Experimental Parasitology 178 (2017) 37–44

Contents lists available at ScienceDirect

Experimental Parasitology

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

# Peroxisome proliferator-activated receptor-γ-mediated polarization of macrophages in *Neospora caninum* infection

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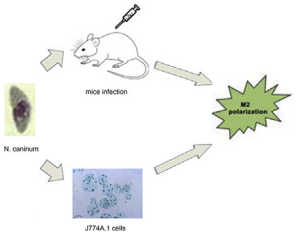
## HIGHLIGHTS

- The expressions of CD206, IL-10 and Arg-1 were significantly increased at 2, 4 and 8 weeks of Neospora caninum infection in mouse peritoneal macrophages.
- The expressions of CD206, IL-10 and Arg-1 ($P < 0.01$) of the M2 marks were increased by activating PPAR-γ in RGZ and Neospora caninum groups.
- Macrophages differentiated into M2 at the late stage of N. caninum infection through mediating PPARγ/NF-κB signaling pathway.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 25 October 2016
Received in revised form
25 April 2017
Accepted 16 May 2017
Available online 17 May 2017

*Keywords:*
*Neospora caninum*
Macrophages
Polarization
PPAR-γ
NF-κB

## ABSTRACT

*Background:* *Neospora caninum* is an apicomplexan parasite closely related *Toxoplasma gondii*, which causes neurological disease and abortion in multiple animal species. Macrophage polarization plays an important role in host immune responses to parasites infection, such as *Toxoplasma gondii*, *Leishmania*, *Trypanosoma cruzi*. However, the dynamics of macrophage polarization, as well as the possible mechanism that regulate macrophage polarization, during *N. caninum* infection remains unclear.
*Methods:* The M1 and M2-phenotypic markers of peritoneal macrophages from mice infected with tachyzoites of *Nc-1* were analyzed by flow cytometry (FCM) analysis. Then J774A.1 cells were respectively treated with GW9662 and RGZ, and stimulated by tachyzoites of *Nc-1*. M1 and M2-phenotypic markers were determined by FCM and ELISA. And the activations of PPAR-γ and NF-κB were determined by Western blotting.
*Results:* In this study, our data showed that macrophages were preferentially differentiated into the M1 type during the acute stage of *N. caninum* infection, while the level of M2 macrophages significantly increased during the chronic stage of infection. In vitro study, compared with the GW9662 group and RGZ group, *N. caninum* can promote M2-polarized phenotype through up-regulate the activity of PPAR-γ and inhibting NF-κB activation.
*Conclusion:* In conclusion, this study demonstrated that macrophages are plastic since M1 differentiated macrophages can express M2 markers with *N. caninum* infection through up-regulating the activity of

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http://dx.doi.org/10.1016/j.exppara.2017.05.002
0014-4894/

PPAR-γ and inhibting NF-κB activation and may be providing new insights for the prevention and treatment of *N. caninum* infection.



## 1. Introduction

*Neospora caninum* (*N. caninum*) and *Toxoplasma gondii* are two closely related apicomplexan protozoans of the family Sarcocystidae, which causes the neosporosis and toxoplasmosis respectively. *N. caninum* is different from *Toxoplasma gondii* in their transmission strategies and zoonotic potentials, which mainly cause neurological disease and abortion in multiple animal species (Dubey et al., 2009). Further reports showed that *N. caninum* has a facultative heteroxenous life cycle involving a definitive canid host and a wide range of intermediate hosts, such as cattle, horse, sheep, goat, deer and rhinoceros and experimentally induced in animal models. (Bartley et al., 2008; Dubey, 2003; Gondim et al., 2004; Reichel and Ellis, 2009). Currently, the majority of research on *N. caninum* has focused on cattle because *N. caninum* can cause abortion of cattle, which causes a range of economic burden on the dairy cattle worldwide (Dubey et al., 2007).

Macrophages, essential component of innate immunity, have multiple functions in inflammation and host defense against various pathogens. Macrophages stimulated with microbial ligands and cytokines, which can be categorized into two subpopulations: classically activated macrophages (M1 macrophages) and alternatively activated macrophages (M2 macrophages). The M1 macrophages could be promoted by a variety of stimuli with lipopolysaccharide (LPS) or interferon gamma (IFN-γ), which are characterized by expression of high levels of pro-inflammatory cytokines, such as tumor necrosis factor (TNF-α), interleukin 12 and 23 (IL-12 and IL-23), and high production of nitric oxide (NO) and reactive oxygen intermediates (ROI), up-regulation costimulatory factors CD80 and CD86, promotion of a Th1 response, and strong microbicide and tumoricidal activity (Biswas and Mantovani, 2010; Gordon and Martinez, 2010; Mantovani et al., 2002; Verreck et al., 2004). In contrast, M2 macrophages polarization could be promoted by a variety of stimuli with Th2 cytokines such as interleukin 4 and 13 (IL- 4, IL-13), glucocorticoids, and Ig complexes in response to promote wound healing and tissue remodeling. The M2 macrophages are characterized by expression of anti-inflammatory cytokines (e.g. IL-10), the upregulation of Arginase-1 (Arg-1), mannose receptor (CD206), scavenger receptor, resistin-like-α (Fizz1) and chitinase 3-like 3 (Ym1), and considered to be involved in parasite containment and promotion of tissue remodeling and tumor progression and have immunoregulatory functions (Gordon and Martinez, 2010; Sica and Mantovani, 2012). Peroxisome proliferator-activated receptors-γ (PPAR-γ), a member of the nuclear hormone receptor family, is a ligand-activated transcription factor expressed in macrophages that has emerged as regulators of lipid metabolism as well as inflammation (Olefsky and Glass, 2010). It has been reported that activation of PPAR-γ plays an important role in regulating the polarization of macrophages (Odegaard et al., 2007).

Some studies reported that macrophages were alternatively activated when infected with virulent *T. gondii* while classically activated when infected with low virulent (cyst-forming) *T. gondii* both in vitro and in vivo (Liu et al., 2013). *Leishmania* parasites can survive and multiply within the host's macrophages through differentiating macrophages into the alternatively activated (M2) macrophages, and the infection becomes chronic (Chan et al., 2012). However, very few studies have been carried out in the dynamics of macrophage polarization during *N. caninum* infection. The purpose of this study was to investigate the dynamics of macrophage polarization during *N. caninum* infection as well as the role played by PPAR-γ and NF-κB in this process.

## 2. Materials and methods

### 2.1. Reagents

RPMI 1640 medium, fetal bovine serum (FBS) and penicillin–streptomycin and sodium pyruvate solution were provided from Hyclone (Logan, UT, USA). The percoll, GW9662 and rosiglitazone (RGZ) were purchased from Sigma. T-PER protein extraction reagent was purchased from Thermo. All enzyme-linked immunosorbent assay (ELISA) kit was purchased from Biolegend (USA). The monoclonal antibodies p65, p-p65 and PPAR-γ used in Western blot were obtained from Cell Signaling Technology Inc (Beverly, MA, USA). The second antibody was acquired by GE Healthcare (Buckinghamshire, UK). Anti-mannose receptor antibody (CD206) and anti-CD80 antibody were purchased from Abcam. All other chemicals were of reagent grade.

### 2.2. N. caninum tachyzoite culture and purification

Tachyzoites of *N. caninum* (Nc-1) strain were passaged in vitro in Vero cell cultures in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 μg/ml streptomycin, 100 U/ml penicillin at 37 °C with 5% $CO_2$. (Lindsay and Dubey, 1989). For the purification of the tachyzoites of *Nc-1*, intact parasites and large debris were removed by centrifugation at 1000r/min for 15 min. After centrifugation, the final pellet was resuspended in cold DMEM and passed through 5, 1-ml syringe and a 27-gage needle in turn, and then purified by 40% Percoll reagent through centrifugation at 3000r/min for 30 min. The fraction containing tachyzoites at the bottom of the tube was collected and washed twice with RPMI 1640 medium by centrifugation at 1000r/min for 15 min.

### 2.3. Mice infection

72 BALB/c mice (4–6 weeks old) which were used for the experiment of macrophage polarization and parasite infection (Baszler et al., 2000) (Chan et al., 2012) were obtained from the Center of Experimental Animals of Jilin University (Jilin, China). The mice were housed individually under a climate-controlled (20–23 °C under a 12-hr light/dark cycle) room and allowed free access to food and water. All cages were washed carefully and sterilized by autoclaving. *N. caninum* infections were performed in BALB/c mice by the intraperitoneal inoculation of 0.5 ml PBS containing $1 \times 10^7$ tachyzoites of *Nc-1*. Mock infected controls were similarly intraperitoneally (i.p.) injected with 0.5 ml of PBS. All the experiments were repeated three times. Animals were sacrificed at 2, 4 and 8 week intervals following experimental inoculation. All animal experiments were performed in accordance with the experimental practices and standards approved by the Animal Welfare and Research Ethics Committee at Jilin University (approval ID, 20111106-2), and all efforts were made to minimize