

TOXOPLASMA GONDII INFECTION ELICITS THE INCREASE OF FAS EXPRESSION ON THE TROPHOBLAST ASSOCIATED WITH THE INCREASE OF TROPHOBLAST APOPTOSIS

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ABSTRACT

Toxoplasma gondii infection causes the increase of trophoblast apoptosis. The mechanism of trophoblast apoptosis in Toxoplasma gondii-infected pregnant mice has not been fully understood. We measured Fas expression on the trophoblast of infected-pregnant mice. The objective of this study was to proof the effect Toxoplasma gondii infection to the increase of Fas expression on pregnant mice trophoblast and the increase of Fas expression on pregnant mice trophoblast associated with increase of trophoblast apoptosis. Forty-eight pregnant Swiss mice were divided into 6 groups. Group I, first week gestation uninfected mice. Group II, first week gestation infected mice. Group III, second week gestation uninfected mice. Group IV, second week gestation infected mice. Group V, third week gestation uninfected mice. Group VI, third week gestation infected mice. Mice were infected with 1×10^3 tachyzoites T. gondii doses by intraperitoneal. Four days post infection, mice were sacrificed, placenta was removed for observation Fas expression by immunohistochemistry staining and TUNEL ASSAY with Apop tag kit was used to observe trophoblast apoptosis index. The data were analysed by ANOVA and Regression. The result showed that Fas expression was significantly difference ($p < 0.00$) between infected mice and uninfected mice trophoblast. Fas expression was increasing on trophoblast of infected mice. Fas expression in first week gestation infected mice increased 7.4 times (from 3.65% to become 27.11%); in second week increased 7.2 times (from 5.37% to become 38.50%) and in the third week increased 3.6 times (from 11.64% to become 41.44%). The increase of Fas expression was significantly elevated when a high trophoblast apoptosis index in infected mice, rather than uninfected mice. We concluded that Toxoplasma gondii infection has effect on the increase of Fas expression on pregnant mice trophoblast and Fas expression associated with the increase of trophoblast apoptosis.

Keywords: Toxoplasma gondii, Fas, trophoblast, apoptosis, pregnancy

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INTRODUCTION

Toxoplasma gondii is an intracellular coccidian belonging to the phylum Apicomplexa. The parasite is globally distributed and can be found within many different species of mammals and birds. *T. gondii* most often causes subclinical infection; however, primary infection during pregnancy can induce fetal pathology and abortion in both humans and animals (Dubey et al. 1998). The pathological consequences for the fetus are dependent upon the trimester during which transmission occurs, and will vary in severity from mild ocular disease to death (Dupouy-Camet 2002; Ghaffar 2001; Weissmann 2003). In general, fetus-severe infection occurring during the early stages of pregnancy than that occurring later, although the risk of transmission during maternal infection increases at later stages of pregnancy. However, recent studies indicate that induction of a strong type 1 cytokine response at the fetal-maternal interface may also result in rejection of the fetus

(Wegmann et al. 1993). Thus, such a response could contribute to spontaneous abortion during acute toxoplasmosis in a pregnant female. It is more indirectly immunobiochemical aspect (such as increase of IFN- γ production, caspase-3 and trophoblast apoptosis) rather than directly parasitological aspect (Sardjono 2005). However, mechanism of trophoblast apoptosis in *Toxoplasma gondii*-infected pregnant mice has not been fully understood.

Apoptosis, or programmed cell death, is a natural mechanism by which the body eliminates unnecessary or potentially dangerous cells in order to maintain normal tissue function. During implantation, apoptosis is important for the appropriate tissue remodelling of the maternal decidua and invasion of the developing embryo (Payne et al. 1999; Galan et al. 2000). Apoptosis has been described in the trophoblast layer of placentas from normal pregnancies throughout gestation, it is higher in third trimester compared to first

trimester placenta (Mor and Abraham 2003; Smith et al. 1997a). This suggests that the regulation of placental apoptosis is essential for the normal physiology of pregnancy. However, the increased apoptosis trophoblast is pathologic conditions and it is a characteristic of trophoblast rejection which caused complications of pregnancy such as abortion, stillbirths and low birth weight infants (Aschkenazi et al. 2002; Smith et al. 1997b).

The Fas/FasL system is one of the main apoptotic pathways controlling trophoblast apoptosis. Trophoblast express both Fas (CD95) and Fas Ligand (FasL; CD95L) (Balkundi et al. 2000; Gruslin et al. 2001; Kauma et al. 1999). The interaction between Fas and FasL or the binding of an agonistic anti-Fas monoclonal antibody (mAb) to the extracellular region of Fas results in the activation of the Fas receptor. Consequently, the Fas-associated death domain (FADD) binds to the cytoplasmic tail of Fas and recruits other cellular proteins, forming the death-inducing signalling complex (DISC), the point at which the TNF death receptor pathways converge. Once assembled, procaspase-8 binds to the DISC and is activated by a series of cleavage steps. Active caspase-8 cleaves a number of effector procaspases including procaspases-3, which results in cleavage and activation of number of substrates resulting in characteristic apoptosis (Gupta 2003).

Under normal conditions, trophoblast cells are resistant to Fas-mediated apoptosis (Payne et al. 1999; Aschkenazi et al. 2002). A number of factors in the placental microenvironment, including hormones and cytokines, modulate the immune response by regulating the expression of FasL and Fas. (Aschkenazi et al. 2002; Balkundi et al. 2003). Cytokines promote sensitivity to Fas-mediated apoptosis and TNF α and IFN γ induce apoptosis. Fas-mediated apoptosis of trophoblast (Balkundi et al. 2003). *T. gondii* infection is a commonly occurring inflammatory state associated with a marked increase in proinflammatory cytokines in the placental microenvironment (Suwanti 2005). In the present study, we tested the hypothesis that *T. gondii* infection elicits the increased of the Fas expression on trophoblast and it is associated with trophoblast Fas-mediated apoptosis.

MATERIALS AND METHODS

Tissue Collection and Processing

Forty-eight pregnant Swiss mice were divided into 6 groups. Group I, first week gestation uninfected mice. Group II, first week gestation infected mice. Group III,

second week gestation uninfected mice. Group IV, second week gestation infected mice. Group V, third week gestation uninfected mice. Group VI, third week gestation infected mice. Mice were infected with 1×10^3 tachyzoites *T. gondii* doses by intraperitoneal. Four days post infection, mice were sacrificed, placenta was removed for observation. Fas expression by immunohistochemistry staining and TUNEL ASSAY with *Apop tag kit* was used to observe trophoblast apoptosis index.

Immunohistochemistry for Fas

Trophoblast Fas expression in paraffin-embedded placental tissues from pregnant mice was determined by immunohistochemistry using the Streptavidine Biotin Kit (Daco). Tissue sections were deparaffinized and rehydrated through graded alcohols using standard procedures. Endogenous peroxidase activity was quenched by a 30-min incubation with 0.3% H₂O₂ in water. Samples were incubated with Fas antibody (Rabbit polyclonal antibody, Dako) by a 30-min at room temperature. Sections were then washed twice with PBS and incubated with a biotinylated secondary antibody.. Sections were then washed twice with PBS and were followed by incubation with preformed streptavidin-biotinylated peroxidase complex. Sections were then washed twice with PBS and were developed with DAB as the chromogen substrate, rinsed, and counterstained with Mayer hematoxylin. Sections incubated with PBS instead of primary antibody served as negative controls

TUNEL ASSAY for Trophoblast Apoptosis

Paraffin-embedded placental tissues from pregnant mice was performed using the ApopTag kit (Daco). Tissue sections were deparaffinized and rehydrated through graded alcohols using standard procedures. Tissue sections were treated with 20 μ g/mL proteinase K for 25 min at room temperature and washed with distilled water, and endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide in PBS for 5 min. Slides were then rinsed with phosphate-buffered saline (PBS) and incubated for 1 h at 37 C in buffer containing digoxigenin-labeled deoxy-UTP and terminal deoxynucleotidyl transferase. Samples were then washed three times with PBS and incubated for 30 min at room temperature with antidigoxigenin antibody-peroxidase conjugate. After rinsing with PBS, slides were incubated at room temperature for 5 min with diaminobenzidine. Slides were counterstained with methyl green. Controls were carried out in which terminal deoxynucleotidyl transferase was omitted from the labeling reaction.

Statistical Analysis

Data were analyzed by Anova two factor to statistically compare the Fas expression on trophoblast between infected pregnant mice and uninfected pregnant mice and linear regression analysis. was performed to determine the correlations between the increase of trophoblast apoptotic indexes and the increase of Fas expression.

RESULTS

T. gondii infection elicits the increased of the Fas expression on trophoblast

To test our hypothesis that *T. gondii* infection elicits the increased of the Fas expression on trophoblast, we performed immunohistochemistry on placental tissues from mice infected *T. gondii* and compared these tissues with mice uninfected. There was a marked increase in Fas expression in the trophoblasts of the infected *T. gondii* placentas pregnant mice (Table 1).

Table 1. Means and SD of Fas Expression on Trophoblast of 1st week, 2nd week and 3rd week Gestation Pregnant Mice

Variable	Treatment	Gestation days		
		1 st week	2 nd week	3 rd week
Fas Expression on Trophoblast	Uninfected	3.65 ^a ± 1.42	5.37 ^a ± 2.19	11.64 ^b ± 4.18
	Infected	27.11 ^c ±13.63	38.50 ^d ±15.23	41.44 ^d ±7.42

Different superscript shows significantly difference ($p < 0.01$)

Fas expression was significantly difference ($p = 0.05$) between infected mice and uninfected mice trophoblast.. Fas expression was increasing on trophoblast of infected mice, rather than uninfected mice. Fas expression in first week gestation infected mice increased 7.4 times (from 3.65% to become 27.11%); in second week

increased 7.2 times (from 5.37% to become 38.50%) and in the third week increased 3.6 times (from 11.64% to become 41.44%). Figure 1 Shows Fas expression on trophoblast mice.

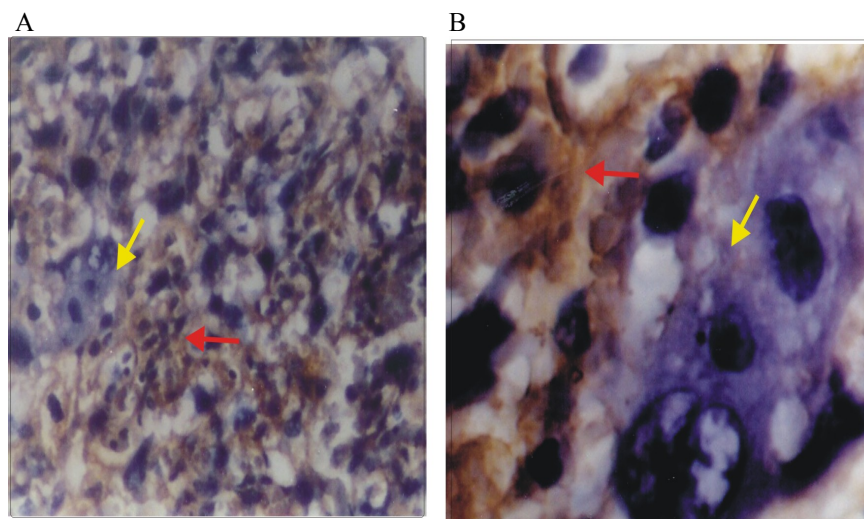


Figure 1. Fas Expression on Trophoblast. Immunohistochemistry using the Streptavidine Biotin Kit. A magnification x 100. B magnification x 400. Yellow arrow is negative cell and red arrow is positive cell.

Association between increase of Fas expression on trophoblast and increase of trophoblast apoptosis

To determine whether apoptosis was a progressive process on trophoblast in infected pregnant mice, the apoptotic index (the percentage of TUNEL-positive nuclei per total nuclei) was compared in infected and uninfected pregnant mice at the first, second and third week of pregnancy. The result showed that trophoblast apoptosis index were significantly difference ($p < 0.00$) between infected and uninfected mice (data not shown).

The linear regression analysis. was performed to determine the correlations between the increase of trophoblast apoptosis index and the increase of Fas expression. The result showed that increase of Fas expression on trophoblast correlate with the increase of trophoblast apoptosis in infected mice. Figure 2 show trophoblast cells apoptotic and Figure 3 shows the association between increase of Fas expression on trophoblast and increase of trophoblast apoptosis

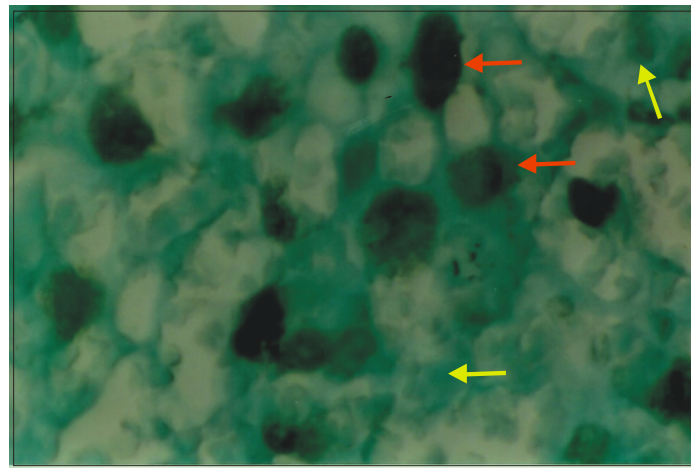


Figure 2. Immunohistochemical staining of a placental *T. gondii*-infected mice by TUNEL. Red arrows are the trophoblast cells apoptotic and yellow arrows are normal trophoblast cells.

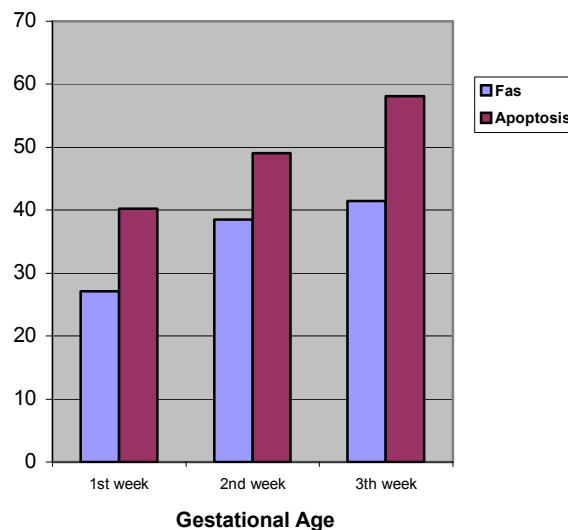


Figure 3. Association between the increase of Fas expression on trophoblast and increase of trophoblast apoptosis

DISCUSSION

This report shows for the first time that *T. gondii* infection elicits the Increase of Fas expression on the mice's trophoblast. The Increase of Fas expression is occurring on the trophoblast of infected pregnant mice at the first, second and third week of pregnancy.

Under normal conditions, trophoblast cells are resistant to Fas-mediated apoptosis (Payne et al. 1999; Aschkenazi et al. 2002). A number of factors in the placental microenvironment, including hormones and cytokines, modulate the immune response by regulating the expression of FasL and Fas. (Aschkenazi et al. 2002; Balkundi et al. 2003). Cytokines promote sensitivity to Fas-mediated apoptosis and TNF α and IFN γ induce apoptosis Fas-mediated apoptosis of trophoblast (Balkundi et al. 2003).

T. gondii infection is a commonly occurring inflammatory state associated with a marked increase in proinflammatory cytokines in the placental microenvironment (Suwanti 2005). So, proinflammatory cytokine in the placental microenvironment in *T. gondii*-infected pregnant mice trigger trophoblast to express Fas.

By the linear regression analysis, we performed to determine the correlations between the increase of trophoblast apoptosis index and the increase of Fas expression. The result showed that increase of Fas expression on trophoblast correlate with the increase of trophoblast apoptosis in infected mice

The Fas/FasL system is one of the main apoptotic pathways controlling trophoblast apoptosis. Trophoblast express both Fas (CD95) and Fas Ligand (FasL; CD95L) (Balkundi et al. 2000; Gruslin et al. 2001; Kauma et al. 1999). The interaction between Fas and FasL or the binding of an agonistic anti-Fas monoclonal antibody (mAb) to the extracellular region of Fas results in the activation of the Fas receptor. Consequently, the Fas-associated death domain (FADD) binds to the cytoplasmic tail of Fas and recruits other cellular proteins, forming the death-inducing signalling complex (DISC), the point at which the TNF death receptor pathways converge. Once assembled, procaspase-8 binds to the DISC and is activated by a series of cleavage steps. Active caspase-8 cleaves a number of effector procaspases including procaspases-3, which results in cleavage and activation of number of substrates resulting in characteristic apoptosis (Gupta 2003).

T. gondii infection also induces Interleukin (IL)-2 secretion by T helper (Danker & Gazienelli 1998). IL-2 induces Natural Killer (NK) cell and CTL (CD8+ T cell) in placenta to express FasL (Golsby et al. 2000).

Interaction FasL belonging NK and CTL cell with Fas belonging trophoblast cause trophoblast apoptosis.

CONCLUSION

In conclusion, our results document for the first time the effect of *T. gondii* infection to expression of Fas on trophoblast and it is trophoblast apoptosis mechanism in toxoplasmosis.

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