Acta Tropica 143 (2015) 1–7

Contents lists available at ScienceDirect

Acta Tropica

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

Polarization of macrophages induced by *Toxoplasma gondii* and its impact on abnormal pregnancy in rats

Lanting Kong[a,1], Qian Zhang[a,1], Jing Chao[b], Huiqin Wen[a], Yihua Zhang[a], He Chen[a], Faustina Pappoe[a], Aimei Zhang[c], Xiucai Xu[c], Yihong Cai[a], Min Li[a], Qingli Luo[a], Linjie Zhang[b,*], Jilong Shen[a,*]

[a] *Anhui Provincial Laboratory of Microbiology and Parasitology, Anhui Key Laboratory of Zoonoses, Department of Parasitology, Anhui Medical University, Hefei 230022, PR China*
[b] *Department of Immunology, Anhui Medical Iniversity, Hefei 230022, PR China*
[c] *The Central Laboratory of Affiliated Provincial Hospital of Anhui Medical University, Hefei 230022, PR China*

ARTICLE INFO

Article history:
Received 25 January 2014
Received in revised form
24 November 2014
Accepted 1 December 2014
Available online 9 December 2014

Keywords:
Toxoplasma gondii
Macrophage polarization
Abnormal pregnancy
Rat

ABSTRACT

Toxoplasma gondii infection is the leading cause of fetal intrauterine growth retardation among the five kinds of pathogens termed as TORCH, including *Toxoplasma*, *Rubella* virus, *Cytomegalo* virus, herpes virus and others during pregnancy. Pathogens infect the fetus through the placenta. *T. gondii* infection may result in congenital toxoplasmosis, miscarriage, stillbirth, and preemie, and increase pregnancy complications. Adaptive immune response induced by *T. gondii* infection stimulates T cells and macrophages to produce high levels of cytokines. Physiologically, the microenvironment of pregnancy was Th2-dominant. Here we set up a pregnant Sprague-Dawley rat model, and reported the polarization of macrophages induced by genotype Chinese 1 strain (Wh6) of *Toxoplasma*, and its adverse impact on pregnancy. The results showed that Wh6 infection pre- or in-gestation both led to abnormal pregnancy outcomes. Peritoneal macrophages in pre-gestation infection were polarized toward classically activated macrophages (M1), while in-gestation infection drove macrophages to polarize toward M2 activation. The Th2-dominant immune response in pregnant rat somewhat inhibits the excessive bias of the macrophages toward M1, and partially, toward M2. Infection of pre- and in-gestation may alter the physiological immune microenvironment in pregnant rats, giving rise to abnormal pregnancy outcomes.

1. Introduction

The apicomplexan protozoan *Toxoplasma gondii* is considered to be a leading cause of death attributed to foodborne disease (Mead et al., 1999; Vaillant et al., 2005). After primary maternal infection by *T. gondii* during gestation, the parasite may enter the fetal circulation by invasion of the placenta (Elbez-Rubinstein et al., 2009), or the immune imbalance induced by *T. gondii*, which is associated with decidual natural killer cells, or the trophoblast cells apoptosis via oxidative injury (Xu et al., 2012) may contribute to abnormal pregnancy outcomes (Xu et al., 2013).

Pregnant women are at risk with 10% of infection in China (Gao et al., 2012) and over 60% in some parts of the world (Pappas et al., 2009). Placental transmission is less frequent when infection is acquired before the tenth week of pregnancy and is rare when acquired before conception. Early maternal infection (during the first and second trimester) may result in severe congenital toxoplasmosis, including fetal death and spontaneous abortion (Wong and Remington, 1994).

Macrophages act as a bridge between innate and adaptive immunity, and serve a number of functions, which are necessary for host protection during *T. gondii* infection (Dunay et al., 2008). High levels of IFN-γ produced by Th1 cells, alone or in concert with microbial products (e.g., lipopolysaccharide, LPS), activate classically activated macrophages (M1) (Hamilton, 2002). M1 is characterized by high capacity to present antigens, high production of toxic intermediates (nitric oxide, NO and reactive oxygen intermediates, ROI), high level of interleukin-12 (IL-12) (Mantovani et al., 2004). NO is known to be a major effector molecule in

* Corresponding authors. Tel.: +86 551 65113863/65161139.
E-mail addresses: zlj33@ahmu.edu.cn (L. Zhang), shenjilong53@126.com (J. Shen).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.actatropica.2014.12.001
0001-706X/

macrophage-mediated cytotoxicity (Brunet, 2001). The M1 cells develop in response to concomitant stimulation with IFN-γ and microbial products such as LPS, and destroy the microorganisms by release of NO. Additionally, M1 induce the expression of CXCL10, CXCL9, and IL-12 (Gao et al., 2012; Mantovani et al., 2004). The macrophage-derived NO is considered a key component of host defense against microbial agents (Fang, 1997). Li et al. (2012) infected rat macrophages with *Toxoplasma* treated with NO inducer IFN-γ+LPS and iNOS inhibitor NG-nitro-L-arginine methylester (L-NAME). The expression of iNOS in rat macrophages stimulated by LPS + IFN-γ was dramatically up-regulated compared with that in control cells. A great number of parasites were found within the NO-attenuated rat peritoneal macrophages treated with L-NAME. These data demonstrate that a high concentration of NO in rat peritoneal macrophages is closely associated with their innate resistance to *T. gondii* infection. Over-production of NO, however, may result in serious immunopathology.

Alternative activated macrophages (M2) stimulated by IL-4 and IL-13 cytokines are produced generally in Th2-type microenvironment (Gordon, 2003). Differently from M1 cells, M2 cells seldom express iNOS, but high levels of arginase 1 (Arg-1), which skews the metabolic pathway of NO to the production of proline. Consequently, these cells fail to produce NO and are significantly compromised in their microbicidal ability for intracellular pathogens (Hesse et al., 2001; Modolell et al., 1995). Alternatively, M2 cells synthesize polyamine and proline that stimulate cell growth, collagen formation, and tissue repair (Hesse et al., 2001).

It has long been known that successful pregnancy under physiological conditions appears to be correlated with Th2 cellular maternal immunity. Acute *T. gondii* infection can drive M1 polarization of macrophages, which produce high level of NO, ROI, and proinflammatory cytokines. It may interfere with the maternal–fetal immunological balance (Shiono et al., 2007), and induce abnormal consequence of pregnancy. Rat macrophages are naturally resistant to *T. gondii* infection (Li et al., 2012) although a detailed characterization of markers of macrophage activation in rat is lacking.

In China, the majority of strains isolated from animals belong to the common clonal lineage of Chinese 1 (ToxoDB#9). Herewith, we built a rat model of acute infection with Wh6 strain (genotype Chinese 1) and explored the featured cytokines of M1 and M2 to observe the impact of macrophages bias on abnormal pregnancy in the infected animals.

2. Materials and methods

2.1. Parasites

T. gondii Wh6 strain (preserved by mouse passage) was used in the experiment. Peritoneal fluids containing tachyzoites was centrifuged at 40 × *g* for 5 min at 4 °C. The supernatant was collected and centrifuged at 2500 × *g* for 15 min at 4 °C, and then the sediments were resuspended in RPMI-1640 medium with 10% FBS for further use after counted.

2.2. Rat models

Nine-to-ten-week-old SD rats (body weight 250–300 g), obtained from the Animal Center of Anhui Medical University, were maintained under standardized conditions of lighting (12 h light:12 h dark) and nutrition (food and water ad libitum) throughout the experiments. All procedures were in strict accordance with the Chinese National Institute of Health Guide for the Care and Use of Laboratory Animals, and approved by the Animal Care and Use Committee of Anhui Medical University (approval no: AMU26-08061). Efforts were made to minimize the number of animals and their sufferings.

For mating purposes, two females were housed overnight with one male starting at 9:00 p.m. Females were checked by 7:00 a.m. The next morning, the presence of a vaginal plug or sperm in the vaginal smear was designated as gestational day (GD) 0.

Thirty two females and eight males were used in the experiment. The female rats were divided into four groups: nonpregnant control (con1), pregnancy control (con2), acute infection on gestation (inf1), and acute infection during pregnancy (inf2). Each rat of the inf1 group was peritoneally injected with 2000 Wh6 tachyzoites at the day before cohabit. The group inf2 was each injected with 2000 tachyzoites on the eighth day of pregnancy. Rats in the two control groups were exposed to only 0.9% sterile saline. Blood samples were collected 2 weeks after infection. All the pregnant rats were sacrificed by euthanasia 14 days post-gestation.

2.3. Cell culture

Rat peritoneal macrophages were obtained by washing the peritoneal cavity with 20 ml of normal saline and separated by centrifugation at 2500 × *g* for 10 min at 4 °C. The cells were suspended in 2 ml RPMI-1640 medium with 10% FBS containing 100 U of penicillin and 100 μg of streptomycin per ml, and cultured for 2 h at 37 °C in six-well plates in an incubator containing 5% CO_2 and 95% air. Non-adhered cells were carefully washed away and fresh medium was added. Most of the remaining cells are considered to be macrophages (Mantovani et al., 2004). Macrophages were cultured for 4 h or 24 h and used for further experiments.

2.4. Measurement of iNOS and arginase activity

Culture supernatants were harvested after 24 h. Nitrite content as a reflection of NO production was determined by the Griess reaction as described previously (Ding et al., 1998). Briefly, 100 μl supernatant or standard solution ($NaNO_2$) was incubated in triplicate with 100 μl of Griess reagent (0.5% sulfanilamide, 0.05% naphthyldiamine dihydrochloride in 5% H_3PO_4) for 10 min. The optical density (OD) was measured at 550 nm in an ELISA reader (Bio-Tek, USA).

Arginase activity of purified macrophage was measured by a colorimetric method as described previously (Mantovani and Locati, 2009). Briefly, 10 mM $MnCl_2$ and 0.5 M L-arginine were successively added to macrophage lysates for 1 h at 37 °C. The reaction was stopped by adding an acid solution (H_2SO_4:H_3PO_4:H_2O = 1:3:7), and the urea generated by arginase was analyzed by addition of α-isonitrosopropiophenone at 100 °C for 45 min. The OD value was measured at 550 nm in an ELISA reader. Arginase activity was determined as the amount of urea produced from total protein of peritoneal macrophages.

2.5. Western blotting

Lysates were prepared from macrophages of the four groups (con1, con2, inf1, and inf2) of rats. Total cell lysates were subjected to SDS-PAGE with 12% separating gel, using SDS-PAGE gel preparation kit (Beyotime) according to the manufacturer's protocol. Then, we transfer proteins onto nitrocellulose membranes (Millipore Corp., Billerica, MA), blocked with 5% skim milk, and conventional immunoblotting was performed using several antibodies. Chemiluminescence was valuated using an ECL kit (Thermo Scientific SuperSignal West Pico). The following primary antibodies were used: β-actin (Cell Signaling, USA), Arg-1 (Abcam, USA), and NOS_2 (Santa Cruz, USA). The results were analyzed using Image J 1.44 software.