Journal of Animal and Veterinary Sciences
2015; 2(1): 6-9
Published online March 30, 2015 (http://www.openscienceonline.com/journal/javs)

The Mechanisms of Adverse Pregnancy Effects Caused by *Toxoplasma gondii* Infection

Yanhua Wang

State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China

Email address

wangyh061001@163.com

To cite this article

Yanhua Wang. The Mechanisms of Adverse Pregnancy Effects Caused by *Toxoplasma gondii* Infection. *Journal of Animal and Veterinary Sciences*. Vol. 2, No. 1, 2015, pp. 6-9.

Abstract

Toxoplasmosis is a disease caused by *Toxoplasma gondii*. Importantly, it is an important biological factor that affects human prenatal and postnatal care. Approximately half of pregnant women who are infected with *T. gondii*, with or without clinical symptoms, develop a maternal-fetal vertical infection, leading to miscarriage and stillbirth and causing congenital fetal defects or malformations (morphological malformations and functional mental retardation). In terms of animal husbandry, infected pregnant animals, especially sheep, also suffer miscarriage. One of the major negative impacts of toxoplasmosis in sheep is related to the treatment cost after disease outbreak and the direct loss due to animal death; there are indirect losses caused by poor productivity and reproductive disorders. This article reviews the pathogenetic mechanisms underlying the adverse pregnancy effects caused by *T. gondii* infection.

Keywords

T. gondii, Adverse Pregnancy, Pathogenetic Mechanisms

1. Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite that has a wide host range and prevails worldwide, causing parasitic zoonoses. Toxoplasmosis, an infection caused by *T. gondii*, is not only significant with regard to public health but also results in huge economic losses in animal husbandry. When our team conducted etiological research and clinically detected serum antibodies to *T. gondii*, a number of farmers from different regions across the country indicated that the miscarriage rates in sheep have continued to rise in recent years. Despite the implementation of a series of measures, the prevention and control effects were not significant. This problem seriously impacted farming efficiency and livestock safety, leaving the majority of farmers vulnerable to economic losses. Therefore, in-depth studies on the molecular mechanisms underlying the adverse effects caused by *T. gondii* infection during pregnancy are urgently needed [1, 2].

2. Structural Damage to the Placenta Caused by *T. gondii* Infection

Early research on the mechanism underlying adverse pregnancy effects caused by *T. gondii* infection has mainly focused on structural damage to the placenta. For example, *T. gondii* infection can cause placental barrier damage, inflammatory pathological damage, necrotizing granuloma in the intervillous space, thickening of the endothelium of inflamed blood vessels, and interstitial fibrosis [3]. Trophoblasts constitute an important component of the placental barrier. Trophoblast cells mediate an endocrine function, and their proliferative and functional dysregulation are closely related to the development and progression of a variety of pregnancy-related diseases [4]. It has been found that *T. gondii* infection can induce a series of morphological changes in trophoblasts, mainly manifesting as evident destruction of trophoblasts, including shrunk cells, condensed cytoplasm, and dense nucleoplasm [5].

3. Effect of *T. gondii* Infection on the Placental Trophoblast Cell Cycle

With further research advances, a series of cellular effects caused by *T. gondii* invasion of cells has been elucidated. To be relevant, research must progress from phenomenon observation to deriving the mechanism that mediates the appearance of the phenomena. Thus, the research object in this case is no longer isolated parasites but instead the complex parasite - host cell interrelationship.

T. gondii has been found to arrest ubiquitin-like, containing PHD and RING finger domains 1 (UHRF1), ct26, and other cell lines in the G2/M phase, thus contributing to the further proliferation and spread of the parasite in host cells [6, 7]. However, when mouse placental trophoblasts are infected with *T. gondii*, their cell cycle is arrested mainly in the S phase rather than in the G2/M phase. The S phase is a critical period of DNA replication and repair in cells. After trophoblasts are infected by *T. gondii*, factors such as the parasite itself and relevant metabolites can cause DNA damage in the cell, whereas arrest of cells in the S phase can extend the time of DNA repair, which is possibly the result of a cellular response to the parasite invasion. With an increasing amount of invading *T. gondii*, cells are significantly arrested in the S phase while the G2/M phase is substantially shortened. When cyclin A expression is decreased or cyclin-dependent kinase-2 (CDK2) can no longer be dephosphorylated, these processes ultimately cause the occurrence of S-phase arrest. Cyclin A/CDK2 is an essential periodicity factor for the transition from the S phase to G2/M phase [8].

4. Effect of *T. gondii* Infection on Trophoblast Apoptosis

For trophoblasts, apoptosis ensures that old or necrotic cells disappear without causing local tissue inflammation, which is a part of the process of cellular metabolism [9-11]. The proliferation, differentiation, and apoptosis of trophoblasts have important roles in the formation and development of the placenta, exerting a vital impact on pregnancy outcome [12,13]. Both *in vivo* and *in vitro* studies have demonstrated that the level of apoptosis in trophoblasts is always elevated after *T. gondii* infection. Additionally, it has been found that apoptosis induced by *T. gondii* infection occurs mainly in non-infected bystander cells, which suggests that apoptosis triggered by *T. gondii* is possibly mediated through soluble molecules [14]. That is, host cell proteins and parasite proteins jointly regulate the apoptotic pathway in infected cells.

The Bcl-2 proto-oncogene is an apoptosis-related gene that is currently a popular topic of study. Bal-2 and Bax are both members of the Bcl-2 family but with completely opposite functions. The former inhibits apoptosis, while the latter promotes apoptosis. An imbalance in the expression of Bal-2 and Bax will cause a reduction in the formation of Bcl-2/Bax heterodimers and lead instead to the generation of Bcl-2/Bcl-2 or Bax/Bax homodimers. If the amount of Bax/Bax homodimers is increased, there will be an increase in the permeability of mitochondria in the cells, ultimately leading to apoptosis. One study found that in BALB/c pregnant mice infected with *T. gondii*, the rate of apoptosis in the placental cells significantly rose. Additionally, Bax was overexpressed in pregnant mouse placental villi and positively correlated with apoptosis, whereas Bcl-2 expression was significantly reduced and negatively correlated with apoptosis. Perhaps an imbalance in the expression of Bax and Bcl-2 in the placental tissue ultimately induced apoptosis. However, the Bcl-2 family is only one of the regulatory elements of apoptosis. Thus, we cannot infer the exact route through which *T. gondii* infection induces apoptosis in placental tissue [15]. Moreover, another study found that infection of pregnant mice with *T. gondii* tachyzoites led to the occurrence of oxidative stress and caused the elevation of the trophoblast apoptosis level. The pathway that induces this apoptosis mainly involves reactive oxygen species (ROS) -mediated endoplasmic reticulum stress, and it is initiated through the activation of caspase12, CHOP, and the c-Jun amino-terminal kinase (JNK) pathways [16]. *T. gondii* can alter the course of apoptosis in host cells through promoting or inhibiting apoptotic signals [17]. The regulation of apoptosis induced by infection with *T. gondii* may be closely related to the virulence factors of the parasite [18], infection status (acute or chronic) of the cells, type of infected cells, and specific experimental observation conditions [19-21].

5. Application of Proteomics in Research on Physiopathological Mechanisms of Pregnancy

According to the existing research data, the possible pathogenetic mechanisms of adverse pregnancy caused by *T. gondii* infection mainly include structural damage to the placenta, placental apoptosis, and oxidative stress injury. It may also involve the interaction between parasite proteins of *T. gondii* and a number of cell adhesion molecules [22]. Nevertheless, the exact mechanism is highly complex, and further research is needed to address this “puzzle”.

No biological activity can be accomplished by a single protein. Instead, processes often require the participation of multiple proteins, which act in parallel or in causal relationships. Especially during mutual adaption and resistance between the parasite and host cell, parasite proteins and host cell proteins form a complex functional network, in which parasite - parasite proteins, parasite - cell proteins, and cell - cell proteins mutually constrain, collaborate, and compete with each other. When invading the host cells, *T. gondii* can cause changes in a number of functional genes during the process of transcription and protein expression in host cells, further causing cellular abnormalities in terms of metabolism, signal transduction, apoptosis, and the cell cycle [23, 24]. These proteins then form the molecular basis for a series of cellular effects occurring within the infected cell. Studying these proteins and associated cell signaling

pathways will contribute to understanding about the infection mechanism. Therefore, researching the interaction between the parasite and host cells is far more important than simply studying the biology and metabolic activities of the cells [25, 26].

Etiological research conducted using proteomic techniques and substantial protein database resources has become an important strategy for understanding the pathogen and pathogen - host interactions. Early proteomics research has mainly focused on the composition of the proteome. With continuous progress of relevant research, qualitative proteomic techniques that can only provide information on protein species and modification can no longer meet the needs of the current research work. The physiological role of a protein not only is determined by the presence or absence of other protein species but also largely depends on the amount of the protein or the extent of protein modification after translation. Quantitative proteomics is an important part of functional proteomics. Accurate quantitative analysis of gene expression at the proteome level by means of an appropriate method or technology is currently a necessary and novel means to study the pathogenesis of diseases [27]. Quantitative proteomic techniques can be used to identify and screen factors that cause variations between different samples. They can also reveal the course and nature of the physiopathological state of cells, as well as their response pathway and cellular regulatory mechanisms to external environmental stimuli. Additionally, these techniques can be used for qualitative and functional analysis on certain key proteins [28]. One of the commonly used absolute quantification techniques at the protein level is the powerful isobaric tags for relative and absolute quantitation (iTRAQ).

As described above, the infection of host cells by the parasite can affect the synthesis of cellular proteins. Moreover, many of the proteins not only undergo quantitative changes but also have abnormal changes regarding the modification status, function, and localization. Compared with the protein expression profiles of normal cells, there will be a number of differential proteins in the infected cells. These differential proteins are specific executors of parasite infection and host cell defense. Identifying these proteins and investigating their relationships with each other are important means of understanding parasite - host interactions. Currently, research on the molecular mechanisms underlying adverse pregnancy caused by *T. gondii* infection is exclusively based on a certain metabolic pathway or signaling pathway. Little is known about the overall alteration of protein expression in placental cells after they are infected by the parasite. Additionally, most studies have employed a mouse model, whereas the fate of host cells is related to multiple factors such as the virulence, infection intensity, and time of the parasite strain, as well as the cell type [19-21, 29]. Hence, many cell activities remain unknown, though they actually affect the life activities of cells. Furthermore, proteomic techniques have been successfully used to study a variety of physiopathological states of pregnancy with placental trophoblasts as a model [30-32]. Therefore, a future study may adopt an *in vitro* constructed placental trophoblast model of *T. gondii* infection and utilize iTRAQ to screen key protein molecules and identify their roles in causing adverse pregnancy effects. This work would have great implications for in-depth interpretation of the replication and infection of *T. gondii* as well as the molecular mechanisms causing its adverse pregnancy effects.

Acknowledgments

This study was supported by grants from the Natural Science Foundation of Gansu Province, China (145RJZA234).

References

[1] Senegas A, Villard O, Neuville A, Marcellin L, Pfaff AW, Steinmetz T, Mousli M, Klein JP, Candolfi E. *Toxoplasma gondii*-induced foetal resorption in mice involves interferon-gamma-induced apoptosis and spiral artery dilation at the maternofoetal interface. Int J Parasitol 2009;39(4):481-7.

[2] Cabañas-Cortés MA, Reyes-Maldonado E, Montiel-Cervantes L, Domínguez-López ML, Jiménez-Zamudio L, García-Latorre E. *Toxoplasma gondii*: effect of maternal infection in the development of lymphoid organs of BALB/c neonates. Exp Parasitol2009;121(3):279-87.

[3] Dubey JP. Lesions in transplacentally induced toxoplasmosis in goats. Am J Vet Res 1988;49(6):905－909.

[4] Sargent IL, Germain SJ, Sacks GP, Kumar S, Redman CW. Trophoblast deportation and the maternal inflammatory response in pre-eclampsia. J Reprod Immunol 2003;59(2): 153-160.

[5] Zhao JY, Huang ZZ, Li YW, Hou YY, Wang XX, He YH, Sun YP. Tachyzoite invasion and pathological lesion in placenta during pregnancy with *Toxoplasma gondii* Infection. J Trop Med 10.2010 1384-1386.

[6] Brunet J, Pfaff AW, Abidi A, Unoki M, Nakamura Y, Guinard M, Klein JP, Candolfi E, Mousli M. *Toxoplasma gondii* exploits UHRF1 and induces host cell cycle arrest at G2 to enable its proliferation. Cell Microbiol 2008;10:908-920.

[7] Gao J,Ye Bin,W Wei H, Kong J. Effects of Toxoplasma gondiitachyzoites on proliferation and cell cycle of mouse colon cancer cells ct26. Chin J Zoonoses 2010; 26(12):1088-1092.

[8] Qi Y, Tu Y, Yang D, Chen Q, Xiao J, Chen Y, Fu J, Xiao X, Zhou Z. Cyclin A but not cyclin D1 is essential for c-myc-modulated cell-cycle progression. J Cell Physiol 2007;210:63-71.

[9] Huppertz, B, Kadyrov, M, Kingdom JC. Apoptosis and its role in the trophoblast. Am J Obstet Gynecol 2006;195(1):29-39.

[10] Kim SM, Park HS, Jun DY, Woo HJ, Woo MH, Yang CH, Kim YH. Mollugin induces apoptosis in human Jurkat T cells through endoplasmic reticulum stress-mediated activation of JNK and caspase-12 and subsequent activation of mitochondria-dependent caspase cascade regulated by Bcl-xL. Toxicol Appl Pharmacol 2009; 241(2):210-220.

[11] Park GB, Kim YS, Lee HK, Song H, Cho DH, Lee WJ, Hur DY. Endoplasmic reticulum stress-mediated apoptosis of EBV-transformed B cells by cross-linking of CD70 is dependent upon generation of reactive oxygen species and activation of p38 MAPK and JNK pathway. J Immunol 2010; 185(12):7274-7284.

[12] De Falco M, Penta R, Laforgia V, Cobellis L, De Luca A. Apoptosis and human placenta: expression of proteins belonging to different apoptotic pathways during pregnancy. J Exp Clin Cancer Res 2005;24(1):25-33.

[13] Soni S, Rath G, Prasad CP, Salhan S, Saxena S, Jain AK. Apoptosis and Bcl-2 protein expression in human placenta over the course of normal pregnancy. Anat Histol Embryol 2010;39:426-431.

[14] Biagioli M, Pifferi S, Ragghianti M, Bucci S, Rizzuto R, Pinton P. Endoplasmic reticulumstress and alteration in calcium homeostasis are involved in cadmium-induced apoptosis. Cell Calcium 2008;43(2):184-195.

[15] Debierre-Grockiego F, Azzouz N, Schmidt J, Dubremetz JF, Geyer H, Geyer R, Weingart R, Schmidt RR, Schwarz RT. Roles of glycosylphosphatidylinositols of *Toxoplasma gondii*. Induction of tumor necrosis factor-alpha production in macrophages. J Biol Chem2003;278(35): 32987-32993.

[16] Xv XC. Roles of oxidative stress and endoplasmic reticulum stress in placental trophoblast apoptosis induced by *Toxoplasma* infection. Anhui Medical University, Hefei, China. P6

[17] Heussler VT, Kuenzi P, Rottenberg S. Inhibition of apoptosis by intracellular protozoan parasites. Int J Parasitol 2001;31(11):1166-1176.

[18] Gavrilescu LC, Denkers EY. IFN-gamma overproduction and high level apoptosis are associated with high but not low virulence *Toxoplasma gondii* infection. J Immunol 2001;167(2):902-909.

[19] Nishikawa Y, Kawase O, Vielemeyer O, Suzuki H, Joiner KA, Xuan X, Nagasawa H. *Toxoplasma gondii* infection induces apoptosis in noninfected macrophages: role of nitric oxide and other soluble factors. Parasite Immunology 2007;29(7):375-385.

[20] Boyle JP, Radke JR. A history of studies that examine the interactions of *Toxoplasma* with its host cell: Emphasis on in vitro models. Int J Parasitol 2009; 39(8): 903-914.

[21] Xi LL, Li W. Progress of research on the pathogenesis, virulence, and genotypes of *Toxoplasma gondii.* J Path Bio 2009;4(11):859-861.

[22] Pfaff AW, Georges S, Abou-Bacar A, Letscher-Bru V, Klein JP, Mousli M, Candolfi E. *Toxoplasma gondii* regulates ICAM-1 mediated monocyte adhesion to trophoblasts. Immunol Cell Bio 2005;83(5):483-9.

[23] Nelson MM, Jones AR, Carmen JC, Sinai AP, Burchmore R, Wastling JM. Modulation of host cell proteome by the intracellular apicomplexan parasite *Toxoplasma gondii*. Infect Immun 2008;76(2):828-844.

[24] Marshall ES, Elshekiha HM, Hakimi MA, Flynn RJ. *Toxoplasma gondii* peroxiredoxin promotes altered macrophage function, caspase-1-de-pendent IL-1βsecretion enhances parasite replication. Vet Res 2011;42(1):80.

[25] Caffaro CE, Boothroyd JC. Evidence for host cells as the major contributor of lipids in the intravacuolar network of toxoplasma-infected cells. Eukaryot Cell 2011;10(8):1095-1099.

[26] Tonkin ML, Roques M, Lamarque MH, Pugnière M, Douguet D, Crawford J, Lebrun M, Boulanger MJ. Host cell invasion by apicomplexan parasites: insights from the co-structure of AMA1 with a RON2 peptide. Science 2011; 333(6041): 463-467.

[27] Bunger MK, Cargile BJ, Ngunjiri A, Bundy JL, Stephenson JL Jr. Automated proteomics of E. coli via top-down electron-transfer dissociation mass spectrometry. Anal Chem 2008;80:1459-1467.

[28] Mann M. Funetional and quantitative Proteomies using SILAC. Nat Rev Mol Cell Biol. 2006;7:952-958.

[29] GE Pu, ZHENG Wei-ping,LI Chun-li,LIU Yu-jiao,YE Bin. Effect of *Toxoplasma gondii* RH strain on cell cycle of mouse placental trophoblasts. Sichuan Journal of Zoology 2012;36:900-904.

[30] Hoang VM, Foulk R, Clauser K, Burlingame A, Gibson BW, Fisher SJ. Functional proteomics: examining the effects of hypoxia on the cytotrophoblast protein repertoire. Biochemistry 2001; 40:4077-4086.

[31] Sawicki G, Dakour J, Morrish DW. Functional proteomics of neurokinin B in the placenta indicates a novel role in regulating cytotrophoblast antioxidant defences. Proteomics 2003;3:2044-2051.

[32] Sun LZ, Yang NN, De W, Xiao YS. Proteomic analysis of proteins differentially expressed in preeclamptic trophoblasts. Gynecol Obstet Invest 2007;64:17-23.