Placenta 34 (2013) 240e247

Contents lists available at SciVerse ScienceDirect

Placenta

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

Trophoblast cells are able to regulate monocyte activity to control Toxoplasma gondii infection

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a r t i c l e i n f o

Article history:
Accepted 12 December 2012

Keywords:
Trophoblast cell
Monocytes
Toxoplasma gondii
Cytokines

a b s t r a c t

Introduction: Toxoplasma gondii is an intracellular parasite that causes severe disease when the infection occurs during pregnancy. Trophoblast cells constitute an important maternaleefetal barrier, with monocytes concentrating around them. Thus, interactions between trophoblasts and monocytes are important for maintaining a successful pregnancy, especially in cases of infection. This study aimed to evaluate the role of trophoblast cells (BeWo line) on monocyte (THP-1 line) activity in the presence or absence of T. gondii infection.
Methods: THP-1 cells were stimulated with supernatants of BeWo cells, previously infected or not with T. gondii, and then infected with parasites. The supernatant of both cells were collected and analyzed for cytokine production and T. gondii proliferation in THP-1 cells was determined.
Results: The results showed that after infection, the pattern of cytokines secreted by THP-1 and BeWo cells was characterized as a pro-inflammatory profile. Furthermore, supernatant of BeWo cells infected or not, was able to change the cytokine profile secreted by infected THP-1 cells, and this supernatant became THP-1 cells more able to control T. gondii proliferation than those that had not been stimulated.
Discussion: This effect was associated with secretion of interleukin (IL)-6 by the THP-1 cells and soluble factors secreted by BeWo cells, such as IL-6 and MIF.
Conclusion: Together, these results suggest that trophoblast cells are able to modulate monocyte activity, resulting in the control of T. gondii infection and subsequent maintenance of pregnancy.

1. Introduction

Toxoplasma gondii is the etiologic agent of toxoplasmosis, a disease that is usually asymptomatic in immunocompetent individuals, but can be severe in immunocompromised patients and cases of congenital toxoplasmosis [1,2]. The main defense mechanism against T. gondii is mediated by a T helper type 1 (Th1)-immune response characterized by secretion of pro-inflammatory cytokines [3]. During pregnancy, the maternal immune response shifts to a T helper type 2 (Th2) profile, with predominant secretion of anti-inflammatory cytokines, which allow fetal tolerance and maintenance of pregnancy [4,5]. However, this anti-inflammatory microenvironment becomes favorable to parasite replication, including T. gondii [6,7].

Several cellular components of the innate immune system are found at the site of embryo implantation. Monocytes constitute 20e30% of this population and remain present at high levels throughout pregnancy [8]. Previous studies demonstrated that trophoblast cells express pattern recognition receptors that recognize the presence of bacteria, viruses, parasites or dying cells, and then secrete cytokines and chemokines able to act on cells of the innate immune system present in the deciduas; for example, inducing the recruitment, differentiation and activity of monocytes [8,9]. Consequently, monocytes within the decidua accumulate around the invading trophoblast cells and acquire an activated phenotype that is important for the clearance of apoptotic cells and cellular debris, thus facilitating trophoblast

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0143-4004
http://dx.doi.org/10.1016/j.placenta.2012.12.006

migration during gestation [10–12]. This evidence suggests that the innate immune cells may play an important role in fine-tuning fetal–maternal immune responses and consequently in successful pregnancy.

Clinical studies, however, have established a strong association between pregnancy complications and intrauterine infections as a result of the interaction between trophoblast cells and innate immune cells [12,13]. Receptors present in trophoblast cells are able to recognize the presence of pathogens, followed by secretion of cytokines that act upon the innate immune cells within the decidua [14]. Therefore, the way that these cells interact with each other can be decisive to the tolerance of allogeneic fetus and the host defense against possible infections.

Monocytes are one of the major cells of the innate immune system responsible for the control as well as dissemination of T. gondii during the acute phase of infection, including at the maternal–fetal interface [15,16]. In this regard, our previous study verified that human villous explants from first trimester pregnancies expressed high levels of intercellular adhesion molecule 1 (ICAM-1) that favored increased adhesion of monocytes to trophoblast cells, favoring dissemination of the infection into the deep placental tissues [16]. Thus, this study aimed to investigate if factors secreted by trophoblasts influence cytokine production and T. gondii control by monocytes, through in vitro experiments using well established cell lines.

2. Materials and methods

2.1. Cell culture

BeWo and THP-1 cell lines obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) were cultured in RPMI-1640 medium (Gibco, Paisley, UK), supplemented with 25 mM HEPES, 100 U/mL penicillin, 100 μg/mL streptomycin (all reagents from Sigma–Aldrich, St. Louis, MO, USA) and 10% heat-inactivated fetal calf serum (FCS) (Cultilab, Campinas, Brazil) – complete medium – in a humidified incubator at 37 °C with 5%CO_2 [17].

2.2. Parasites

Tachyzoites of the T. gondii 2F1 RH strain, which constitutively express cytoplasmic β-galactosidase, were a gift from Dr. Vern Carruthers, Medicine School of Michigan University (USA). The parasites were propagated in BeWo cells cultured in RPMI 1640 medium supplemented with penicillin, streptomycin and 2%FCS at 37 °C and 5%CO_2.

2.3. Treatment of THP-1 cells with supernatants of BeWo cells

BeWo cells were cultured in 96-well plates (1 × 10^5 cells/200 μL/well) in complete medium for 24 h at 37 °C and 5% CO_2. Cells were then infected with tachyzoites of T. gondii at a 3:1 (parasites: host cell) ratio or incubated only with medium (control). After incubation for 24 h at 37 °C and 5%CO_2, the plates were centrifuged (720 g, 10 min) and the cell-free supernatants were collected and stored at −80 °C until used for cytokine detection and treatment of THP-1 cells.

THP-1 cells were cultured in 96-well plates (1 × 10^5 cells/200 μL/well) in complete medium for 24 h at 37 °C and 5%CO_2 and then treated with the uninfected or infected BeWo supernatants (conditioned medium). As a control, THP-1 cells were cultured only with complete medium. After 24 h of treatment, the cell supernatants were removed and complete medium was added to the wells. THP-1 cells were then infected with tachyzoites (3:1; parasites: cells) and incubated for 24 h at 37 °C and 5%CO_2. The plates were centrifuged (720 g, 10 min) and the cell-free supernatants were collected and stored at −80 °C for cytokine detection.

2.4. Measurement of cytokines secreted by THP-1 and BeWo cells

2.4.1. Enzyme-linked immunosorbent assay (ELISA)

Human cytokines (IL-12, TGF-β1 and MIF) were measured in the supernatants of THP-1 and BeWo cells by sandwich ELISAs according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). The sensitivity limits of these assays were 7.8 pg/mL for IL-12, and 25 pg/mL for TGF-β1 and MIF.

2.4.2. Cytometric bead array (CBA)

Human cytokines (IL-2, IFN-γ, TNF-α, IL-6, IL-4, IL-10 and IL-17A) were measured in the supernatants of THP-1 and BeWo cells by cytometric bead array® (CBA, BD Biosciences, San Jose, CA, USA), using the Th1/Th2/Th17 kit (BD Bioscience), according to the manufacturer's instructions. The samples were analyzed under BD® flow cytometry (FACSCalibur, BD Company, San Diego, CA, USA) and the data were calculated using a specialized software (BD® Cell Quest and CBA software).

2.5. T. gondii proliferation assay in THP-1 cells

THP-1 cells were cultured in 96-well plates (2 × 10^4 cells/200 μL/well) at 37 °C and 5%CO_2 for 24 h and then stimulated with the uninfected or infected BeWo supernatants or with complete medium alone for 24 h. Subsequently, the supernatants were removed and the THP-1 cells were infected with tachyzoites of T. gondii (3:1; parasites: cell) and incubated for 24 h at 37 °C and 5%CO_2. The plates were centrifuged (720 g, 10 min), the supernatants removed and the cells analyzed for T. gondii intracellular proliferation using a colorimetric β-galactosidase assay as previously described [18].

In a second set of experiments, THP-1 cells were cultured for 24 h as described above. Next, T. gondii tachyzoites were added to cells (3:1) and, after 4 h of infection, the cells were washed and treated with polyclonal rabbit anti-human IL-6 antibody (Peprotech Inc., RockyHill, USA) at 10 μg/mL. As controls, infected cells were treated with medium or 10 μg/mL of an irrelevant polyclonal rabbit IgG antibody (Jackson Immuno Research, West Grove, USA). After 24 h of treatment T. gondii intracellular proliferation was determined [18].

In another set of experiments, THP-1 cells were cultured into 96-well plates (2 × 10^4 cells/200 μL/well) for 24 h at 37 °C and 5%CO_2. The cells were then stimulated with supernatants of BeWo cells, infected or not, previously neutralized with 10 μg/mL of anti-IL-6 (Peprotech), anti-MIF (R&D Systems) or anti-TGF-β1 (R&D Systems) antibodies for 2 h at 37 °C and 5%CO_2. After 24 h of stimulation, THP-1 cells were infected with T. gondii tachyzoites (3:1) for additional 24 h. As controls, THP-1 cells were stimulated with non-neutralized BeWo cell supernatant or neutralized with 10 μg/mL of an irrelevant polyclonal rabbit IgG antibody (Jackson Immuno Research), and then infected with T. gondii. After 24 h of infection, T. gondii intracellular proliferation was determined [18].

Finally, THP-1 cells were cultured into 96-well plates (2 × 10^4 cells/200 μL/well) for 24 h at 37 °C and 5%CO_2. Cells were washed three times with incomplete medium and then treated separately with human recombinant cytokines: rIL-6 (PeproTech) at 25, 50 and 100 pg/mL, rMIF (BDBioscience) at 2000, 4000 and 8000 pg/mL and rTGF-β1 (R&D Systems) at 500, 1000 and 2000 pg/mL for additional 24 h at 37 °C and 5%CO_2. Next, T. gondii tachyzoites were added to the cells (3:1) and, after 3 h of infection, the cells were washed with medium and again treated with the cytokines in the same concentrations. As controls, cells were infected and treated with medium only. After 24 h of treatment, parasite proliferation was measured [18].

T. gondii intracellular proliferation data were expressed as number of tachyzoites calculated in relation to the reference curve of 2F1 strain tachyzoites, ranging from 1 × 10^6 to 15.625 × 10^3 total parasites.

2.6. Analysis of cytokine signatures

Complementary "non-conventional" analysis of cytokine data was applied to evaluate the cytokine profile in the supernatant from each experimental condition, using the general concept of "low" and "high" cytokine levels as previously proposed by Luiza-Silva et al. [19]. For this purpose, the whole data universe of cytokine levels in the supernatants (pg/mL) recorded for all experimental groups was used to calculate the global median value for each cytokine. The global median values were used as the cut-off edge to tag each individual supernatant as it presents "low" or "high" levels of cytokines. Following, the frequency (%) of supernatant showing "high levels of cytokine" (above the global median cut-off) was calculated in order to determine the percentage of experimental conditions displaying high levels of cytokines. The "cytokine signature" for each experimental condition was then assembled as an ascendant curve of frequencies of high cytokine levels for each experimental condition. The comparative analysis of cytokine signatures was performed using the 50th percentile as a limit to identify the cytokines considered relevant on each experimental condition. In order to highlight the relevant differences between experimental groups, we have used gray rectangles to stretch the distinctive cytokines icon from each cytokine signature. This approach showed to be relevant to detect subtle changes in the cytokine signatures not detectable by conventional statistical approaches. The value of this non-conventional "qualitative-observational" method was emphasized during data analysis, as the patterns of cytokine signature were consistent with the parasite growth observed in parallel experiments.

2.7. Statistical analyses

Statistical analyses were carried out using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). Data were expressed as mean ± standard deviation (SD) of two to three independent experiments performed in triplicate. Data were compared using the Student's t test. Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. THP-1 and BeWo cells infected with T. gondii preferentially secrete pro-inflammatory cytokines

The cytokine profiles secreted by THP-1 and BeWo cells, infected or not with T. gondii, are illustrated in Fig. 1. The infected THP-1 and BeWo cells secreted higher levels of pro-inflammatory cytokines (MIF, IL-12 and IL-6) (Fig. 1AeC) and lower levels of the anti-inflammatory cytokine (TGF-b1) (Fig. 1E) than uninfected cells. However, the cytokine profile was different for IL-10 and IL-17A, with lower IL-10 levels secreted by infected THP-1 cells (Fig. 1F) and lower IL-17A levels secreted by infected BeWo cells (Fig. 1D) compared to their respective uninfected cells.

The cytokine signatures emphasize the predominance of pro-inflammatory responses in THP-1 and BeWo cells infected with T. gondii (Fig. 2). Thus, uninfected THP-1 cells showed an anti-inflammatory response profile, with relevant frequency of IL-10 and TGF-b1 and, after infection, these cells changed to a pro-inflammatory response, with relevant frequency of IL-12, MIF and IL-17A (Fig. 2A). In uninfected BeWo cells, the cytokines with relevant frequency were TGF-b1 and IL-17A. After infection, the predominant cytokines were IL-12, MIF, IL-6 and IL-10 (Fig. 2B).

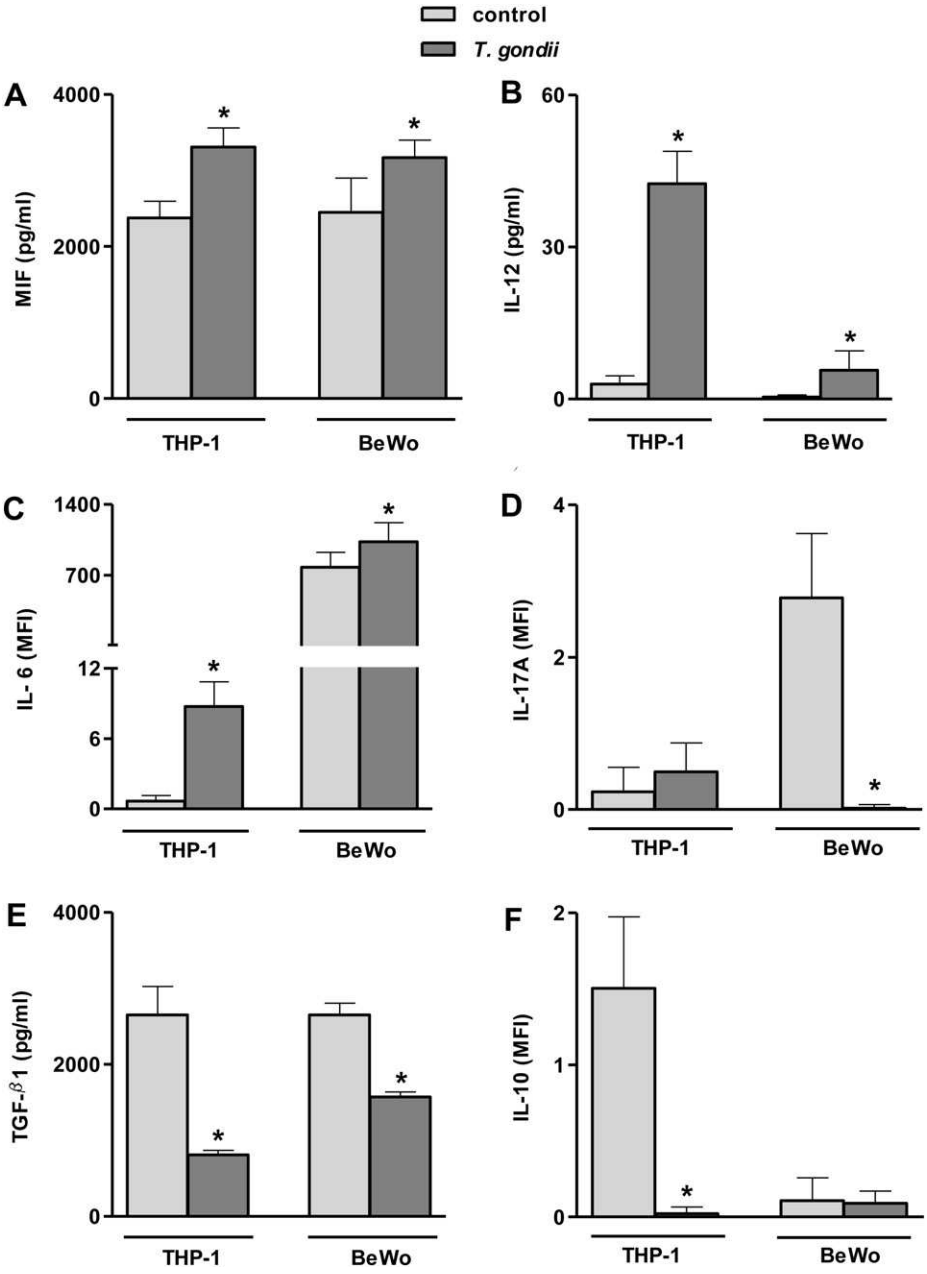

Fig. 1. Secretion of cytokines by THP-1 and BeWo cells infected with T. gondii. The results are expressed in pg/mL when cytokines were measured by ELISA: (A) MIF, (B) IL-12 and (E) TGF-b1; and in MFI (mean of fluorescence intensity) when measured by flow cytometry: (C) IL-6, (D) IL-17A and (F) IL-10. THP-1 and BeWo cells not infected with T. gondii served as control. Data are expressed as mean ± SD of two independent experiments performed in triplicate. *Significant differences in relation to the control in each cell line ($P < 0.05$; Student's t test).

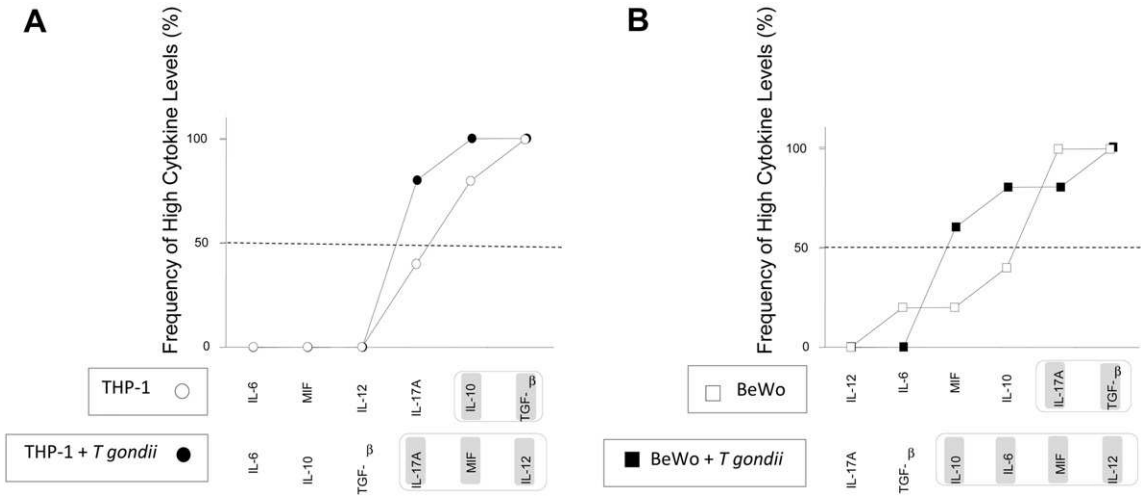

Fig. 2. Cytokine signatures secreted by THP-1 and BeWo cells infected or not with T. gondii. The results are expressed as frequency of high cytokine levels (%) calculated from the global medians for each cytokine, which were then used as cut-off. Frequencies were considered relevant when higher than 50%

3.2. BeWo cells are able to regulate the secretion of pro- and anti-inflammatory cytokines by THP-1 cells

THP-1 cells, infected or not, secreted higher levels of MIF and IL-12 when stimulated with supernatant from infected than uninfected BeWo cells (Fig. 3Ae B). In infected THP-1 cells, however, the secretion of MIF and IL-12 was even higher when the cells were not stimulated in comparison to the cells stimulated with both supernatants from BeWo cells (Fig. 3Ae B).

THP-1 cells, infected or not, secreted more IL-6 after stimulus with the supernatants from BeWo cells, especially with the infected BeWo cell supernatant (Fig. 3C). The secretion of IL-17A by uninfected THP-1 cells was not altered by any stimulus (Fig. 3D), but after infection, THP-1 cells secreted higher levels of IL-17A when stimulated with supernatant of infected than uninfected BeWo cells (Fig. 3D).

THP-1 cells, infected or not, secreted lower levels of TGF-b1 when stimulated with the supernatants of BeWo cells than the control, mainly with the infected BeWo cell supernatant (Fig. 3E). In contrast, the secretion of IL-10 was higher in THP-1 cells, infected or not, when stimulated with the BeWo cell supernatants than the control, and this secretion was even higher after stimulus with infected BeWo cell supernatant (Fig. 3F).

The analysis of the cytokine signatures revealed that uninfected THP-1 cells, when stimulated with supernatant from infected BeWo cells, showed a predominantly pro-inflammatory profile, with higher frequencies of IL-6 and IL-12 compared with the other stimuli, which induced an anti-inflammatory response (IL-10 and TGF-b1) (Fig. 4A). On the other hand, infected THP-1 cells exhibited a pro-inflammatory cytokine profile regardless of the stimulus, with relevant frequency of IL-12, IL-17A, IL-6 and MIF after stimulus with infected BeWo cell supernatant, MIF and IL-6 after stimulus with uninfected supernatant, and IL-12, MIF and IL-17A in the absence of stimulus (Fig. 4B).

The levels of IL-2, IL-4, TNF-a and IFN-g were also analyzed by flow cytometry, but the results were below the detection limit of the assays (data not shown).

3.3. THP-1 cells are more able to control T. gondii proliferation when stimulated with BeWo cell supernatants

When stimulated with uninfected or infected BeWo cell supernatants, THP-1 cells were more able to control T. gondii proliferation than non-stimulated cells (Fig. 5A). Furthermore, the proliferation of T. gondii was even lower in THP-1 cells stimulated with infected than uninfected BeWo cell supernatants (Fig. 5A).

3.4. IL-6 and parasitism are inversely associated in THP-1 cells stimulated with BeWo cell supernatants

To investigate if the control of T. gondii proliferation was associated with the cytokine profile secreted by the THP-1 cells, the relationship between the number of tachyzoites and each cytokine was analyzed. Among the analyzed cytokines, IL-6 showed a relevant inverse association with the number of tachyzoites, since THP-1 cells infected after stimulus with supernatants from infected or non-infected BeWo cells secreted higher levels of IL-6 and were more able to control T. gondii proliferation than the control cells (Fig. 5B). For the remaining cytokines, no relationship between the parasitism and cytokine profile secreted by THP-1 cells was found (data not shown). These findings were corroborated when IL-6 was neutralized with a specific antibody, since THP-1 cells pretreated with anti-IL-6 antibody showed a higher number of tachyzoites than untreated THP-1 cells (Fig. 5C) or treated with an irrelevant antibody (data not shown).

3.5. MIF and IL-6 secreted by BeWo cells are involved in the control of parasitism in THP-1 cells

As MIF, IL-6 and TGF-b1 were the most secreted cytokines by BeWo cells, the supernatants of BeWo cells, infected or not with T. gondii, were first neutralized with each specific antibody and the parasitism in THP-1 cells treated with these supernatants was evaluated (Fig. 6A). THP-1 cells stimulated with uninfected or infected BeWo cell supernatants and neutralized with anti-IL-6 or anti-MIF antibodies showed higher number of tachyzoites than untreated controls or those neutralized with irrelevant antibody (Fig. 6A). Differently, THP-1 cells stimulated with uninfected or infected BeWo cell supernatants and neutralized with anti-TGF-b1 antibody had a parasitism index similar to the untreated control and to the cells neutralized with an irrelevant antibody (Fig. 6A). These findings were corroborated when THP-1 cells were treated with rIL-6 or rMIF, showing that parasitism was reduced with the highest concentration of IL-6 (100 pg/mL) and the lowest concentration of MIF (2000 and 4000 pg/mL) compared with the control (Fig. 6B). Furthermore, THP-1 cells treated with TGF-b1 at 500 pg/

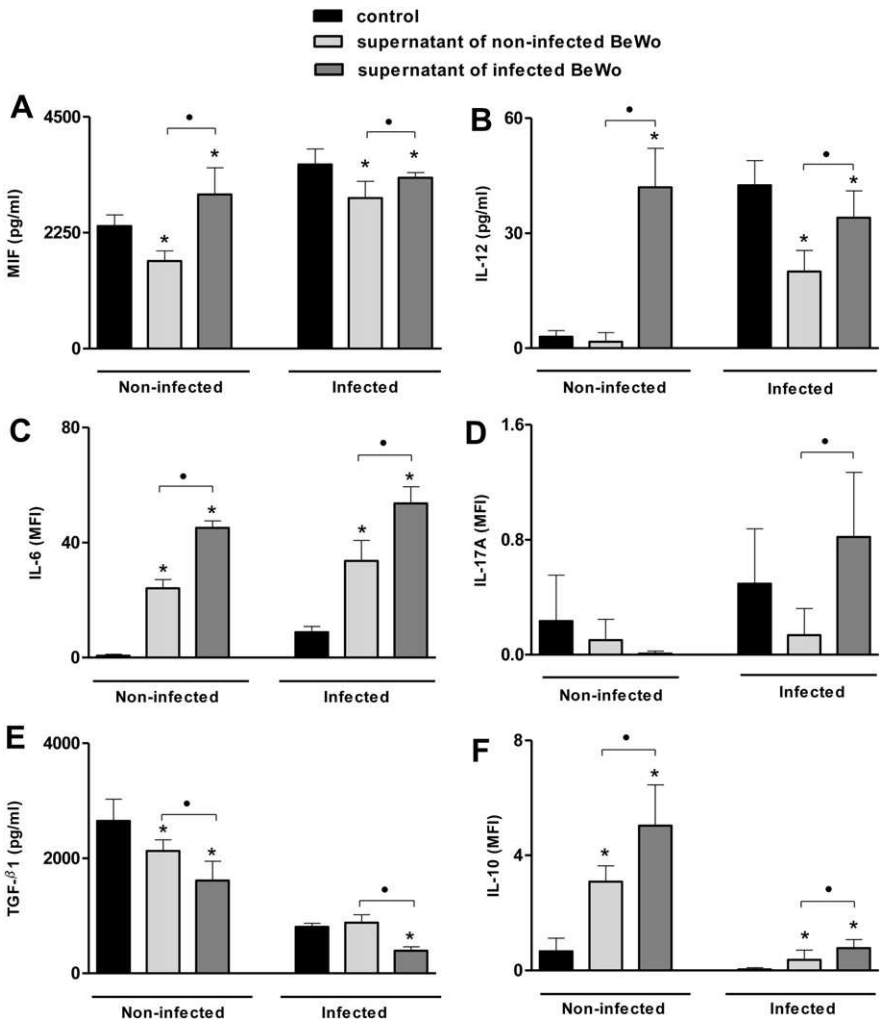

Fig. 3. Secretion of cytokines by THP-1 cells, infected or not with T. gondii, after stimulation with supernatants from uninfected or infected BeWo cells. The results are expressed in pg/mL when cytokines were measured by ELISA: (A) MIF, (B) IL-12 and (E) TGF-b1; and in MFI (mean of fluorescence intensity) when they were measured by flow cytometry: (C) IL-6, (D) IL-17A and (F) IL-10. THP-1 cells, infected or not with T. gondii, without stimulus with supernatants from uninfected or infected BeWo cells served as control. Data are expressed as mean ± SD of two independent experiments performed in triplicate. (*) Significant differences in relation to the control in each condition; (•) significant differences between non-infected and infected BeWo cell supernatants in each condition ($P < 0.05$; Student's t test).

mL and 2000 pg/mL also showed higher number of tachyzoites than the control (Fig. 6B).

4. Discussion

During pregnancy, immunological changes are necessary to maintain its normal course [8]. Previous studies demonstrated that trophoblast cells promote fetal acceptance under normal conditions, but may initiate signals promoting fetal rejection in the presence of infection by agents such as T. gondii [20,21]. Monocytes are the main cells used by T. gondii for its dissemination and, during pregnancy, these cells are attracted to the placental microenvironment by trophoblast cells [10]. Therefore, it is possible that the presence of monocytes at the maternal-fetal interface can facilitate the vertical transmission of this parasite for the embryos [15].

In the present study, we showed that T. gondii-infected THP-1 and BeWo cells secreted increased levels of pro-inflammatory cytokines (MIF, IL-12 and IL-6) and had reduced production of anti-inflammatory cytokines (TGF-b1). Thus, this pro-inflammatory profile may represent a mechanism associated with the control of the infection as observed in other studies [22–24]. We also showed that THP-1 cells, when stimulated with supernatants from non-infected BeWo cells, secreted less MIF than non-stimulated cells, suggesting that trophoblast cells downregulate the secretion of MIF by monocytes in the absence of infection, contributing to the establishment of a favorable microenvironment for the maintenance of pregnancy. In contrast, THP-1 cells, when stimulated with supernatant of infected BeWo cells, secreted higher levels of MIF than the controls (no stimulus or uninfected BeWo cell supernatant), suggesting that the parasite in trophoblast cells induces release of soluble factors that are able to upregulate the secretion of MIF by monocytes. Additionally, THP-1 cells infected without stimulus secreted more MIF than those infected after stimulus with supernatants from infected or uninfected BeWo cells, suggesting that trophoblast cells, even when infected, induce infected monocytes to secrete less MIF, thereby helping to minimize the pro-inflammatory response induced by the parasite. This could help to control the parasitism and maintain the pregnancy, since MIF, at appropriate concentrations, induces inflammatory responses that can provide protection against infections and ensure rapid and efficient elimination of parasites, as required for the establishment of pregnancy [25].

Our results also showed that IL-12 production by THP-1 cells was increased after stimulation with infected BeWo cell supernatants,

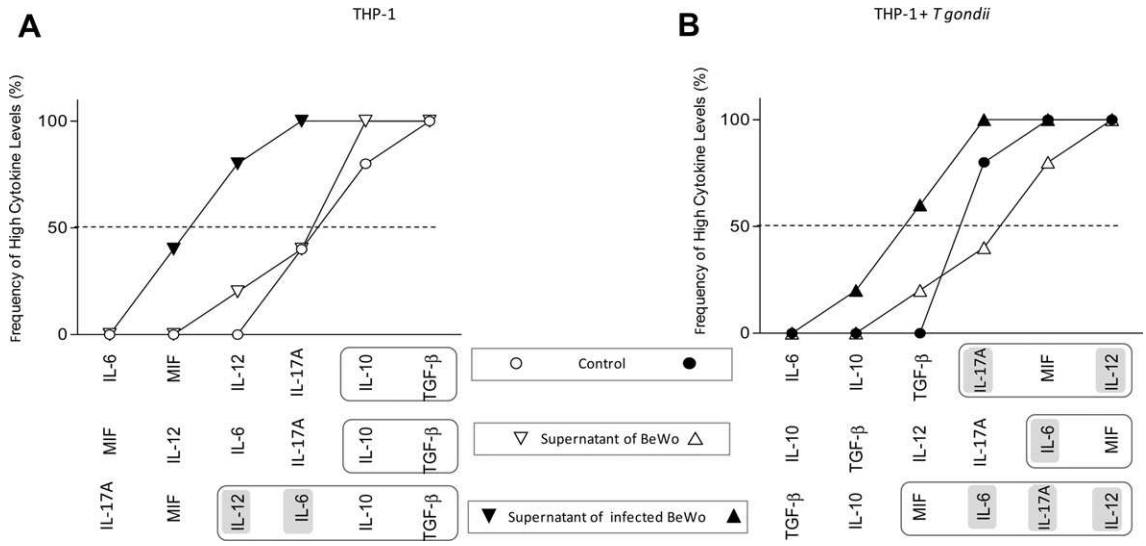

Fig. 4. Cytokine signatures secreted by THP-1 cells, infected or not with T. gondii, after stimulation with supernatants of uninfected or infected BeWo cells. The results are expressed in frequency of high cytokine levels (%) calculated from the global medians for each cytokine, which were then used as cut-off. Frequencies were considered relevant when higher than 50%

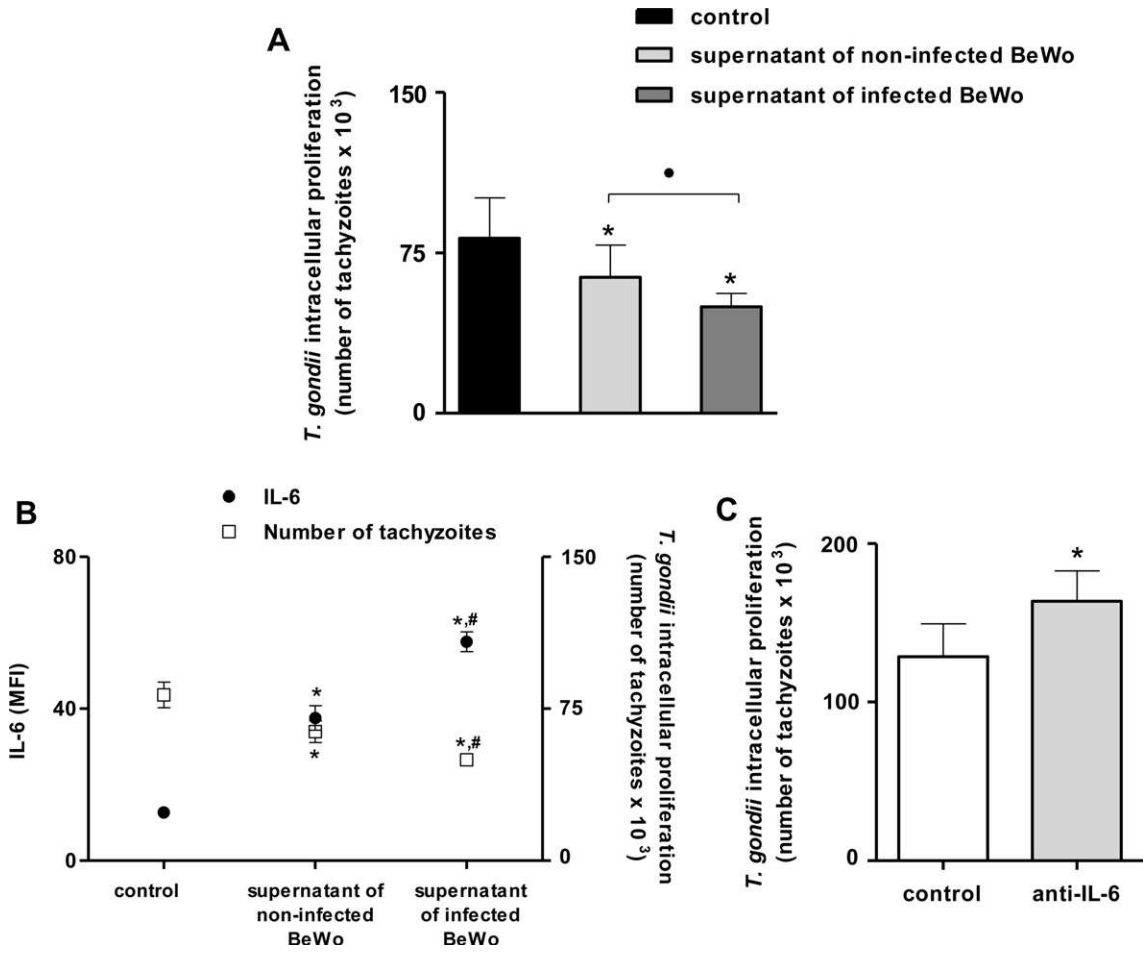

Fig. 5. (A) Toxoplasma gondii intracellular proliferation in THP-1 cells after stimulation with supernatants from uninfected or infected BeWo cells. Infected THP-1 cells without treatment with supernatants from uninfected or infected BeWo cells served as control. Data are expressed as mean ± SD of the number of parasites in three independent experiments performed in triplicate. (*) Significant differences in relation to the control; (○) significant differences between non-infected and infected BeWo cell supernatants ($P < 0.05$; Student's t test). (B) Relationship between the secretion of IL-6 by THP-1 cells and the proliferation of T. gondii in cells non-stimulated (control) or stimulated with supernatants of uninfected or infected BeWo cells. (*) Significant differences in relation to the control. (#) Significant differences between non-infected and infected BeWo cell supernatants ($P < 0.05$; Student's t test). (C) T. gondii intracellular proliferation in THP-1 cells treated with anti-IL-6 antibody. THP-1 cells infected with T. gondii and not treated with anti-IL-6 antibody served as control. (*) Significant differences in relation to the control ($P < 0.05$; Student's t test).

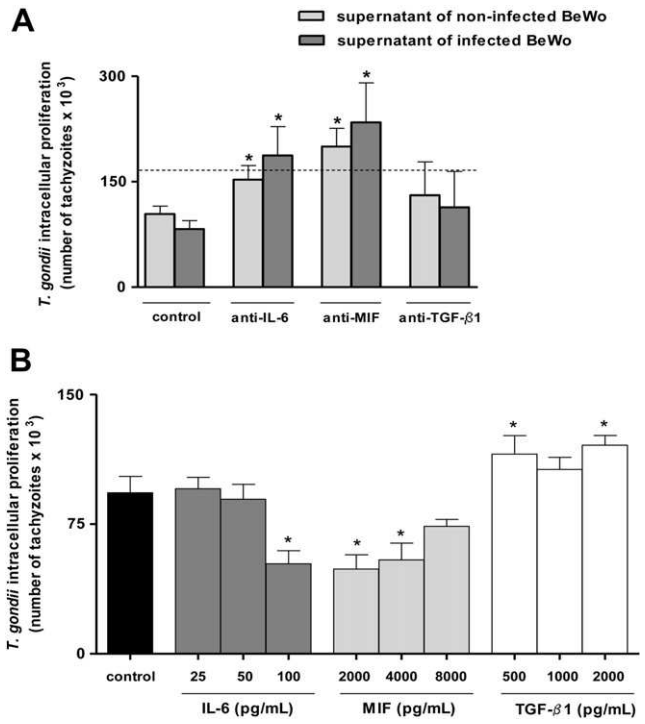

Fig. 6. (A) Toxoplasma gondii intracellular proliferation in THP-1 cells after stimulation with neutralized supernatants from uninfected or infected BeWo cells. The supernatants were separately neutralized with 10 mg/mL of anti-IL-6, anti-MIF or anti-TGF-b1 antibodies. THP-1 cells stimulated with non-neutralized supernatants served as control. Data are expressed as mean ± SD of the number of parasites in three independent experiments performed in triplicate. The dotted line represents the number of tachyzoites in THP-1 cells without any stimulus. (*) Significant differences in relation to the control ($P < 0.05$; Student's t test). (B) T. gondii intracellular proliferation in THP-1 cells treated with rIL-6 (25, 50 and 100 pg/mL), rMIF (2000, 4000 and 8000 pg/mL) or rTGF-b1 (500, 1000 and 2000 pg/mL). THP-1 cells cultured without any treatment served as control. Data are expressed as mean ± SD of the number of parasites in three independent experiments performed in triplicate. (*) Significant differences in relation to the control ($P < 0.05$; Student's t test).

but not with uninfected supernatants, suggesting the participation of the parasite and soluble factors secreted by BeWo cells in this effect. In infected THP-1 cells, however, the stimulus with BeWo cell supernatants, infected or not, downregulated the secretion of IL-12 by THP-1 cells, suggesting an important mechanism of interaction between trophoblast cells and monocytes to establish a favorable microenvironment for the development and maintenance of pregnancy even in the presence of infection.

Interleukin-6 is fundamental in the process of embryo implantation and in the early stages of pregnancy, since IL-6$^{-/-}$ mice showed reduced fertility and a decrease in viable implantation sites [26]. IL-6 can also act at the maternale fetal interface to alter many of the parameters that influence fetal growth, including nutrient transfer, anoxia and vascular permeability [27,28]. In this study we demonstrated that THP-1 cells, infected or not with T. gondii, secreted higher levels of IL-6 after stimulation with uninfected or infected BeWo cell supernatants than those without stimulus. As this cytokine may facilitate the growth and invasion of the trophoblast through the decidual tissue [10], these results demonstrating that trophoblast cells stimulate the secretion of IL-6 by THP-1 cells suggest that these innate immune cells may play a modulatory role at the implantation site, promoting appropriate signals that facilitate trophoblast growth and invasion. The presence of T. gondii in BeWo cells also stimulates them to release soluble factors that can upregulate the production of IL-6 by THP-1 cells. Additionally, IL-17A was also secreted at increased levels by infected THP-1 cells stimulated with infected BeWo cell supernatant. These data reinforce the role of IL-17A in the control of T. gondii, since this cytokine is important in the combat of several kinds of pathogens [29,30].

IL-10 and TGF-b1 are regulatory cytokines essential for tolerance of the fetus during pregnancy [31]. Our results showed that BeWo cells secrete soluble factors that downregulate the secretion of TGF-b1 by THP-1 cells. This downmodulation was potentiated in THP-1 cells infected by T. gondii, suggesting that TGF-b1 levels decreased as a consequence of the action of pro-inflammatory cytokines induced by the parasite and required to control the infection. In contrast, BeWo cell supernatants were able to upregulate the secretion of IL-10 by THP-1 cells even in the presence of T. gondii, contributing to the establishment of a typically anti-inflammatory microenvironment [32].

The present study also showed that factors secreted by BeWo cells were able to regulate THP-1 cell activity, rendering them more able to control T. gondii growth. Furthermore, the production of IL-6 by THP-1 cells was associated with parasitism control. Similar results have been seen in other studies, demonstrating that IL-6 participates in the protective immune response against Giardia duodenalis [33] and Trypanosoma cruzi [34], and plays a critical role in limiting microbial multiplication during early infection with Listeria monocytogenes [35], Vaccinia virus [36] and Candida albicans [37]. Others authors demonstrated that IL-6$^{-/-}$ mice infected with T. gondii showed impaired development of the innate pro-inflammatory response, which is essential for limiting parasite replication [24]. Another study using IL-6$^{-/-}$ mice also showed a protective effect of IL-6 in cases of encephalitis caused by T. gondii infection [38]. IL-6 is also important in the inflammation resolution, since this cytokine can stimulate the secretion of IL-10 [39]. This important relationship was observed in studies of human Plasmodium falciparum showing that elevated IL-6/IL-10 ratios in the plasma due to relative IL-10 deficiencies led to a fatal outcome of severe malaria [40]. Taken together, these cytokines can balance the intensity of immune responses.

In an attempt to identify which soluble factors secreted by BeWo cells would be regulating THP-1 cell functional activity, we performed experiments with neutralized BeWo cell supernatants or added recombinant cytokines. We verified that IL-6 and MIF secreted by BeWo cells, as well these added recombinant cytokines, were able to regulate the THP-1 cell response, rendering them more competent to control T. gondii growth. In contrast, TGF-b1 secreted by BeWo cells did not show any effect on T. gondii proliferation in THP-1 cells. Some mechanisms of MIF and IL-6 actions can be hypothesized to explain how these cytokines can control T. gondii infection.MIF is critical for host resistance to infection, and our group has investigated the role of this cytokine in the fetale maternal interface over the last years [16,22]. However, the role of its signaling pathway during the infection is not completely understood so far. MIF can suppress the activity of p53 trough the activation of ERK (extracellular signal-regulated kinases) that results in an inhibition of the apoptotic process and consequently promotes a long inflammatory response [41]. MIF is also responsible for the upregulation of the toll-like receptor 4 (TLR4) and the activation of transcription factors like Elk1 and Ets, which have an important action by mediating the innate immunity against pathogens [42]. Moreover, MIF can activate NF-kB (Nuclear Factor Kappa-B), a transcriptional factor involved in regulating expression of many pro-inflammatory cytokines and this signaling pathway participates in the defense against several infections [43]. Interleukin-6, in turn, induces IDO (indoleamina 2,3 dioxigenase) expression trough JAK/STAT pathway [44]. As IDO is an enzyme responsible for the degradation of tryptophan, which is an essential amino acid to T. gondii proliferation, IL-6 can control T. gondii growth due to IDO upregulation. However, future studies are

necessary to confirm these hypotheses. Thus, our results reinforce the idea that soluble factors present in a specific microenvironment alter the activity of immune cells in this microenvironment [45].

In conclusion, the present work demonstrated that trophoblast cells are able to regulate the functional activity of THP-1 cells, altering the pattern of cytokines secretion by these cells of the innate immune system as well as their susceptibility to infection by T. gondii at the maternale fetal interface. Together, our data suggest that similar phenomena may occur in vivo; thus, trophoblast cells may interact with monocytes present in the decidua and regulate their activity to ensure the maintenance of pregnancy. Therefore, the control of T. gondii infection in the placental microenvironment depends on the delicate relationship between innate immune cells and trophoblast cells.

Acknowledgments

This work was supported by Brazilian Research Agencies (FAPEMIG, CNPq and CAPES).

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