Experimental Parasitology 168 (2016) 9–15

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Full length article

Caspase-8 activity is part of the BeWo trophoblast cell defense mechanisms against *Trypanosoma cruzi* infection

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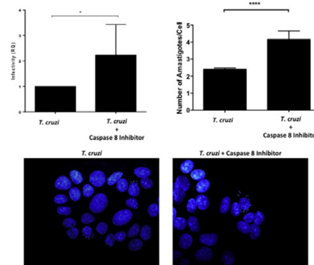
HIGHLIGHTS

- *Trypanosoma cruzi* induces caspase 8 activity in a trophoblast cell line (BeWo).
- Caspase 8 inhibition increases *T. cruzi* infection in BeWo cells.
- Caspase 8 activity might be part of BeWo cell defenses.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 May 2016
Accepted 17 June 2016
Available online 18 June 2016

Keywords:
Caspase-8
Trophoblast defense mechanism
Congenital Chagas disease

ABSTRACT

Congenital Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi* that must cross the placental barrier during transmission. The trophoblast constitutes the first tissue in contact with the maternal-blood circulating parasite. Importantly, the congenital transmission rates are low, suggesting the presence of local placental defense mechanisms. Cellular proliferation and differentiation as well as apoptotic cell death are induced by the parasite and constitute part of the epithelial turnover of the trophoblast, which has been suggested to be part of those placental defenses. On the other hand, caspase-8 is an essential molecule in the modulation of trophoblast turnover by apoptosis and by epithelial differentiation. As an approach to study whether *T. cruzi* induced trophoblast turnover and infection is mediated by caspase-8, we infected BeWo cells (a trophoblastic cell line) with the parasite and determined in the infected cells the expression and enzymatic activity of caspase-8, DNA synthesis (as proliferation marker), β-human chorionic gonadotropin (β-hCG) (as differentiation marker) and activity of Caspase-3 (as apoptotic death marker). Parasite load in BeWo cells was measured by DNA quantification using qPCR and cell counting.

Our results show that *T. cruzi* induces caspase-8 activity and that its inhibition increases trophoblast cells infection while decreases parasite induced cellular differentiation and apoptotic cell death, but not

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http://dx.doi.org/10.1016/j.exppara.2016.06.008
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cellular proliferation. Thus, caspase-8 activity is part of the BeWo trophoblast cell defense mechanisms against *T. cruzi* infection. Together with our previous results, we suggest that the trophoblast turnover is part of local placental anti-parasite mechanisms.

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1. Introduction

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*) and transmitted by triatominae vectors, constitutes a devastating health problem in Latin America. Moreover, Chagas disease is one of the "Top 10" in the ranking of major neglected tropical diseases by disability-adjusted life years (DALYs) (Hotez et al., 2015). Due to the extensive global migration of asymptomatic people, Chagas disease has also become an emerging disease in non-endemic countries. The congenital transmission allows *T. cruzi* to expand within a developed country where the vector bugs are not common or where human contact with them is limited (Perez-Molina et al., 2015; Smith Darr and Conn, 2015). Interestingly, in spite of the high serologic prevalence, that may reach 80% among pregnant woman in endemic countries, the transmission rate to fetuses is low (≈1–12%) (Perez-Molina et al., 2015; Rendell et al., 2015).

Congenital transmission of pathogens is the consequence of complex interactions between the pathogen, maternal and fetal/newborn immune responses as well as the placenta, being the latter the least-studied factor. It has been proposed, that the epithelial turnover of the villous trophoblast, the first tissue of the placenta in contact with maternal-blood circulating pathogens, could be part of local defense mechanisms of the human placenta (Liempi et al., 2014). The trophoblast is a lining epithelium, that suffers a continuous turnover (Bernischke et al., 2006). The epithelial (Murphy, 2011) as well as the trophoblast turnover (Liempi et al., 2014) are considered part of the innate immune system, since pathogens must adhere to cells prior invasion and those attached to a lining epithelium are continuously eliminated with the superficial cells (Liempi et al., 2014) (Murphy, 2011).

The turnover in the trophoblast implies a precise orchestration of various cellular processes including cellular proliferation of the basal cytotrophoblast (CTB), differentiation or syncytial fusion by incorporating CTB cells into a non-replicative superficial syncytiotrophoblast layer (STB) and cell death (Berniscke et al., 2006; Gauster et al., 2009a; Ji et al., 2013). We have previously shown that *T. cruzi* induces cellular proliferation, differentiation and cell death in the trophoblast (Droguett AL et al., 2013; Duaso et al., 2011; Liempi et al., 2014), suggesting that the trophoblast turnover could be a local antiparasite mechanism.

CTB differentiation and fusion is regulated by multiple factors including apoptotic cell death involved caspases (aspartate-specific cysteine proteases) (Gauster et al., 2009b). These enzymes are not only known for their key roles in programmed cell death but also for being involved in other biologic processes such as cellular differentiation (Shalini et al., 2015). Particularly, caspase-8, an apoptosis initiator caspase, has been proposed to regulate trophoblast differentiation and fusion. For instance, caspase-8 is activated in highly differentiated CTB cells just prior to fusion and has not been found in proliferating CTB cells (Huppertz and Borges, 2008; Pidoux et al., 2012). Moreover, fusion of the trophoblast can be visualized by localizing caspase-8 (Gauster et al., 2009b).

Here we studied if the *T. cruzi* induced trophoblast turnover and capacity of infection is mediated by caspase-8. In BeWo cells (a trophoblastic cell line) we determined the expression and enzymatic activity of caspase-8, DNA synthesis (as proliferation marker), β-human chorionic gonadotropin (β-hCG) (as differentiation marker) and activity of Caspase-3 (as apoptotic death marker). Parasite load in BeWo cells was measured by DNA quantification by qPCR and cell counting. Our results show that *T. cruzi* induces caspase-8 activity and that its inhibition increases the infection of BeWo cells by the parasite while impairing cellular differentiation and cell death.

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) (Villalta and Kierszenbaum, 1982). BeWo cells (ATCC CCL-98) were grown in DMEM-F12K medium supplemented with 10% FBS, L-glutamine and antibiotics (penicillin-streptomycin) (Drewlo et al., 2008). Cells were grown at 37 °C in a humidified atmosphere with 5% CO_2, with replacement of the culture medium every 24–48 h.

2.2. Infection of VERO cells with T. cruzi and trypomastigote harvesting

After confluence, 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 h, amastigotes transform back into trypomastigotes, which lyses the host cells. The parasites were recovered by low-speed centrifugation (500*g*), which produces trypomastigotes in the supernatant and amastigotes in the sediment (Villalta and Kierszenbaum, 1982).

2.3. Infection of BeWo cells with T. cruzi trypomastigotes

2×10^5 BeWo cells were incubated in presence or absence of *T. cruzi* trypomastigotes and in presence or absence of 5 μM of the Caspase-8 activity inhibitor IETD-CHO (BD-Biosciences®). FBS (10%), Forskolin (50 μM) (Sigma-Aldrich®) or Staurosporine (2 μM) (Sigma-Aldrich®) were used as positive controls for cellular proliferation, cellular differentiation and apoptotic cell death, respectively. For cellular proliferation analysis, BeWo cells were previously synchronized by serum deprivation as described previously (Langan and Chou, 2011). The cells were analyzed at 48 h post-infection. For *T. cruzi* amastigote identification, infected cells were processed for DAPI (4, 6-diamidino-2-phenylindole, Molecular Probes; 1 μg DAPI/ml) nuclear staining and amastigotes were recognized by their nuclear size and presence of kinetoplast (Fig. 4C) and quantified using MATLAB® software (Liempi et al., 2015).

2.4. Western blotting

BeWo cells were incubated in RIPA buffer (50 mM Tris, 150 mM NaCl, 0.1% SDS, 0.5% Na Deoxycholate, 1% Triton X100,

1 mM PMSF and a protease inhibitor cocktail [Complete Mini, Roche®]); afterwards, cells were sonicated for 1 min and maintained on ice for 20 min. The cell lysates were centrifuged at 15.000*g* for 20 min to remove debris. Protein concentration was measured using the Bradford assay, with Sigma bovine serum albumin as the protein standard. Then, 30 μg of protein was separated on a 10% sodium dodecyl sulfate polyacrylamide gel, blotted onto a nitrocellulose membrane, and probed with monoclonal antibodies against caspase-8 (sc-56070, Santa Cruz Technologies®; 1:1000 v/v) and against cleaved caspase 8 (Asp391) (#9496, Cell Signalling®; 1:1000 v/v). To correct for loading, the membranes were stripped and re-probed with an anti-human GAPDH (sc-51905 Santa Cruz Technologies®; 1:500 v/v) antibody. Immunoreactive proteins were detected using enhanced chemiluminescence reagents according to the manufacturer's instructions (Amersham Biosciences® UK, Ltd.). The films were scanned, and NIH-Image v1.6 (NIH, Bethesda, MD) was used for the densitometric analysis of the bands.

2.5. Enzyme activity

Caspase-3 (Caspase-Glo®3/7 Assay, Promega®) and caspase-8 (Caspase-Glo®8 Assay, Promega®) enzyme activities were determined by commercial kits according to the manufacturer's instructions. Briefly, Caspase-3 and 8 activities were determined by luminescence using luminogenic Caspase-3 (Ac-DEVD-pNA) or 8 (Ac-LETD-pNA) substrates, which reacts after cleavage by Caspase-3 or caspase-8 with a thermostabile luciferase. Luminescence was read in a microplate reader (Varioskan® Flash). Data were normalized with respect to the values obtained in control conditions. Intra-assay coefficients of variation were less than 10%.

2.6. DNA amplification by real-time PCR (qPCR)

Genomic DNA was extracted from BeWo cells with a Wizard Genomic DNA Purification Kit (Promega®, USA) according to the manufacturer's instructions. The resulting DNA was quantified with a μDrop Plate DNA quantification system in a Varioskan Flash Multimode Reader (Thermo Scientific, USA). For amplification of human and parasite DNA, two specific primer pairs were used. A 100 bp human GAPDH sequence was amplified using the primers hGDH-F (5′-TGATGCGTGTACAAGCGTTTT-3′) and hGDH-R (5′-ACATGGTATTCACCACCCCACTAT-3′), which were designed using Primer Express software (version 3.0; Applied Biosystems®). For *T. cruzi* DNA detection, a 182 bp sequence of satellite DNA was amplified using the primers TCZ-F (5′-GCTCTTGCCCACAMGGGTGC-3′) and TCZ-R (5′-CAAGCAGCGGATAGTTCAGG-3′) (Castillo et al., 2012; Cummings and Tarleton, 2003). Each reaction mix contained 200 nM of each primer (forward and reverse), 1 ng of DNA, 12.5 μL of SensiMix® SYBR Green Master Mix (Bioline®, USA) and $H_2$0 for a total volume of 25 μL. The amplification was performed in an ABI Prism 7300 sequence detector (Applied Biosystems®, USA). The cycling program was as follows: an initial incubation at 20 °C for 2 min, a denaturation step at 95 °C for 10 min and 40 amplification cycles of 95 °C (15 s), 60 °C (15 s) and 72 °C (30 s). The final step was a dissociation stage that ranged from 60 to 95 °C (105 s). The relative quantification analysis of the results was expressed as an RQ value determined using the comparative control (ΔΔCt) method (Castillo et al., 2012; Pfaffl, 2001).

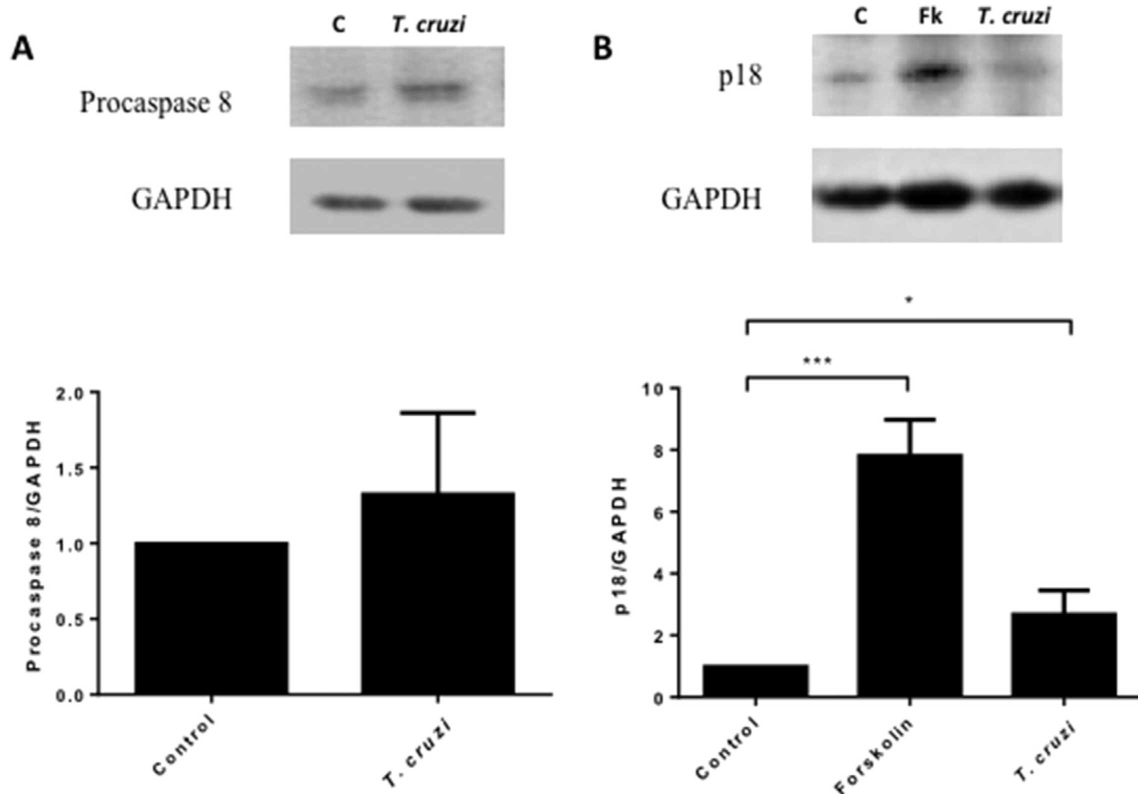

Fig. 1. ***T. cruzi*** **trypomastigotes induce caspase-8 cleavage in BeWo cells**. BeWo cells were incubated in the presence and absence of *T. cruzi* trypomastigotes for 24 h at a cell:parasite ratio of 1:0.1 or with Forskolin (50 μM) as a positive control. A (left panel), representative Western blots for caspase-8 and GAPDH from BeWo cells incubated in presence and absence of the parasite. The bar graph represents the ratios of Caspase-8 to GAPDH normalized in terms of the values obtained in control cells. B (right panel), representative Western blots for cleaved caspase-8 (p18) and GAPDH from BeWo cells under the different experimental conditions. The bar graph represents the ratios of cleaved caspase-8 to GAPDH normalized in terms of the values obtained in control cells. All values are given as the means ± S.D., and they correspond to at least 3 independent experiments that were performed in duplicate or triplicate. Data were normalized in terms of the control values and analyzed by Student *t*-test (A) or ANOVA followed by Dunnett's post-test (B). All values, given as the means ± S.D., correspond to at least 3 independent experiments performed in triplicate. $^{*}p \leq 0.05$; $^{***}p \leq 0.001$.

2.7. BrdU incorporation assay

BrdU incorporation in DNA was measured by spectrophotometry (B2851-20, USBiological®) according manufacturer's instructions. Data were normalized with respect to the values obtained in control conditions. Intra-assay coefficients of variation were less than 10%.

2.8. Detection of β-hCG in the culture medium

The concentrations of β-hCG in the cell culture supernatants were determined using the β-hCG ELISA Kit (DRG®βHCG ELISA (EIA1911)) according to the manufacturer's instructions. Data were normalized with respect to the values obtained in control conditions. Intra-assay coefficients of variation were less than 10%.

2.9. Statistics

All experiments were performed in triplicate. Results are expressed as means ± S.D. The significance of differences was evaluated using Student's t-test for paired data or ANOVA followed by Dunnett's post test as indicated.

3. Results

3.1. *T. cruzi* trypomastigotes induce caspase-8 activity in BeWo cells

BeWo cells were incubated in presence and absence of *T. cruzi* trypomastigotes at a cell:parasite ratio of 1:0.1 and in presence or absence of the caspase-8 activity inhibitor (IETD-CHO, 5 μM) for 48 h. The parasite does not induce a significant increase of pro-caspase-8 expression (Fig. 1A), but induces a significant increase (2.69 ± 0.757 ($p \leq 0.05$)) of the cleaved and therefore activated p18 fragment of the enzyme (Fig. 1B). To confirm the activation of caspase-8, its enzymatic activity was determined after *T. cruzi* infection. Indeed, the parasite induces an increase in caspase-8 activity (1.71 ± 0.263; $p \leq 0.01$, Fig. 2) that is similar to the observed activity in the positive control (1.66 ± 0.269; $p \leq 0.01$, Fig. 2). Inhibition of caspase-8 with the specific activity inhibitor prevents the parasite-induced activity.

3.2. Inhibition of caspase-8 activity increases *T. cruzi* BeWo cell infection

BeWo cells were incubated with *T. cruzi* trypomastigotes at a cell:parasite ratio of 1:0.1 and in presence or absence of the above mentioned caspase-8 activity inhibitor for 48 h. The inhibition of caspase-8 activity significantly increases (2.23 ± 1.20; $p \leq 0.05$) the parasite DNA load in BeWo cells respect to infected control cells (Fig. 3). Additionally, we determined the percentage of infected cells as well as the number of amastigotes (white arrows, Fig. 4C) (intracellular replicative form of the parasite) per BeWo cell (Fig. 4). Interestingly, there is no significant difference in the percentage of infected cells comparing caspase-8 activity inhibitor treated cells and control ones (Fig. 4A and C). However, the number of amastigotes per BeWo cell increases significantly from 2.40 ± 0.072 to 4.15 ± 0.501 ($p \leq 0.0001$) (Fig. 4B and C).

3.3. Inhibition of caspase-8 activity decreases the parasite-induced cellular differentiation and apoptotic cell death, but not the cellular proliferation, in BeWo cells

Since the trophoblast turnover has been proposed to be a possible local anti-parasite mechanism (Liempi et al., 2014), we analyzed the effect of caspase-8 activity inhibition on trophoblast BeWo cells proliferation, differentiation and cell death.

BeWo cells were incubated in presence and absence of *T. cruzi* trypomastigotes at a cell:parasite ratio of 1:0.1 and in presence or absence of the caspase-8 activity inhibitor for 48 h. FBS, Forskolin and Staurosporine were used as positive control for cellular proliferation, differentiation and apoptotic cell death, respectively. For cellular proliferation analysis, BeWo cells were previously synchronized by serum deprivation. Caspase-8 inhibition does not affect DNA synthesis parasite-exposed cells as measured by BrdU incorporation (Fig. 5A).

On the other hand, the inhibition of caspase-8 activity prevents significantly the parasite-induced cellular differentiation as measured by determining the secretion of β-hCG, the main biochemical trophoblast differentiation marker, (Fig. 5B). The parasite increases 2.48 ± 0.217 ($p \leq 0.001$) the amount of β-hCG, in presence of the inhibitor the increase of hormone secretion is not statistically significant respect to the control not treated BeWo cells.

Finally, we analyzed the effect of caspase-8 inhibition on parasite induced apoptotic cell death by measuring caspase-3 like activity (effector caspase). *T. cruzi* increases significantly the caspase-3 like activity (1.45 ± 0.098 ($p \leq 0.01$)) respect to control levels. Caspase-8 activity inhibition prevent the significant increase of parasite-induced caspase-3 activity (0.84 ± 0.047 (Fig. 5C)).

4. Discussion

The low congenital transmission rates of *T. cruzi* (Duaso et al., 2011; Perez-Molina et al., 2015; Rendell et al., 2015) together with the absence of typical amastigote nests (obligate intracellular

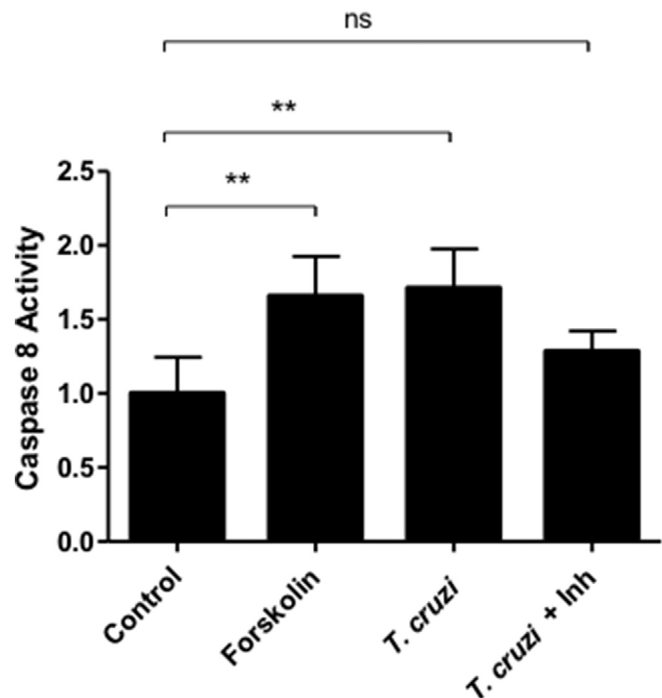

Fig. 2. *T. cruzi* trypomastigotes induce caspase-8 activity in BeWo cells. BeWo cells were incubated in the presence and absence of *T. cruzi* trypomastigotes for 24 h at a cell:parasite ratio of 1:0.1 in presence and absence of caspase-8 activity inhibitor IETD-CHO (5 μM) or with Forskolin (50 μM) as a positive control. Caspase-8 activity was measured through luminescence using commercial kits, according to the manufacturer's instructions. BeWo cells incubated with the parasite show a significant increase in caspase-8 activity, which is prevented by the enzyme inhibitor. Data were normalized in terms of the control values and analyzed by ANOVA followed by Dunnett's post-test. All values, given as the means ± S.D., correspond to at least 3 independent experiments performed in triplicate. $^{**}p \leq 0.01$.

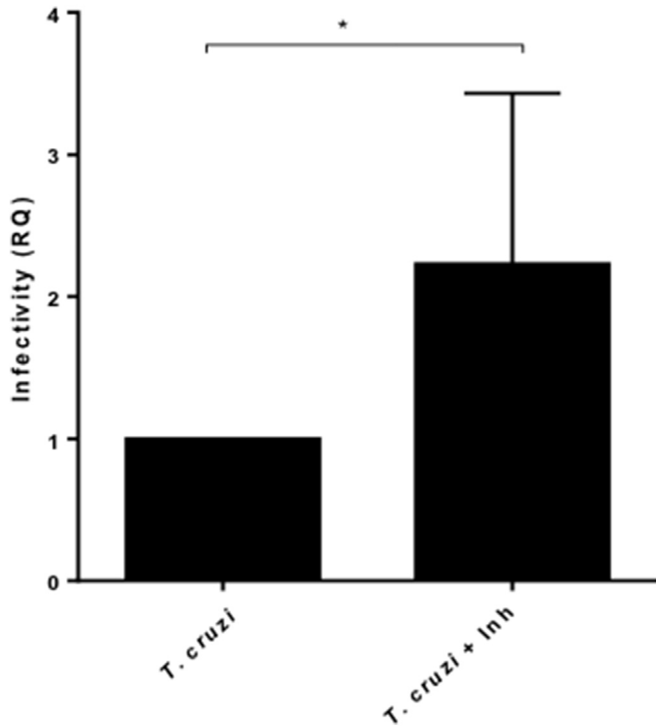

Fig. 3. Inhibition of caspase-8 increases the amount of *T. cruzi* DNA in BeWo cells. BeWo cells were incubated in the presence and absence of *T. cruzi* trypomastigotes for 24 h at a cell:parasite ratio of 1:0.1 in presence and absence of caspase-8 activity inhibitor IETD-CHO (5 μM). Data are a comparison of parasite DNA in 1 ng of total DNA isolated from infected BeWo cells. Real-time quantification by qPCR was performed using ΔΔCt method. Data represents means ± SD and were analyzed by Student *t*-test. $^{*}p < 0.05$.

replicative form of the parasite) in *ex vivo* infected HPCVE (Duaso et al., 2010) and in placentas from woman with chronic Chagas' disease (Duaso et al., 2012) have lead to postulate that local placental anti-parasitic mechanism may exist (Duaso et al., 2011; Liempi et al., 2014). Moreover, amastigote nests can only be seen in *ex vivo* infected explants when the trophoblast is enzymatically removed with trypsin (Lujan et al., 2004). Therefore, the trophoblast seems to be a fundamental barrier to *T. cruzi* placental invasion. In this context, the induction of a higher trophoblast turnover by the parasite may be part of a local defence mechanism probably related to maintain the placental barrier (Liempi et al., 2014).

We have previously shown that *T. cruzi* induces cellular proliferation, cellular differentiation (Liempi et al., 2014) as well as apoptotic cell death (Duaso et al., 2011) in the trophoblast. Differentiation and apoptosis are both cellular processes that are closely related in the trophoblast (Gauster and Huppertz, 2010) and also is caspase-8 (Gauster and Huppertz, 2010; Huppertz and Gauster, 2011; Longtine et al., 2012). For instance, activation of caspase-8 induces the phosphatidylserine exposure on the external leaf of the plasma membrane, which is a key signal for syncytial fusion (Gauster and Huppertz, 2010) and for apoptotic cell death (Savill, 1998). Here we demonstrate that *T. cruzi* induces activity of caspase-8 (Figs. 1 and 2) and that this activation is related to the infection of BeWo cells with the parasite (Figs. 3 and 4).

Caspase-8 is also involved in cellular proliferation, in particular related to tissue repair and regeneration (Connolly et al., 2014). Therefore we analyzed if the parasite-induced caspase-8 activity is related to the previously described increase in trophoblast proliferation (Droguett AL et al., 2013). As shown in Fig. 5A, the inhibition of caspase-8 does not affect DNA synthesis. This result is in accordance to the fact, that in the villous trophoblast, caspase-8 is expressed and active in terminal differentiated, not proliferating,

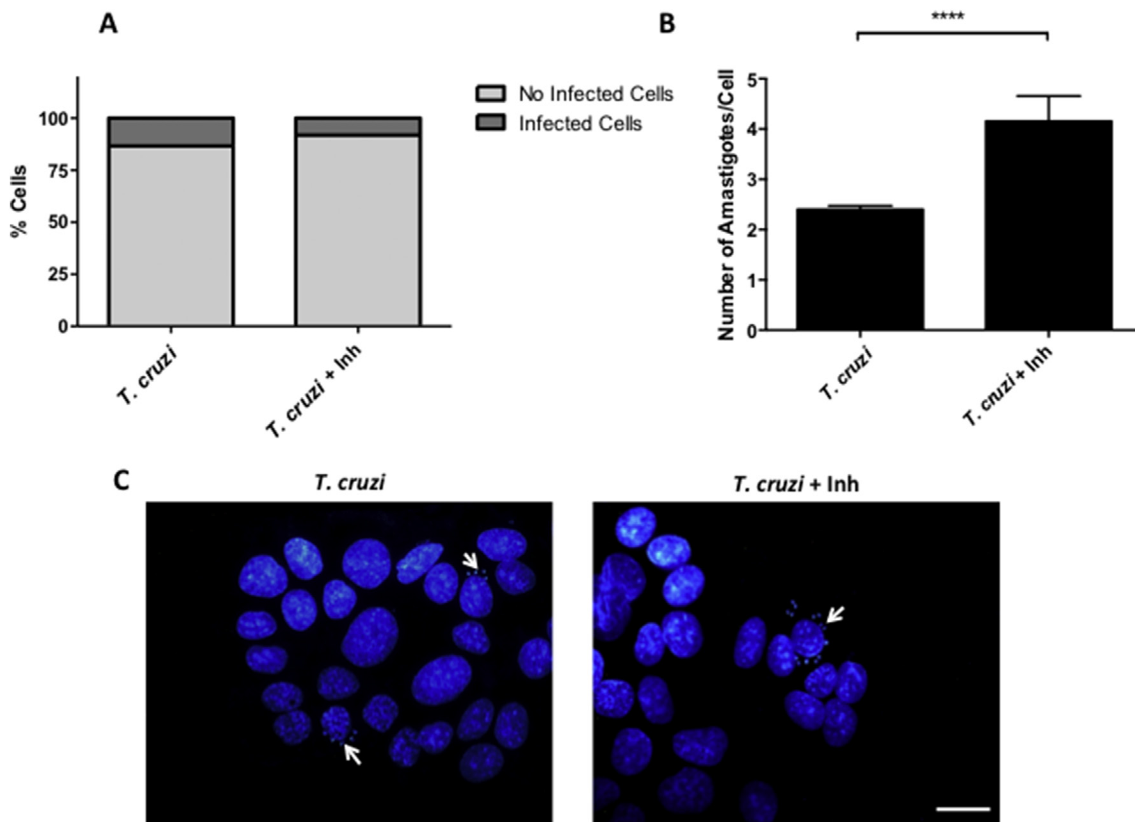

Fig. 4. Inhibition of caspase-8 does not affect the percentage of infected BeWo cells, but increases the number of intracellular parasites. BeWo cells were incubated in the presence and absence of *T. cruzi* trypomastigotes for 24 h at a cell:parasite ratio of 1:0.1 in presence and absence of caspase-8 activity inhibitor IETD-CHO (5 μM). A (left upper panel), the bar graph shows percentage of infected (dark gray) and non-infected cells (light gray). B (upper right panel), the bar graph shows the number of intracellular parasites in infected cells. Data represents means ± SD and were analyzed by Student *t*-test. $^{*}p < 0.05$. C, shows representative images of BeWo cells that were processed for DAPI staining. White arrows show intracellular amastigotes in BeWo cells. In presence of the caspase-8 inhibitor the number of intracellular parasites is increased. Scale bar: 10 μm.

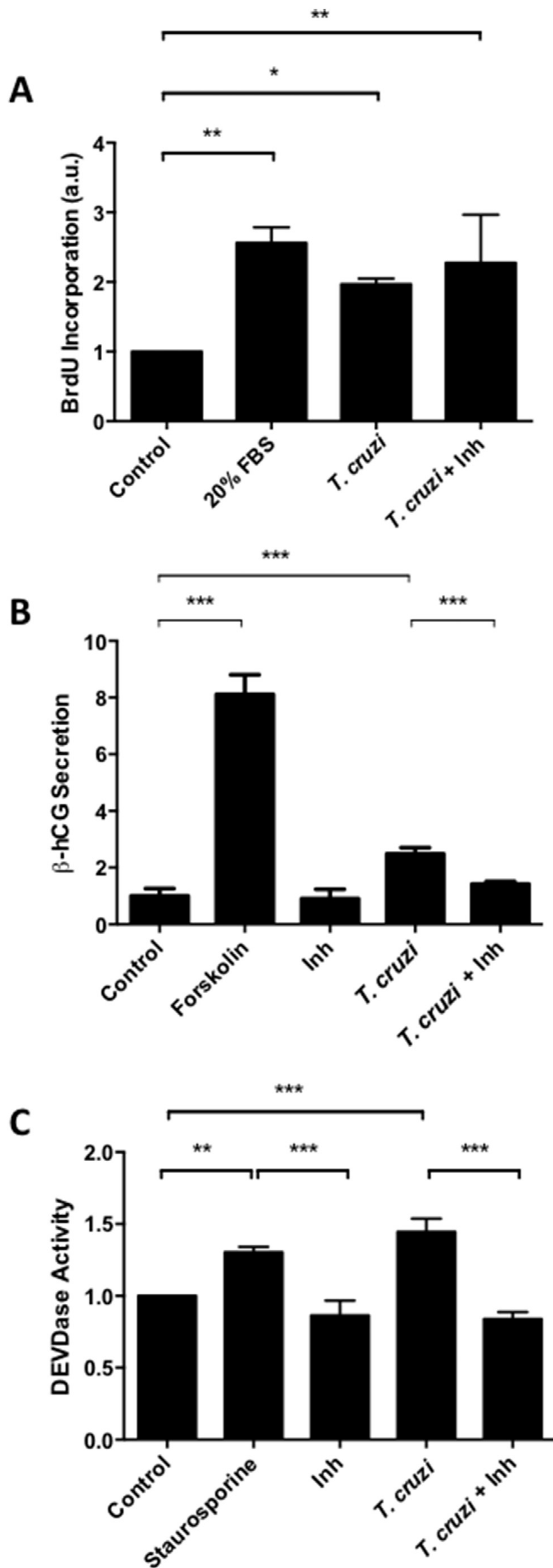

Fig. 5. Inhibition of caspase-8 does not prevent *T. cruzi*-induced DNA synthesis in BeWo cells. BeWo cells were synchronized by serum deprivation and, subsequently, incubated in the presence and absence of *T. cruzi* trypomastigotes for 24 h at a cell:-parasite ratio of 1:0.1 in presence and absence of caspase-8 activity inhibitor IETD-CHO (5 μM) or with FBS (20%) as a positive control. BrdU incorporation was measured through spectrophotometry using commercial kits, according to the manufacturer's instructions. Data were normalized in terms of the control values and analyzed with an ANOVA followed by Dunnett's post-test. All values, given as the means ± S.D., correspond to at least 3 independent experiments performed in triplicate. $^{*}p \leq 0.05$; $^{**}p \leq 0.01$.

CTB cells just prior fusion with the STB (Huppertz and Borges, 2008; Pidoux et al., 2012).

On the other hand, the inhibition of caspase-8 activity does prevent the cellular differentiation of the trophoblast (Fig. 5B), as measured by secretion of β-hCG, as differentiation marker. We have previously demonstrated, that *T. cruzi* induces not only β-hCG and syncytin expression (Liempi et al., 2014), both major biochemical markers of trophoblast differentiation (Berniscke et al., 2006), but also cell fusion and desmoplakin re-distribution (Liempi et al., 2014). Caspase-8 is also responsible for cytoskeleton rearrangement previous to cell fusion by cleaving α-fodrin (Huppertz and Gauster, 2011; Rote et al., 2010). α-fodrin belongs to the spectrin protein family of sub-membranous cytoskeletal proteins that carry binding sites for phosphatidylserine. The spectrin network maintains the curvature of the plasma membrane and its degradation affects membrane curvature facilitating fusion (Martens and McMahon, 2008). The expression of α-fodrin is diminished in highly differentiated CT and is entirely missing in the ST (Huppertz and Gauster, 2011). Therefore, *T. cruzi* induced caspase-8 activity might explain, at least partially, the parasite induced trophoblast differentiation (Liempi et al., 2014).

Apoptotic cell death is considered as a final differentiation of the trophoblast that culminates with the release of syncytial knots, which counterbalance the continuous incorporation of CTB cells into the STB, maintaining therefore the epithelial homeostasis (Berniscke et al., 2006). Inhibition of parasite induced-caspase-8 activity also prevents caspase-3 activity (Fig. 5C). Caspase-3 is one of the effector caspases, proteolytically cleaved by caspase-8 (Fliss and Brune, 2012). Caspase-3, together with other effector caspases, acts over a range of substrates, facilitating chromatin condensation by processing nuclear envelope laminins and mediating the collapse of the cytokeratin network, leading to the dismantling of the dying cell (Kurokawa and Kornbluth, 2009).

Interestingly, programmed cell death contributes to the innate immune control over intracellular pathogens, eliminating infected cells and restricting dissemination within the host organism (Guo et al., 2015). Moreover, several pathogens are able to modulate caspase-8. For instance, *Mycobacterium tuberculosum* and *Candida albicans* (Uchiyama and Tsutsui, 2015) as well as *Salmonella enterica* (Man et al., 2013) activate caspase-8 leading to the production of pro-inflammatory cytokines. On the other hand, several virus, including cytomegalovirus, are able to inhibit caspase-8 assuring in that way their spread into the host cell and body (Fliss and Brune, 2012).

Interestingly, it has been shown in mice that *T. cruzi* activates caspase-8 as well as the effector caspase 9 in thymocytes, causing their apoptotic cell death. Moreover, inhibition of both caspases prevents thymocyte death following *T. cruzi* infection, explaining partially the thymocyte depletion observed during parasitaemia peaks (Farias-de-Oliveira et al. 2013). During infection in mice with other trypanosomatid parasite, *Leishmania major*, the inhibition of caspase-8 reduced the proportion of CD8 T cells and IFN-gamma expression in both CD4 and CD8 T cells in lymph nodes, suggesting another non-apoptotic role of caspase-8 activity during T cell-

mediated immune response (Pereira et al., 2008).

Toxoplasma gondii (*T. gondii)*, other protozoan parasite also activates caspase-8 in the trophoblast. This parasite also increases caspase-8 and 3 levels and activity in first trimester trophoblast primary cell cultures and in BeWo cells leading to apoptotic cell death (Zhao et al., 2013). However, we cannot rule out, that the induced apoptosis may serve to evade host defenses that acts to limit the infection. Particularly, in the case of *T. gondii*, which is a more successfully congenital transmitted parasite invading parasite in the placenta than *T. cruzi* (Robert-Gangneux et al., 2011).

5. Conclusion

Caspase-8 activity is part of the BeWo trophoblast cell defense mechanisms against *T. cruzi* infection. Together with our previous results, we suggest that the trophoblast turnover is part of local placental anti-parasite mechanisms.

Acknowledgements

This work was supported by ERANET-LAC grant ELAC2014/HID-0328 (to UK and NG) and "Fondo Nacional de Desarrollo Científico y Tecnológico" (FONDECYT, Chile) grants 1120230 (to UK), 1130189 (to JM) and 1130113 (to NG).

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