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Antiparasitic Mechanisms of the Human Placenta

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<http://dx.doi.org/10.5772/intechopen.73569>

Abstract

Trypanosoma cruzi, during vertical transmission, crosses the placental barrier. The trophoblast, a continuous renewing epithelium, is the first tissue of this anatomical barrier to have contact with the parasite. The epithelial turnover, including the trophoblast, is part of the innate immune response due to the fact that pathogens attach to the surface of cells prior invasion. Cellular processes such as proliferation, differentiation, and apoptotic cell death are part of the trophoblast turnover. Interestingly, *T. cruzi* induces all of them. In addition, the placenta expresses TLRs, whose activation leads to the secretion of pro-inflammatory and immunomodulating cytokines. *T. cruzi* is recognized by TLR-2, TLR-4, TLR-7, and TLR-9. In the present review, we analyze the current evidence about the trophoblast epithelial turnover, the induction of a specific cytokine profile as a local placental innate immune response, as well as other possible defense mechanisms against the parasite.

Keywords: *Trypanosoma cruzi*, placenta, epithelial turnover, TLRs, cytokine profile

1. Introduction

Congenital Chagas disease, caused by *Trypanosoma cruzi* (*T. cruzi*), is associated with premature labor, low birth weight, and stillbirths [1, 2]. 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” [3, 4] but is essential in determining the probability of transmission since it forms the primary barrier between the maternal and fetal compartments throughout pregnancy [5].

Mother and developing fetus are protected against environmental challenges by the immune system; the placenta is able to modulate fetal as well as maternal immune responses. Maternal

immune system presents an enhanced capacity of cellular and molecular recognition and communication between each other. Therefore, during normal pregnancy, the maternal immune defenses assure the health of the mother and developing child. Moreover, the fetus during its development also acquires immune defenses that are able to modulate the maternal immune system. Considering this facts, the immune system responses are unique and particularly effective [6].

The maternal and the fetal developing innate and adaptative immune systems determine the probability of fetal/neonatal infection. Fetal infection is related to diverse pregnancy disorders such as abortion, preterm labor, intrauterine growth retardation, and preeclampsia [7]. Particularly, congenital *T. cruzi* infection can cause abortion, stillbirth, and intrauterine growth restriction [1, 2, 8].

The innate immune system presents a main role in protecting the developing child against *T. cruzi* infection. Thus, increase of pro-inflammatory cytokines is present in the sera of uninfected babies born to infected mothers [9]. However, in newborns who suffer from congenital infection, the levels of inflammation markers as well as active NK cells are low [10]. Therefore, the innate immune response is effective in uninfected newborn from chagasic mothers. The adaptive immune system is also relevant; for instance, maternal anti-*T. cruzi* antibodies are transferred through the placenta to the fetus and where they reduce the parasitemia [9].

Importantly, congenital transmission rates for *Trypanosoma cruzi* (*T. cruzi*) are relatively low (3.9–5.6%) [11, 12]. Moreover, the typical amastigote nests (intracellular parasites) cannot be observed in placentas from mothers with chronic Chagas disease [13] nor in human placental chorionic villi explants (HPCVE) infected in vitro with the parasite [14]. In the latter, only a few parasite antigens and DNA can be identified [14, 15]. In addition, other infections of the placenta are not commonly observed [11]. All these evidences suggest the presence of systemic and local defense mechanisms against pathogen and that the placenta is a key factor against *T. cruzi* infection.

2. Antiparasitic mechanisms of the placenta

Importantly, during congenital transmission, the parasites must cross the placental barrier [8, 16].

2.1. Placenta

The placenta is a temporary organ that provides nutrition and gas exchange for the developing fetus, ensuring normal embryo-fetal growth and development and supporting pregnancy-related changes in maternal physiological systems [17]. The human placenta is classified as discoidal, villous, and hemochorial and consists of a fetal portion, which originates from the *Chorion frondosum*, and a maternal portion, or basal decidua, which originates from the endometrium. The functional units are the floating chorionic villi, formed by the trophoblast, and

the villous stroma. The trophoblast comes into contact with maternal blood in the intervillous space (IVS) and is delimited by a basal lamina from the villous stroma, which is the fetal connective tissue containing the fetal capillaries. The placental barrier is formed by the trophoblast, basal laminae, villous stroma, and fetal capillary endothelium (**Figure 1**) [4, 8].

The placenta may contain as much as 500 mL of maternal blood, in the IVS, exposing the trophoblast to pathogens that might be present in it [5]. Therefore, the trophoblast is a key factor against congenital infection since it is the first fetal tissue that comes into contact with pathogens circulating in the maternal blood [11]. On the other hand, the placenta, as an immune regulatory organ, acts as a modulator of fetal as well as maternal immune responses [6]. The placenta, in particular the trophoblast, is also part of a local innate immune response. Three types of defense mechanisms in innate immunity have been described: (i) anatomical barriers, such as the placental barrier (**Figure 1**), (ii) cellular innate immune responses, and (iii) humoral innate immune responses. During tissue invasion, pathogen breaks the anatomical barriers, and innate immune cells are activated and secrete cytokines and chemokines to control pathogen replication [18, 19].

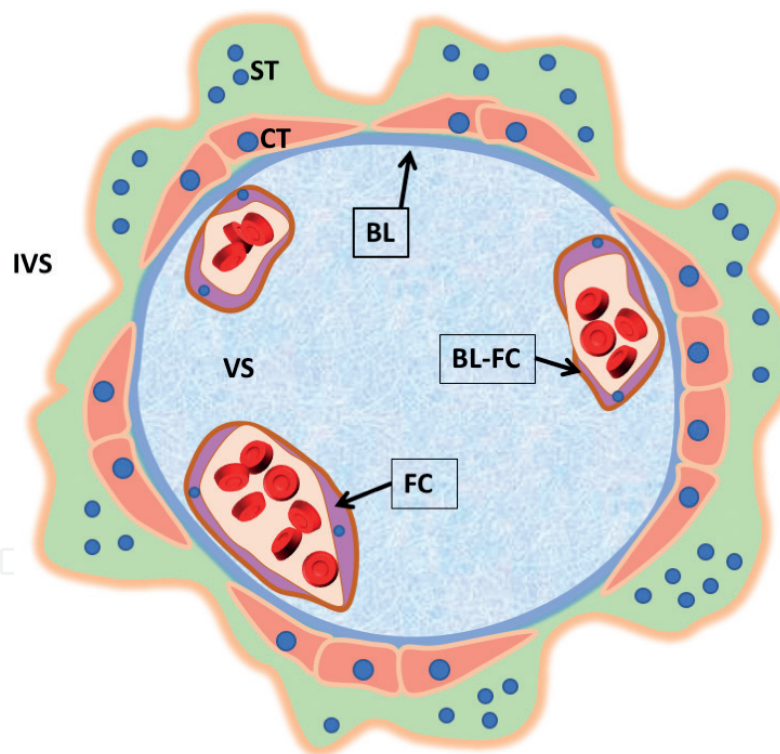


Figure 1. The placental barrier: the placental barrier is formed by the trophoblast composed by the superficial syncytiotrophoblast (ST) that contacts the maternal blood in the intervillous space (IVS) and the cytotrophoblast (CT) which corresponds to the germinative layer of the epithelium. The trophoblast is supported by the fetal connective tissue of the villous stroma (VS) that contains the fetal capillaries (FC). The placental barrier presents also two basal membranes (BM): (1) between villous stroma and trophoblast and (2) around fetal endothelium. The cells of the CT proliferate and afterward differentiate into the ST. The continuous incorporation of CT cells into the ST is counterbalanced by the formation of apoptotic ST knots, which are released into the IVS.

2.2. The trophoblast

The trophoblast is a bistratified epithelium composed of the superficial syncytiotrophoblast (ST) and the basal cytotrophoblast (CT). There is strong evidence that the ST layer is resistant to numerous pathogens, including *T. cruzi* [20, 21]. However, damage of the syncytium might allow the parasite access to the villous core, increasing parasite infection [5, 22].

2.3. The trophoblast epithelial turnover

The basal CT cells are the only ones of the trophoblast with proliferative capacity. The superficial multinucleated ST layer is highly differentiated and is unable to proliferate. Importantly, the ST contacts directly with the maternal blood [23–25], where in case of *T. cruzi* infection, the parasite circulates [11, 26]. The ST is a typical syncytium that is continuous and normally uninterrupted and covers all villous trees of the human placenta. The ST is formed and maintained by the continuous incorporation of CT cells through syncytial fusion, meaning that the CT cells suffer cellular differentiation. The continuous incorporation of CT cells into the ST is counterbalanced by the formation of apoptotic knots that are released into the maternal blood present in the IVS [24, 25]. The normal epithelial turnover assures the integrity of diverse anatomical barriers, including the placental one. The maintenance of the integrity of anatomical barriers is part of the innate immune system due to the fact that pathogens, prior to cell invasion, must attach to the surface of cells. As these cells are continuously eliminated, the attached pathogens are removed with them [3]. Thus, the trophoblast turnover should be considered as a defense mechanism against pathogens, including *T. cruzi*.

2.3.1. Cell proliferation

We have previously shown that the parasite induces, in the trophoblast, cellular proliferation. These experiments were performed in HPCVE and in the trophoblastic cell line BeWo; both models are commonly used in trophoblast studies [27]. In HPCVE *T. cruzi* increases DNA synthesis as well as the PCNA proliferation marker [11]. On the other hand, in BeWo cells the parasite also induces significant DNA synthesis (as determined by BrdU incorporation), increase of the percentage of cells in the ST and G₂/M cell cycle phases, and the expression of the widely used proliferation markers AgNORs, PCNA, and Ki67 [28]. Importantly, it should be taken into account that PCNA acts also as a molecular coordinator in multiple other cellular functions such as DNA damage repair, cell cycle control, cell survival, and gene expression [29]. Therefore, the increase of PCNA expression could also be a response to *T. cruzi*-induced cell and tissue damage. We have previously demonstrated that *T. cruzi* induces during ex vivo infection tissue disorganization of HPCVE [14] as well as apoptosis [30]. However, Ki67, a more specific proliferation marker that can be observed only during the active phases of the cycle [24], was significantly increased together with the other proliferation markers.

2.3.2. Cell differentiation

As described above, CT cells differentiate continuously and fuse with the ST [24, 25]. *T. cruzi* induces cell differentiation in the trophoblast in HPCVE and BeWo cells. Thus, the parasite

increases the protein expression of the major biochemical markers of trophoblast differentiation [31]: β -human chorionic gonadotropin (β -hCG) and syncytin [3]. Moreover, *T. cruzi* induces cell fusion in BeWo cells as demonstrated by a two-color fusion assay and by the analysis of the redistribution of the intercellular adhesion protein desmoplakin [3]. Previously, we have shown that *T. cruzi* activates the ERK1/ERK2 MAPK pathway [32]. Interestingly, the induction of trophoblast differentiation is mediated by the activation of the ERK1/ERK2 MAPK and other MAPK signal transduction pathways [33].

2.3.3. Apoptotic cell death

T. cruzi also induces apoptotic cell death in the trophoblast. The ST releases continuously apoptotic knots into the IVS [24]. In HPCVE, the induction of apoptosis has been demonstrated by the determination of the presence of pyknotic nuclei, induction of DNA fragmentation, caspase-3 like activity, and presence of caspase-3 and cleaved cytokeratin 18 [30]. Cellular processes related to apoptosis are also regulating cell differentiation (fusion) in the trophoblast. For instance, CT cell differentiation is regulated by caspases [34, 35]. Particularly, caspase-8, an apoptosis initiator caspase, regulates trophoblast differentiation and fusion. Caspase-8 is activated in highly differentiated CT cells just prior to fusion and escorts the fusing cell content including the nucleus into the ST, and it has not been found in proliferating CT cells [23, 36]. Moreover, the fusion of the trophoblast has been visualized by localizing caspase-8 [34]. *T. cruzi* induces in BeWo cells as well as HPCVE the expression and activation of caspase-8 [11, 37]. Moreover, the inhibition of caspase-8 increases the amount of parasite DNA and the number of intracellular parasites in BeWo cells [37]. The inhibition of caspase-8 decreases parasite-induced cellular differentiation and apoptotic cell death, but not cellular proliferation [11, 37].

2.4. The trophoblast and the innate immune cellular response against *T. cruzi*

The innate immune response against pathogens is initiated by pathogen pattern recognition receptors (PRRs), which include *Toll*-like receptors (TLRs) that recognize and bind highly conserved sequences known as pathogen-associated molecular patterns (PAMPs). The human trophoblast expresses all ten of the known functional TLRs [7], and *T. cruzi* is recognized by TLR-2, TLR-4, TLR-7, and TLR-9. Surface TLRs (TLR-2 and TLR-4) recognize glycosylphosphatidylinositol (GPI)-anchored mucin-like glycoproteins from *T. cruzi* surface [16, 38, 39]. We have shown that *T. cruzi* infection is related to TLR-2, but not to TLR-4 and TLR-9, expression, and activation [16]. The binding of TLR-2 to its ligands leads to activation of signaling pathways and upregulation of genes involved in the innate immune response including cytokines and chemokines [7, 16]. *T. cruzi* induces the secretion of IL-1 β , IL-6, IL-8, IL-10, and TNF- α in HPCVE [16]. Interestingly, IL-1 β , IL-6, and TNF- α secretions are also associated with cellular proliferation and differentiation in the trophoblast [40, 41], and inhibition of TLR-2 impairs trophoblast turnover (manuscript under review in "*Placenta*"). However, up to now, we do not know whether the activation of TLRs occurs mainly in the trophoblast or if other placental cells are also involved in this matter that should be addressed in the future. Importantly, as a consequence of our results, the TLR-2-initiated cytokine profile should also be considered as a local placental defense mechanism.

2.5. Other placental defense mechanisms against *T. cruzi*

The placenta, and particularly the trophoblast, expresses many noncoding RNAs including microRNAs (miRNAs) that regulate placental development function. Moreover, different miRNAs exhibit specialized functions during normal and pathological pregnancies. Placental miRNAs, packaged within exosomes and other vesicles or bound in protein complexes, are capable of communicating distinctive signals to maternal and fetal tissues [5]. Placenta-specific and trophoblast-derived miRNAs, encoded in the chromosome 19 miRNA cluster (C19MC), are released within exosomes and confer resistance to viral infection in other mammalian cells [42]. Preliminary results from our laboratory show that *T. cruzi* induces in HPCVE a specific C19MC-encoded miRNA profile. Some of those miRNAs are involved in the regulation of immune functions, particularly those of TLR-mediated pathways [43]. Studies on *T. cruzi*-induced miRNAs and exosomes are currently ongoing, being of particular interest since miRNA pathways are potential diagnostic tools and targets for therapeutic control of parasitic diseases [44] and other pathologies, including placenta-derived ones [5]. Targeting miRNAs constitute a promising possibility for the treatment of different diseases due to the facts that (i) miRNAs are regulators of gene expression, (ii) are relatively easy to manipulate, (iii) can be administrated in vivo, and (iv) present an apparent lack of adverse effects when administered intravenously. Moreover, *miRNAs are detectable in biological fluids, thus offering real potential as noninvasive biomarkers*, providing new diagnostic and therapeutic options during pregnancy and for several diseases as well [45, 46].

In summary, the studies about the placental defense mechanism that determines the probability of infection, together with parasite, maternal, and fetal/newborn factors, are of outstanding interest since they are potential diagnostic, prognostic, and therapeutic tools.

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