: HPCVE were co-incubated with 10^5 *T. cruzi* trypomastigotes or *T. gondii* tachyzoites for 2 h; LPS and CpG-DNA were used as positive controls. Relative expression of TLR-2 (panel A), TLR-4 (panel B), and TLR-9 (panel C) was assayed by Western blot. Values were corrected to GAPDH as loading controls, and data were normalized with respect to control values and analyzed by ANOVA followed by Dunnett's posttest. All values are given as the mean±S.D. and correspond to at least three independent experiments carried out in duplicates or triplicates. **P*<.05, ****P*≤.001, *****P*≤.0001

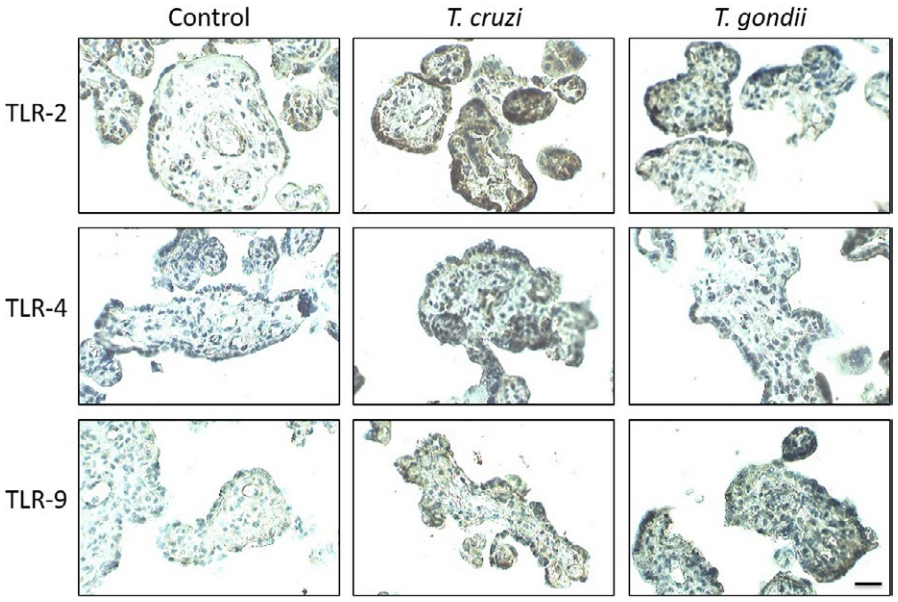

FIGURE 2 *Trypanosoma cruzi* and *Toxoplasma gondii* induce different protein expression of TLR-2, TLR-4, and TLR-9 in HPCVE: HPCVE incubated with *T. cruzi* showed an increase in TLR-2 and TLR-4 immunoreactivity in the trophoblast as well as in the villous stroma compared to control non-treated explants. In the same tissue compartments, HPCVE incubated with *T. gondii* showed an increase in TLR-9 compared to control samples. Bar scale: 25 μm

TLR-9 (375±5.40% *P*≤.01) significantly increased the amount of *T. gondii* DNA in the placental tissue (Figure 3B).

3.4 | *T. cruzi* and *T. gondii* induce differential secretion of TNFα, IL-1β, IL-6, IL-10, IL-8, and IFNγ in HPCVE

HPCVE were co-incubated with 10^5 *T. cruzi* trypomastigotes or *T. gondii* tachyzoites for 2 hours as well as in the presence and absence of LPS as positive controls. Cytokines were measured in the culture supernatant. *T. cruzi* induced significant increases in TNF-α (2706.18±377.9%; *P*≤.05, Figure 4A), IL-1β (2706.18±2954.03% *P*≤.0001, Figure 4B), IL-6 (63.09±4.03%; *P*≤.05, Figure 4C), IL-8 (1001.88±76.83%; *P*≤.0001, Figure 4D), and IL-10 (440.34±14.32%; *P*≤.0001, Figure 4E). In contrast, *T. gondii* induced a significant increase in IL-8 (2315.79±470.68%; *P*≤.0001, Figure 4D) secretion but did not affect the other cytokines that were induced by *T. cruzi*. Interestingly, neither parasite induced a significant increase of IFNγ (Figure 4F).

4 | DISCUSSION

The interaction between the host and pathogens (in this case, parasites) is the most important factor in determining whether an infection is successful or not. Host–parasite interaction includes invasion of the host through primary barriers (such as the placental barrier), evasion

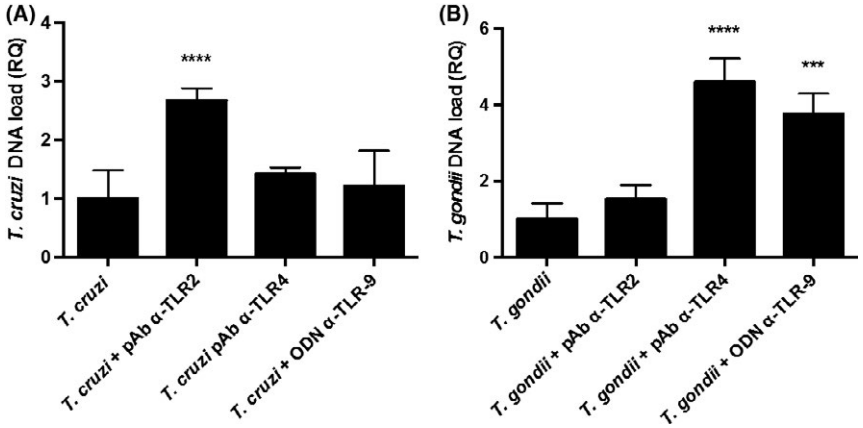

FIGURE 3 Ex vivo infection of *Trypanosoma cruzi* and *Toxoplasma gondii* in HPCVE is mediated by different TLRs: HPCVE were co-incubated with 10^5 *T. cruzi* trypomastigotes (panel A) or T. gondii tachyzoites (panel B) for 2 h. For TLR inhibition, explants were incubated prior to infection with neutralizing antibodies (PAb-hTLR2; PAb-hTLR4); or inhibitory oligonucleotides (ODN-TTAGGG). Parasite load on explants was assayed by qPCR. Data were normalized with respect to control values and analyzed by ANOVA followed by Dunnett's posttest. All values are given as the mean±SD and correspond to at least three independent experiments carried out in duplicates or triplicates. ***$P \leq .001$, ****$P \leq .0001$

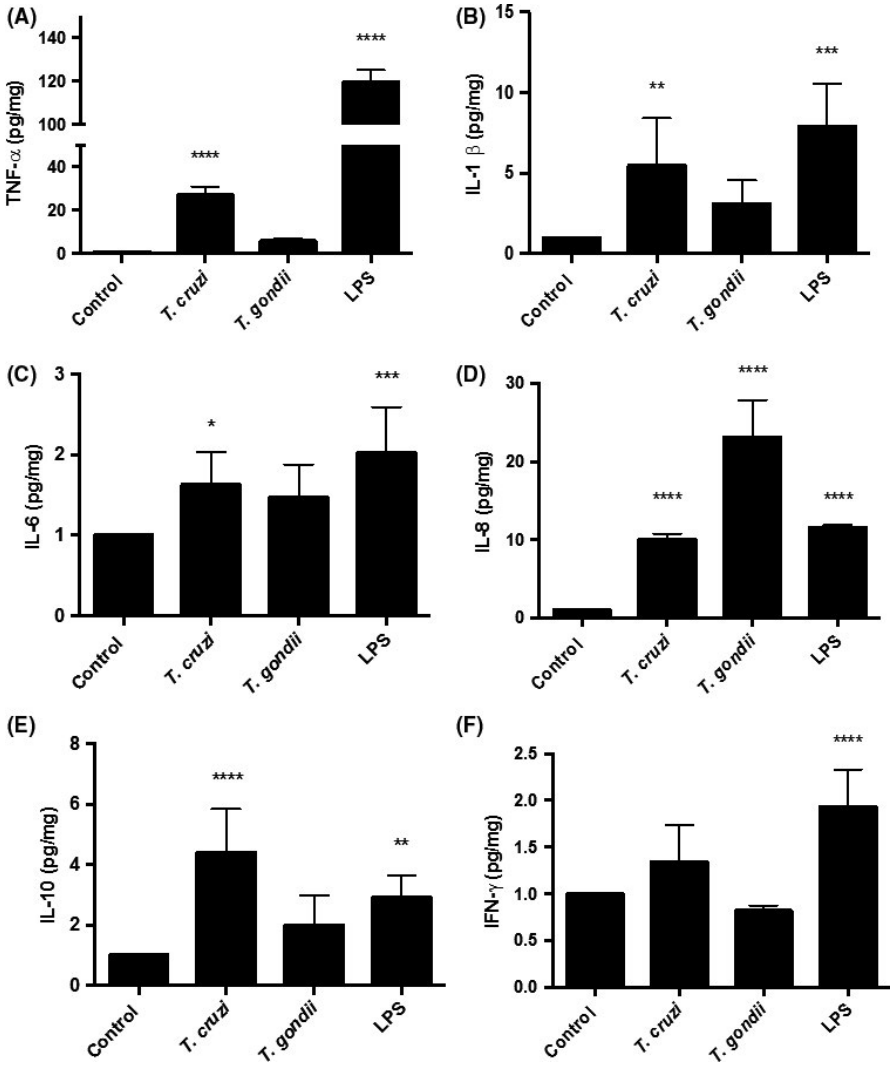

FIGURE 4 *Trypanosoma cruzi* and *Toxoplasma gondii* induce differential secretion of TNF-α, IL-1β, IL-6, IL-10, IL-8, and IFN-γ in ex vivo infected HPCVE: HPCVE were co-incubated with 10^5 *T. cruzi* trypomastigotes or T. gondii tachyzoites for 2 h; LPS was used as positive control. Culture media supernatants were collected, and presence of TNF-α (panel A), IL-1β (panel B), IL-6 (panel C), IL-8 (panel D), IL-10 (panel E), and IFN-γ (panel F) was assayed by ELISA with commercial kits. Data were normalized with respect to control values and analyzed by ANOVA followed by Dunnett's posttest. All values are given as the mean±SD and correspond to at least three independent experiments carried out in duplicates or triplicates. *$P < .05$, ** $P \leq .01$, ***$P \leq .001$, ****$P \leq .0001$

of host defenses, pathogen replication in the host, and immunological capacity of the host to control or eliminate the pathogen.[24] TLRs are PRRs that participate in the initiation of the innate immune response against pathogens. Because the two parasites *T. cruzi* and *T. gondii* present both extracellular and intracellular forms during their respective life cycles, different TLRs recognizing these different morphological

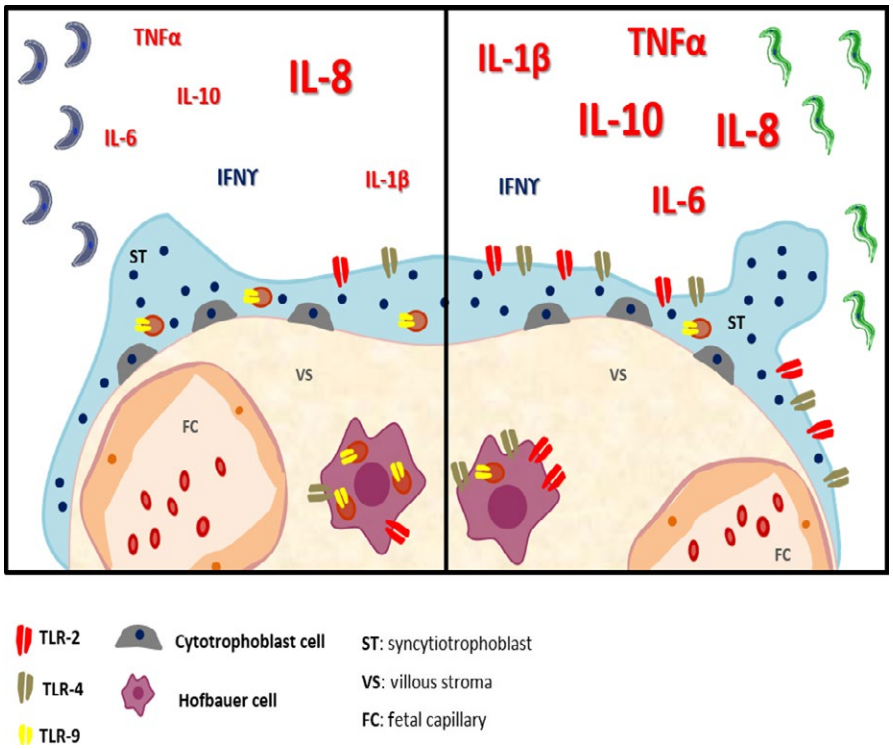

FIGURE 5 *Trypanosoma cruzi* and *Toxoplasma gondii* induce differential expression of TLR-2, TLR-4, and TLR-9 as well as different cytokine/chemokine profiles in ex vivo-infected HPCVE: *T. cruzi* induces, in ex vivo-infected HPCVE, increases of TLR-2 and TLR-4 protein expression as well as of secretion of IL-1β, IL-6, TNF-α, IL-10, and IL-8 (right panel). In contrast, *T. gondii* induces increases of TLR-9 protein expression and IL-8 secretion (left panel). The differential TLR activation and cytokine/chemokine profile might explain, at least partially, the different susceptibility of the placenta to both parasites

stages are activated during infection,[14] particularly TLR-2, TLR-4, TLR-7, and TLR-9.[12–14]

Dendritic cells are the most important host immune cells involved in the activation of the innate and adaptive immune responses against parasites. Dendritic cells undergo maturation via TLRs, and recognize, capture and process antigens in peripheral tissues; once activated, dendritic cells present antigens and stimulate T cells to produce cytokines such as IL-12, IL-10, TNF-a, and IL-6.[25] However, both *T. cruzi* and *T. gondii* are able to infect as well as activate TLRs in non-phagocytic cells leading to activation of the innate immune response against them. Since pathogens express multiple PAMPs that act as TLR agonists, the result is a unique, complex and overlapping Th1–Th2 cytokine/chemokine profile.[26] Therefore, the pro- or anti-inflammatory microenvironments induced by both parasites depend on the specific cytokines present.

Here, we have shown that *T. cruzi* induced TLR-2 and TLR-4, but not TLR-9, protein expression in HPCVE (Figure 1-2). In cardiomyocyte culture, *T. cruzi* induces TLR-2 protein expression, and it is postulated that the increase in this particular PRR is related to cardiomyocyte survival. In the same study, an increase of TLR-4 expression was not observed.[27] Moreover, in both white and brown adipose tissue, TLR-2 and TLR-9 expression is increased while TLR-4 expression is exclusively increased in white adipose tissue.[28]

In the present study, *T. gondii* induced a significant increase of TLR-9 protein expression, but did not affect TLR-2 or TLR-4 expression in HPCVE (Figure 1-2). In murine intestinal epithelial cells, *T. gondii* increases the expression of TLR-2, TLR-4 and TLR-9.[29] However, it has been shown that TLR-9 is not required for the development of *T. gondii*-induced ileitis in mice but that TLR-9 mediates distinct inflammatory changes in intestinal and extra-intestinal compartments including the brain.[30] Likely, is a more effective immune response against *T. gondii* in the digestive epithelia since it is the main infection route of this parasite in the intermediate hosts.[31] Another protozoan, *Leishmania infantum*, induces significant downregulation of TLR- 4 and TLR-9 transcription in lymph nodes, but a significant upregulation of TLR-9 in skin from infected dogs.[32] Patients with Visceral Leishmaniasis, caused by *Leishmania donovani*, have high levels of expression of TLR-4 and TLR-9 in blood cells.[33] Therefore, the differences observed in the responses against parasites depend not only on the pathogen but also, at least partially, on the tissue or organ invaded.

The activation of TLRs leads to the production of cytokines and antimicrobial factors via a common intracellular signaling pathway.[11] The fact that the ex vivo infection of both parasites is mediated by different TLRs could be explained, at least partially, by the particular cytokine/chemokine profile induced by each of them (Figure 3)

In HPCVE, *T. cruzi* induces a significant increase of cytokine levels observed in the culture media, specifically significant increases of IL-1β, IL-6, TNF-α, IL-8, and IL-10. In contrast, *T. gondii* only induces a significant increase of IL-8 secretion and does not affect any of the cytokines that are induced by *T. cruzi* (Figure 4). IL-1β, IL-6, IL-8, and TNF-α are pro-inflammatory cytokines whereas IL-10 is anti-inflammatory.[34] In HPCVE, the cells, responsible of parasite-induced cytokine production, are those of the trophoblast as well as the macrophages (or Hofbauer cells) in the villous stroma.[8] The pro-inflammatory cytokines are involved in pathogen clearance and, if their action is prolonged, tissue damage. IL-10 dampens the effects of the pro-inflammatory cytokines, but has also been shown to be involved in proliferation and anti-apoptotic activities of several cancers.[35] We

have previously shown that *T. cruzi* induces tissue damage in HPCVE,[9] and placental antiparasitic mechanisms, such as trophoblast epithelial turnover, lead to the induction of cellular proliferation, differentiation and death.[36] However, *T. gondii* induces more severe tissue damage than *T. cruzi*. Such greater damage by *T. gondii* might be explained by the fact that this parasite does not induce the IL-10 anti-inflammatory cytokine, and this is probably partially responsible for the impaired induction of local placental anti-parasite response.[37] Accordingly, it has been demonstrated that sera of uninfected newborns (*T. gondii*) from seropositive mothers have higher IL-10 levels.[38]

Interestingly, neither of the parasites induced a significant increase of IFNγ in our experimental model (Figure 4). IFNγ is critical for coordinating protective immunity against infection with intracellular pathogens, in particular *T. gondii*.[39,40] However, it has been demonstrated that IFNγ is unable to control *T. gondii* infection in the trophoblast-derived cell line BeWo.[41] This is in accordance with the fact that infants with congenital Chagas disease or Toxoplasmosis present lower IFNγ levels in their sera than healthy, non-infected infants.[38] In normal pregnancies, semiallogeneic trophoblast cells are not subject to transplant rejection reactions by the maternal immune system due in part to intrinsic regulatory mechanisms that prevent IFNγ-induced expression of MHC II molecules. Moreover, gestational complications, including fetal loss, have been linked to elevation in IFNγ levels.[42]

5 | CONCLUSION

Ex vivo infection of HPCVE with *T. cruzi* or *T. gondii* elicits differential TLR activation and cytokine/chemokine profiles, which might explain, at least partially, the different susceptibility of the placenta to both parasites (Figure 5).

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