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Toxoplasma gondii triggers secretion of interleukin-12 but low level of interleukin-10 from the THP-1 human monocytic cell line

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Abstract

Previous studies using both in vitro and in vivo mouse models have demonstrated that a subtle balance between pro- and anti-inflammatory cytokines, among which interleukin-12 (IL-12) and interleukin-10 (IL-10), respectively is crucial to control *Toxoplasma* infection. However, the few studies performed with human cell lines highlighted important host-related differences in the immune response to *Toxoplasma gondii*. The goal of our work was thus to study the production of both IL-12 and IL-10 by the THP-1 human monocytic cell line in response to *Toxoplasma.* We demonstrated that infection by live parasites (RH strain) triggers secretion of IL-12, but low level of IL-10. IL-12 secretion appeared within 8 h, up to 48 h. We also showed that infection by live parasites is not mandatory since heat-killed parasites, crude tachyzoite lysate as well as excreted/secreted antigens induced significant, yet reduced production of IL-12.

Keywords: Interleukin-12; Interleukin-10; *Toxoplasma gondii*; THP-1; Human

1. Introduction

The intracellular protozoan parasite *Toxoplasma gondii* is ubiquitous and infects both human beings and animals. Although toxoplasmosis can be dramatic in both pregnant women and immunodeficient patients, between 30% and 80% of the human population carries latent, asymptomatic infection.

In immunocompetent individuals, infection is first characterized by an acute phase, which results from dissemination of the rapidly dividing tachyzoites throughout the body. Chronic infection is associated with dormant bradyzoites residing in tissue cysts, which are maintained indefinitely in the central nervous system and other host tissues if no modification of host immunity appears. In immunocompetent individuals, toxoplasmosis is thus an infection which is efficiently controlled by the host immune system [1].

Macrophages play an important role in the first step of immune defences against *Toxoplasma*. Indeed, when stimulated by parasites, macrophages secrete several cytokines such as tumor necrosis factor α (TNFα), interleukin-1 (IL-1) and interleukin-12 (IL-12). Upon stimulation with IL-12, natural killer (NK) cells produce high levels of interferon gamma (IFNγ) which in turns, enhances both macrophage IL-12 secretion and effector functions, leading to a powerful first line of defence against the parasite, prior to the establishment of the adoptive T cell response [2]. Early stimulation of macrophages also plays an important role in directing cell mediated immunity since IL-12 promotes Th1-type acquired immunity, which is essential to control *Toxoplasma* infection [3].

Most of the experiments performed to dissect the immune mechanisms developed during *Toxoplasma* infection, involved mouse models. Several studies reported that mouse macrophages, dendritic cells and neutrophils secrete

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IL-12 when directly infected or exposed to soluble *Toxoplasma* antigens [2,4,5]. IL-12 is a key cytokine in the induction of IFNγ, TNFα and protective immunity against *Toxoplasma* infection: IL-12p40 as well as IL-12p35-deficient mice fail to develop a protective immune response and succumb during acute *Toxoplasma* infection [6]. However, Mordue et al. [7] demonstrated that lethal infections are associated with massive over-stimulation of Th1 cytokines, which probably contributes to pathology and lethality. Although Th1 cytokines, including IL-12, are essential to control parasite proliferation and dissemination during the early phase of infection, this early cytokine response must be regulated to prevent tissue damage and lethal immunopathology.

Interleukin-10 (IL-10) is a cytokine well known for its down-regulatory effects on IL-12 synthesis and macrophages are considered as an important source of IL-10 [8]. IL-10 deficient mice succumb to *Toxoplasma* infection, which is characterized by over-production of IL-12, IFNγ and TNFα [9]. The balance between IL-12 and IL-10 is thus essential to control *Toxoplasma* infection.

In mice, the induction of secretion of both IL-12 and IL-10 by macrophages does not require infection by live parasites since in vitro exposure of macrophages to tachyzoite soluble fraction triggers high levels of these cytokines [10,11].

If mouse models have provided a wealth of information on the host immune response raised consecutively to *Toxoplasma* infection, nevertheless, one should be extremely cautious when applying observations and conclusions derived from mouse models to humans. Indeed, important differences exist between mouse and human immune responses, among which for example, the involvement of TNFα. Indeed, while the protective effect of TNFα secreted by mouse macrophages was demonstrated in response to *Toxoplasma* infection, secretion of TNFα has never been observed during human infection [12]. Investigations on the interaction between *Toxoplasma* and human cell lines are required to determine accurately the immune mechanisms developed by human beings in response to the parasite. The goal of the present study was thus to investigate if the THP-1 human monocytic cell line secretes pro- and/or anti-inflammatory cytokines in response to infection by *Toxoplasma* or stimulation with parasite antigens and if so, what is the secretion kinetics.

2. Materials and methods

2.1. Parasites

Tachyzoites of the *T. gondii* RH strain were multiplied in vitro in Human Foreskin Fibroblasts (HFF) in Dulbecco's modified minimal essential medium (DMEM) (Invitrogen, SARL, Cergy-Pontoise, France) supplemented with 10% fetal bovine serum (FBS) (Gibco™, Invitrogen France), 2 mM L-glutamine (Gibco™), 50 U/ml penicillin and 50 μg/ml streptomycin (Gibco™), respectively. Twice a week, after lysis of the monolayer by *T. gondii*, 5% of culture supernatant containing tachyzoites was collected and used to infect a fresh monolayer.

Heat-killed tachyzoites were obtained after incubation at 56 °C for 50 min. Viability was obtained with acridine-orange.

2.2. Production of Toxoplasma antigens

Excreted/secreted antigens (ESA) were produced as previously described [13]. Briefly, freshly lyzed RH tachyzoites (1.2×10^8) were incubated at 37 °C for 3 h under mild agitation in test tubes containing 1.5 ml DMEM supplemented with 10% (v/v) heat-inactivated FBS. After centrifugation for 10 min at 1000*g*, the ESA-containing supernatant was aliquoted and stored at −70 °C until used. To verify the absence of parasite lysis, proteins were resolved by SDS–PAGE, transferred to nitrocellulose, and probed with the monoclonal antibody anti-SAG1 as previously reported [14].

Tachyzoite lysate antigen (TLA) was prepared from in vitro-cultured RH tachyzoites, as previously described [15]. Briefly, freshly lyzed parasites were counted and passed through a 3 μm pore-size polycarbonate membrane to remove host cells debris. Parasites were washed twice in phosphate-buffered saline (PBS) (bioMérieux, France), centrifuged at 5000*g* for 3 min and disrupted by three cycles of freezing at −70 °C and thawing at 37 °C. Finally, the lysate was sonicated four times for 20 s using a Sonoclean cell disintegrator (Labo-moderne, France) and stored at −70 °C until used.

2.3. Human monocytic THP-1 cells

THP-1 is a promonocytic cell line derived from a patient with acute lymphocytic leukemia (ATCC, American Type Culture Collection, Manassas, USA) and already used in *Toxoplasma* model [12]. The THP-1 cell line was cultured in RPMI 1640 medium (Gibco™) supplemented with 10% FBS, 100 UI/ml penicillin, 100 μg/ml streptomycin and 1 mM of L-glutamine, at 37 °C, in a humidified atmosphere of 5% CO_2. THP-1 cells cultured in 24-well plates were washed with PBS before infection or stimulation.

2.4. Infection or stimulation of THP-1 cells by live parasites, killed tachyzoites, TLA or ESA

Freshly lyzed RH tachyzoites were used to infect THP-1 cells (10^6 per well in 24-well tissue culture plates, BD Biosciences, Pont de Claix, France) at the ratio of 10 parasites to 1 THP-1 cell. Cell culture supernatants were collected at 3 h, 8 h, 18 h, 24 h or 48 h post infection (pi) and stored at −20 °C.

THP-1 cells were stimulated by adding 10^6, 10^7 or 10^8 heat-killed parasites; quantities of either ESA or TLA corresponding to 10^6, 10^7 or 10^8 parasites, respectively (10^6, 10^7 or 10^8 parasite-equivalents). One microgram of LPS