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Trophoblast-macrophage crosstalk on human extravillous under *Toxoplasma gondii* infection

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ABSTRACT

Introduction: The interaction between human extravillous trophoblasts and macrophages has an important role in implantation and placentation. However, any dysfunction in this communication system is associated with pregnancy pitfalls, and a *Toxoplasma gondii* infection can be a potential problem in this crosstalk. Therefore, the aim of this study was to assess the influence of infected macrophages on cytokine production and the incidence of apoptosis in *T. gondii*-infected extravillous trophoblast cells.
Methods: HTR-8/SVneo cells were treated with supernatant from macrophages infected or not by *T. gondii* (conditioned medium) in order to analyze apoptosis and cytokine production in comparison to uninfected control conditions.
Results: The IL-6 secretion by HTR-8/SVneo cells increased synergistically by treatment with conditioned medium and *T. gondii* infection. The apoptosis index of HTR-8/SVneo cells was also upregulated by treatment with conditioned medium and infection. In addition, a low expression of Fas/CD95 and a high soluble FasL release were observed during infection, although no significant change was observed in the proliferation of *T. gondii*.
Discussion: The parasite modulates the high apoptosis index in HTR-8/SVneo cells in order to favor its establishment inside its host cells. On the other hand, the conditioned medium from uninfected macrophages restores the apoptosis rates, although the effect of the infection seems to be stronger. In conclusion, our results showed that *T. gondii* infection in human extravillous trophoblasts is able to modulate the trophoblast-macrophage crosstalk.

1. Introduction

The physiology of the extravillous trophoblast is essential for the establishment of the maternal-fetal interface capable of sustaining proper fetal growth and development. At this interface, complex cellular interactions and paracrine factors are involved not only to ensure the success of pregnancy, but also to modulate the susceptibility to infection by intracellular pathogens, such as *Toxoplasma gondii* [1,2].

T. gondii is an obligate intracellular protozoan parasite with a high capacity to propagate asexually in the nucleated cells of warm-blood vertebrate hosts, including humans [3]. When a woman acquires the primo infection during pregnancy, there is a risk of vertical transmission of the parasite to the fetus through transplacental passage [4,5]. Maternal infection during the third trimester of pregnancy induces a higher risk of vertical transmission, and often this situation

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results in asymptomatic newborns that might develop neurologic and ocular damage in childhood. Although infection during early pregnancy is associated with a low risk of fetal transmission, the consequences can be more severe including mental retardation and miscarriage [4,6].

The immune response to *T. gondii* is associated with a T helper 1 (Th1) profile and requires the production of pro-inflammatory cytokines, such as interleukin (IL)-12 and interferon (IFN)-γ [7], which are responsible for activating macrophages, natural killer (NK) cells, $CD4^+$ and $CD8^+$ T cells in order to control the infection [7,8]. Macrophages are an important cell population necessary for the host protection against *T. gondii*. In response to the infection, these cells upregulate factors known to control parasite growth, such as nitric oxide (NO), IL-12 [8] and tumoral necrosis factor (TNF) alpha [9,10].

On the other hand, successful pregnancy is associated with a predominant Th2 profile, however, a pro-inflammatory condition is also required especially in the first trimester of pregnancy for the process of implantation and trophoblast invasion [11]. During pregnancy, placenta turnover depends on the functional loss of trophoblast cells by apoptosis, a physiological process by which the trophoblast cells are removed and a younger population is replaced [12,13]. An exaggerated or inhibited apoptosis has also been associated with deficient trophoblast invasion into maternal decidua, a critical process for a successful pregnancy [13,14]. Apoptosis deregulation has been associated with several pregnancy disorders including preeclampsia, intrauterine growth restriction, miscarriage, preterm birth and placental abruption [15].

Additionally, apoptosis is an important mechanism of defense against parasitic infection, in order to eliminate infected cells and avoid microbial dissemination [16]. *T. gondii* infection influences the incidence of apoptosis in trophoblast cells. The production of cytokines and the modulation of apoptosis in the placental microenvironment are fundamental for the success of pregnancy, as well as for the control of *T. gondii* infection. We have previously demonstrated that *T. gondii* infection is able to interfere in trophoblast apoptosis depending on parasite virulence [1,17]. More recently, studies have demonstrated that cytokines, such as IL-6 and TNF-α produced by trophoblast cells, are able to modulate the vertical transmission of *T. gondii* [13,14].

Macrophages represent a major cellular component of the implantation site, suggesting their involvement in specific functions [2]. These cells are essential to spiral artery remodeling and in the support of trophoblast invasion in the maternal decidua [18]. The crosstalk between macrophages and extravillous trophoblasts is a key process in the control of trophoblast apoptosis [13]. Macrophages produce pro-angiogenic factors, cytokines and growth factors, and induce the release of FasL to directly promote survival or apoptosis of the trophoblast, depending on the microenvironment at maternal-fetal interface [2,13]. Thus, macrophages are essential in the modulation of apoptosis in trophoblast cells in order to maintain the homeostasis and adequate invasion of this cell population.

Considering the importance of the trophoblast-macrophage interaction at the maternal-fetal interface, the present study aimed to investigate whether the interaction between macrophages and extravillous trophoblasts is affected by *T. gondii* infection. For this purpose, we evaluated the incidence of apoptosis and cytokine production in a human extravillous trophoblast cell line (HTR-8/SVneo cells) exposed to conditioned medium collected from macrophages infected or not by *T. gondii*.

2. Materials and methods

2.1. Cell culture

The human extravillous trophoblast cell line (HTR-8/SVneo cells) was originally generated from villous explants at early pregnancy [19] and was a kind gift from Dr. Estela Bevilacqua (University of São Paulo, SP, Brazil). The human myelomonocytic cell line (THP-1 cells) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Both cells were cultured in RPMI-1640 medium (Cultilab, Campinas, SP, Brazil), 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) – complete medium – in a humidified incubator at 37 °C with 5% CO_2.

2.2. Parasites

Tachyzoites of the *T. gondii* 2F1 RH strain, which constitutively express cytoplasmic β-galactosidase, were a kind gift from Dr. Vern Carruthers (Medicine School of Michigan University, USA). The parasites were propagated in human epithelial uterine cells (HeLa cells) cultured in RPMI 1640 medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin and 2% FCS at 37 °C and 5% CO_2.

2.3. Differentiation of THP-1 in macrophages (MØ) and production of conditioned medium

THP-1 cells were cultured in 75 cm^2 flasks (1×10^6 cells/15 mL) in complete medium at 37 °C and 5% CO_2. THP-1 cells were differentiated into macrophages with 10 ng/mL of phorbol 12-myristate 13-acetate (PMA) (Biogen Com. Dist. Ltda, Sumarezinho, SP, Brazil) for 48 h at 37 °C and 5% CO_2 [20]. The macrophages (obtained as described above) 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. Next, the cells were infected with *T. gondii* tachyzoites in the proportion of three parasites per cell (3:1) or incubated only with medium. After 24 h, the plates were centrifuged (720 × *g*, 10 min) and the cell-free supernatants were collected and stored at −80 °C until further analyses by cytokine detection assays or treatment of HTR-8/SVneo cells. The supernatants from uninfected or infected macrophages were referred to as conditioned medium.

2.4. Treatment with conditioned medium and T. gondii infection of HTR-8/SVneo cells

The HTR-8/SVneo cells were cultured in 6-well plates (1×10^6 cells/2000 μL/well) in complete medium for 24 h at 37 °C and 5% CO_2 and then treated with conditioned media (500 μL complete medium + 1500 μL supernatant from macrophages) collected from uninfected or infected macrophages for an additional 24 h. Parallel cultures of HTR-8/SVneo cells were maintained only with complete medium.

After this incubation, the medium was removed and the cells were infected or not with *T. gondii* tachyzoites (3:1) and incubated for a additional 24 h at 37 °C and 5% CO_2 in complete medium. Then, the plates were centrifuged (720 × *g*, 10 min), the cell-free supernatants were collected and stored at −80 °C for cytokine and soluble FasL detection. The cells were collected to analyze the apoptosis index, Fas/CD95 expression and *T. gondii* intracellular proliferation.

2.5. Cytokine and soluble FasL measurements

Human cytokines (MIF, IL-10, TGF-β1, IL-6 and IFN-γ) were measured using sandwich ELISAs according to the manufacturer's instructions (BD Biosciences, San Diego, CA, USA). The sensitivity limits of these assays were 62.5 pg/mL for MIF; 7.8 pg/mL for IL-10; 125 pg/mL for TGF-β1 and 4.7 pg/mL for IL-6 and IFN-γ. The data were expressed in pg/mL.