

RESEARCH ARTICLE

Immunocompetent host develops mild intestinal inflammation in acute infection with *Toxoplasma gondii*

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Abstract

Toxoplasma gondii (*T. gondii*) is the causative agent of toxoplasmosis, common zoonosis among vertebrates and high incidence worldwide. During the infection, the parasite needs to transverse the intestinal barrier to spread throughout the body, which may be a trigger for an inflammatory reaction. This work evaluated the inflammatory alterations of early *T. gondii* infection in peripheral blood cells, in the mesenteric microcirculation, and small intestinal tissue by measurement of MPO (myeloperoxidase) activity and NO (nitric oxide) level in rats. Animals were randomly assigned into control group (CG) that received saline orally and groups infected with 5,000 oocysts for 6 (G6), 12 (G12), 24 (G24), 48 (G48) and 72 hours (G72). Blood samples were collected for total and differential leukocyte count. Intravital microscopy was performed in the mesentery to evaluate rolling and adhesion of leukocytes. After euthanasia, 0.5cm of the duodenum, jejunum and ileum were collected for the determination of MPO activity, NO level and PCR to identify the parasite DNA and also the mesentery were collected to perform immunohistochemistry on frozen sections to quantify adhesion molecules ICAM-1, PECAM-1 and P-Selectin. The parasite DNA was identified in all infected groups and there was an increase in leukocytes in the peripheral blood and in expression of ICAM-1 and PECAM-1 in G6 and G12, however, the expression of P-selectin was reduced in G12. Leukocytes are in rolling process during the first 12 hours and they are adhered at 24 hours post-infection. The activity of MPO increased in the duodenum at 12 hours, and NO increased in the jejunum in G72 and ileum in G24, G48 and G72. Our study demonstrated that *T. gondii* initiates the infection precociously (at 6 hours) leading to a systemic activation of innate immune response resulting in mild inflammation in a less susceptible experimental model.

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Introduction

Toxoplasma gondii (*T. gondii*) is an intracellular parasite that has the ability to infect warm-blooded animals but only the felines are the definitive hosts where they can sexually reproduce. Domestic and wild animals, and humans are considered intermediate hosts, however, the parasite does not complete its life cycle [1,2].

Epidemiological studies in Brazil have shown that the prevalence of *T. gondii* infection in the general population ranges from 20% to 84% [3]. It is estimated that about half a billion people worldwide exhibit antibodies against this parasite [4]. Studies in Brazil, which verified seropositivity and risk factors for infection in cattle, determined that in the northern region of Brazil, only 5.3% were found [5]. However, this percentage might reach up to 71% in other regions where there is a higher production of cattle [6]. Thus being an important route of contamination.

T. gondii is transmitted primarily by ingestion of oocysts present in contaminated water and food or tissue cysts present in raw or undercooked meat [7]. During the acute infection, tachyzoites multiply rapidly and reach different host cells. Subsequently, bradyzoites form tissue cysts, preferentially in nervous and muscle tissue, characterizing the chronic phase of infection [8,9].

After oral infection, the parasite transposes the intestinal barrier and spreads through the body initiating an inflammatory response [10]. Polymorphonuclear leukocytes (PMN), the first line of defense against infectious agents, initially roll and adhere to the wall vessels of the blood microcirculation. This process is known as leukocytes rolling [11,12]. The firm adherence occurs later, by the contact of leukocytes with endothelial immunoglobulins [13]. After adherence the leukocytes transmigrate into the extravascular space towards the injury focus in attempt to combat, or at least stop the parasite [14].

Previously it has been shown that *T. gondii* causes intense inflammation in the intestine [15–17]. Inflammatory infiltrate was observed in the intestinal wall of domestic cats [18], birds [19] and rats [20] infected with *T. gondii* showing the presence of eosinophils [19]. However, there are few studies evaluating the kinetics of acute infection by *T. gondii* at different time-points. Therefore, it is important to understand the parasite-host interaction that occurs during the transposition of intestinal barrier by *T. gondii*. This study evaluated the inflammatory changes of early *T. gondii* infection in peripheral blood cells, in the mesenteric microcirculation, and small intestinal tissue by measurement of MPO activity and NO level.

Material and methods

Experimental design

The experimental protocol was approved by the Ethics Committee on Animal Experiments of the Universidade Estadual de Maringá, Brazil (protocol number 079/2013). The experiments were performed in accordance with the guidelines of the Brazilian Control and Experimentation Committee.

Four-week-old male Wistar rats received orally antiparasitic treatment with metronidazole (500mg/kg/5 days) and fenbendazole (50mg/kg/single dose). Seven days after the end of the treatment, stool testing was performed to confirm the absence of any parasite. The rats were subsequently randomly allocated into a control group (CG) and the infected groups with *T. gondii* for 6 hours (G6), 12 hours (G12), 24 hours (G24), 48 hours (G48) and 72 hours (G72).

Orally each animal in the infected group received 5,000 sporulated oocysts of *T. gondii* (ME-49 strain, genotype II) that were resuspended in 1 mL sterile saline (S1 Fig). Oocysts were obtained from the Parasitology Veterinary Laboratory, at the Universidade Estadual de Londrina, Brazil. CG received only saline. These animals were kept under standard conditions in

controlled temperature ($24 \pm 2^\circ\text{C}$) and photoperiod of 12 hours (6 a.m.–18 p.m.), receiving standard rodent chow (Nuvilab, Colombo, PR, BR) and water *ad libitum*.

White blood cell counts

Immediately before euthanasia, blood samples were obtained from the tail and total leukocytes were counted using a Neubauer chamber. The differential leukocyte count was determined in blood smears stained by May-Grunwald-Giemsa using light microscopy. The data obtained were expressed as mean \pm S.E.M. The percentage values were calculated by the ratio of the mean number of leukocytes in the group divided by the mean of the control group times 100.

Euthanasia and tissue collection

After the experimental period, the rats were euthanized by deep anesthesia with halothane vapor [21]. Then, vertical laparotomy was performed and the duodenum, jejunum and ileum were excised.

Detection of the parasite in the intestine using qPCR

T. gondii DNA was detected by real-time quantitative PCR in all infected animals used in the experiments. One centimetre of the whole wall from duodenum, jejunum and ileum were collected for this assay. For DNA extraction and precipitation by sodium acetate and ethanol was performed as described previously [22]. The amplification of *T. gondii* DNA was held using the method described by Homan et al. (2000). Primers Tox4 (CGCTGCAGGGAGGAAGAC GAAAGTTG) and Tox5 (CGCTGCAGACACAGTGCAT CTGGATT) were used and these flanked a 529 bp fragment (GenBank No. AFI46527) of *T. gondii* DNA. The DNA was quantified using Quant-iT™ dsDNA Assay Kit, broad range, (Life technology).

Intravital microscopy analysis in the mesenteric microcirculation

The procedures were adapted from Baez (1973)[23]. The rats (140–220g; n = 4–10) were anesthetized with a combination of 10 mg.kg⁻¹ xylazine (Calmium Agener-Union Animal Health) and 50 mg.kg⁻¹ Ketamine (Francotar®—Virbac Animal health) by intramuscular route. After trichotomy a midline abdominal incision was made and a loop of the ileal mesentery was exteriorized and their mesenteric was exposed for observation of the microcirculation vessels *in situ* by microscopy. The animals were maintained on a heated plate (37°C) provided with a transparent area over which the mesentery was fixed. The preparation was kept moist and warm by irrigation with Ringer-Locke solution (37°C, pH 7.2–7.4). Post-capillary venules with diameters ranging from 18–25 μm were analyzed (S2 Fig).

The interaction of circulating leukocytes with the surface of the endothelium was determined by counting of the number of rolling and adherent leukocytes in a segment of 100 μm vessel for 5 minutes. Leukocytes that were stationary for at least 30s were considered adherent to venular endothelium. Two numerical determinations were made per animal and each vascular segment was examined only once. After the end of each experiment, euthanasia was performed by deep anesthesia. Data were expressed by the number of rolling and adherent cells/100 μm of venule over a period of 5 minutes.

Immunohistochemical analyses of adhesion molecules in mesentery

For immunohistochemistry, only the groups CG, G6, G12 and G24 were included. After euthanasia, the mesentery was immediately removed and frozen in nitrogen-hexane solution. Cryosections (10 μm) were obtained and fixed in cold acetone for 10 min. Nonspecific sites were then

blocked by incubation at room temperature with Tris-buffered saline/Tween₂₀ containing bovine serum albumin (BSA) (0.5%) for 15 min. The sections were incubated for 2 h at 37°C with one of the following antibodies a mouse monoclonal anti-P-selectin antibody (CD62P; Abcam, USA), mouse monoclonal anti-ICAM-1 antibody (CD54; Santa Cruz Biotech., USA) and finally a mouse monoclonal anti-PECAM-1 antibody (CD31; Novus Biologicals, USA). The antibodies were diluted at a concentration of 1:100 in TBST (Tris-buffered Saline) containing 1% BSA. Endogenous peroxidase was blocked using 2% hydrogen peroxide (30 w/w) for 15 min at room temperature. After washing the slides with TBST, the sections were incubated with secondary antibodies (ADVANCE™ HRP Link, Dako [PECAM-1] or Histostain® – Plus Invitrogen™ [P-selectin and ICAM-1]) for 2 h at 37°C, rinsed in TBST, and then incubated with HRP-conjugated antibodies for 30 min at room temperature. After three washes, the samples were developed with the HRP substrate 3-amino-9-ethylcarbazole (AEC; Vector Lab., Burlingame, CA, USA) or 3, 3'-Diaminobenzidine (DAB; Invitrogen™) for 10 min and counterstained with Mayer's hematoxylin. The background reaction was determined in sections incubated in the absence of primary antibody (negative control). The slides were mounted, and images were captured using an Olympus BX50 microscope equipped with a 3CCD Pro-series camera. The objects stained with AEC and DAB on the walls of mesenteric vessels were identified after threshold determination, and the stained area was quantified using an image analyzer (NIS-elements; Nikon, Tokyo, Japan [24]).

Determination of myeloperoxidase activity on intestinal tissues

Myeloperoxidase (MPO) activity was determined using homogenate supernatant of 0.5 cm from the duodenum, jejunum and ileum. The samples were placed in 50 mM potassium phosphate buffer solution (pH 6.0) that contained 0.5% hexadecyl trimethyl ammonium bromide (1 mL/50 mg of tissue; Sigma®) in a potter homogenizer. The homogenate was vortexed and centrifuged for 5 min. 10 µl of the supernatant was added to each well of a 96-well microplate in duplicate. 200 µl of the substrate solution (16.7mg o-dianisidine dihydrochloride (Sigma®), 90 mL double-distilled water, 10 mL potassium phosphate buffer, and 50 µl of 1% H₂O₂) were added. The enzyme reaction was stopped by the addition of 30 µl of sodium acetate. The optical density was measured at 460 nm using a microplate spectrophotometer (Asys Expert Plus).

Measurement of total nitrite on intestinal tissues

The level of nitric oxide was determined by Griess method, which determines the nitrite production as a measure of the gas production [25]. The supernatant from the duodenum, jejunum and ileum homogenate (50 µl) was placed in a 96-well microplate in triplicate, and then, Griess solution was added (1% sulfanilamide in 5% phosphoric acid and 0.1% dihydrochloride N-1-naftyletilonodiamine in water) at room temperature. After 10 min, a reading was performed using an ELISA plate reader at a wavelength of 550 nm [26]. NO concentrations were calculated from a sodium nitrite standard curve. The results were expressed as µM. Statistical analysis

The data were expressed as mean ± SEM for each experimental group. The results were statistically evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. The software used was GraphPad Prism version 5.01, GraphPad Software, Inc. Differences were considered significant at $p < 0.05$.

Results

T. gondii DNA was detectable in infected rats

The rats did not present clinical manifestations during the experiment. We performed real-time PCR to analyze the presence of the parasite DNA in the duodenum, jejunum and ileum.

Table 1. Real-time quantitative PCR assay in the small intestine of *T. gondii* infected rats.

Groups	Animals	Duodenum		Jejunum		Ileum	
		Ct	fg/ μ L	Ct	fg/ μ L	Ct	fg/ μ L
GC	1	-	-	-	-	-	-
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
G6	1	36.2	71.0	-	-	37.8	31.0
	2	29.9	1093.0	37.7	3.2	35.7	14.0
	3	35.5	105.0	-	-	34.5	20.0
	4	34.9	17.0	34.6	19.0	28.0	22570.0
G12	1	-	-	33.6	6.0	-	-
	2	-	-	33.8	40.0	-	-
	3	-	-	-	-	-	-
	4	34.7	22.0	36.6	6.0	-	-
G24	1	37.5	189.0	34.0	247.0	-	-
	2	35.4	12.0	34.7	19.0	34.7	19.0
	3	31.3	954.0	37.1	44.0	-	-
	4	34.2	26.0	33.9	31.0	35.1	18.0
G48	1	-	-	-	-	-	-
	2	35.1	15.0	36.3	8.0	-	-
	3	-	-	-	-	-	-
	4	35.7	10.0	-	-	35.8	9.0
G72	1	36.9	5.0	35.3	14.0	34.2	-
	2	34.5	210.2	34.8	18.0	35.0	26.1
	3	34.3	24.0	34.9	18.0	34.5	17.0
	4	35.0	18.0	33.8	32.0	-	21.0

Real-time PCR assay performed by using the primers Tox4 and 5 on 1 cm piece of duodenum, jejunum and ileum of control group (CG) and rats infected with *Toxoplasma gondii* (ME-49) for 6 (G6), 12 (G12), 24 (G24), 48 (G48) and 72 hours (G72). Ct, threshold cycle; (-)—not detected.

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The results demonstrated that the *T. gondii* DNA was present in all infected groups, except in the ileum of G12 (Table 1), however the parasite DNA was observed later on the G24.

***T. gondii* infection induces recruitment of predominantly PMN cells through ICAM-1 and PCAM-1**

We evaluated the mobilization of leukocytes in the systemic circulation towards to the tissue. Firstly, we counted the number of total leukocytes in the peripheral blood which were increased only between 6h and 12h. Total leukocytes were bigger in 72% at 6h (G6: $12,928 \pm 933 \text{ mm}^{-3}$) and in 69% at 12h post infection (G12: $12,544 \pm 1,495 \text{ mm}^{-3}$) when compared to CG ($7,516 \pm 494 \text{ mm}^{-3}$) ($p < 0.05$). Additionally, when we investigated the differential leukocyte counting, a massive increase of PMN rather than MN was observed. PMN were 125% bigger at 6h (G6: $3,331 \pm 302 \text{ mm}^{-3}$) and 163% at 12h post infection (G12: $3,880 \pm 869 \text{ mm}^{-3}$) in relation to CG, while MN were 59% at 6h (G6: $9,597 \pm 723 \text{ mm}^{-3}$) and 43% at 12h post infection (G12: $8,665 \pm 829 \text{ mm}^{-3}$) ($p < 0.05$) (Fig 1) also in relation to CG. These results showed that the PMN cell type are mostly recruited in early infection of this experimental model. Furthermore, the number of these cells was normalized after 24h post infection (S1 Table.).

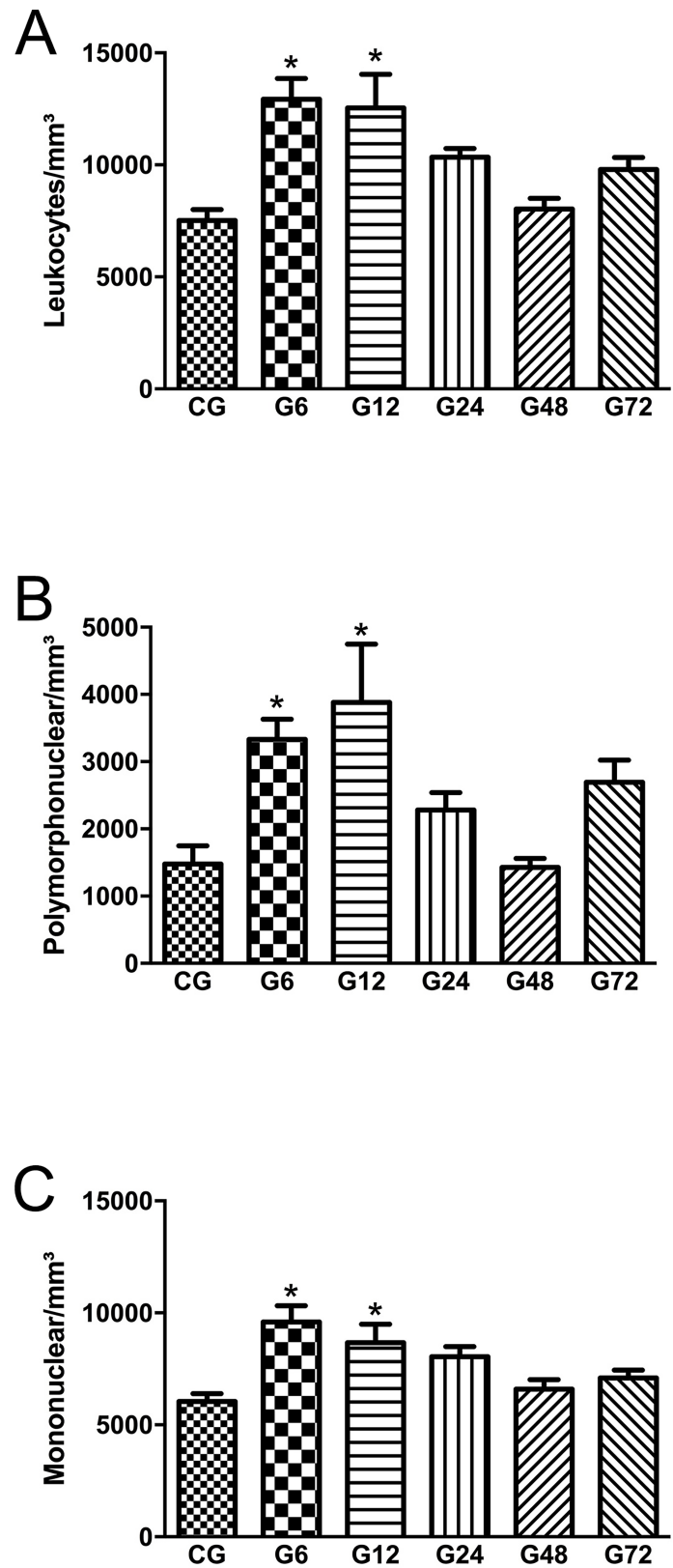


Fig 1. Total leukocyte count (A), Polymorphonuclear (B), and Mononuclear (C) leukocytes count in the peripheral blood of Wistar rats infected with oocysts of *T. gondii* at different times. Data are mean \pm S.E.M., * $p < 0.05$, compared

to the control group (ANOVA, Tukey's test). CG (Control group), G6, G12, G24 G48 and G72 (rats infected for 6, 12, 24, 48 or 72 hours with oocysts of ME 49 strain of *T. gondii*, respectively).

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In order to evaluate the recruitment of leukocytes from the peripheral blood towards to intestinal tissue, we performed the mesenteric microcirculation *in situ*. The *T. gondii* infection caused a marked increase in the rolling leukocytes number from 6h (G6: 161.2 ± 20.4 leukocytes.5 min⁻¹) up to 12h (G12: 175.6 ± 13.5 leukocytes.5 min⁻¹) post infection ($p < 0.05$) when compared to the CG (101.0 ± 7.4 cells/5 min) (Fig 2A). Whilst, no significant differences was observed for rolling leukocytes in the G24 (110.1 ± 3.9 cells/5 min), G48 (104.0 ± 14.1 cells/5 min) or G72 (106.0 ± 3.7 cells/5 min) ($p > 0.05$). Furthermore, the infection resulted in an increase of adherent cells only in the G24 (18.5 ± 2.2 cells/100 μm) ($p < 0.05$) when compared to CG (5.0 ± 2.0 cells/100 μm). The number of adherent cells remained unchanged in the G6 (6.4 ± 1.42 cells/100 μm), G12 (7.7 ± 1.4 cells/100 μm), G48 (6.6 ± 2.3 cells/100 μm) and G72 (9.2 ± 0.7 cells/100 μm) in relation to CG (Fig 2B; $p > 0.05$). These numerical changes are illustrated in the Fig 3.

We evaluated the adhesion molecules in the mesenteric endothelium involved in the migration of leukocytes (Fig 4) and we observed that the expression of ICAM-1 and PECAM-1 were statistically augmented in the G6 and G12 groups compared to control ($p < 0.05$). It means that ICAM-1 and PECAM-1 were the main adhesion molecules involved in the migration process of leukocytes during the *T. gondii* infection. On the other hand, we observed that P-selectin was decreased only in the G12 ($p < 0.05$).

***T. gondii* infection impaired MPO activity and increased the release of NO**

After the migration of leukocytes to the gut tissue, we evaluated the activity of immune cells through MPO and release of NO. As shown below, NO demonstrated to be more relevant to combat the parasite.

The level of NO production was monitored by measuring the nitrite level in the small intestine by Griess reaction in order to evaluate the release of NO from resident/migrated cells in the acute *T. gondii* infection. The nitrite quantification showed an increased NO production in the jejunum of G72 (7.882 ± 0.995 μM) and in ileum of the groups G24 (5.043 ± 1.804 μM), G48 (4.974 ± 1.640 μM), and G72 (6.627 ± 1.165 μM) in comparison to the CG (2.680 ± 1.709 for jejunum and 1.701 ± 1.033 μM for ileum respectively) ($p < 0.05$) (Fig 5A, 5B and 5C).

We assayed the MPO activity to confirm the presence/activation of neutrophils in the small intestine. The results showed a significant increase of MPO activity in the duodenum only at 12h post infection (G12: 0.1856 ± 0.0143) ($p < 0.05$) compared to the control group (0.1516 ± 0.0078). On the other hand, the infection did not modify the MPO activity in the jejunum and ileum of the rats ($p > 0.05$) (Fig 5D, 5E and 5F). These results show that the main immune response to *T. gondii* is not through MPO activity.

Discussion

Information about the innate response in immunocompetent hosts at the beginning of *T. gondii* infection is limited. Here we used a rat model of oral *T. gondii* infection to study some inflammatory alterations at the site of infection, in the small intestine and mesentery.

Our data showed an increase in leukocyte cells in peripheral blood at the beginning of infection. Furthermore, on differential leukocyte count, it is clear that this increase was mainly by PMN, showing that these cells are the most recruited during early infection (at 6 hours) in this experimental model. Studies have shown that depletion of PMN promotes an increased

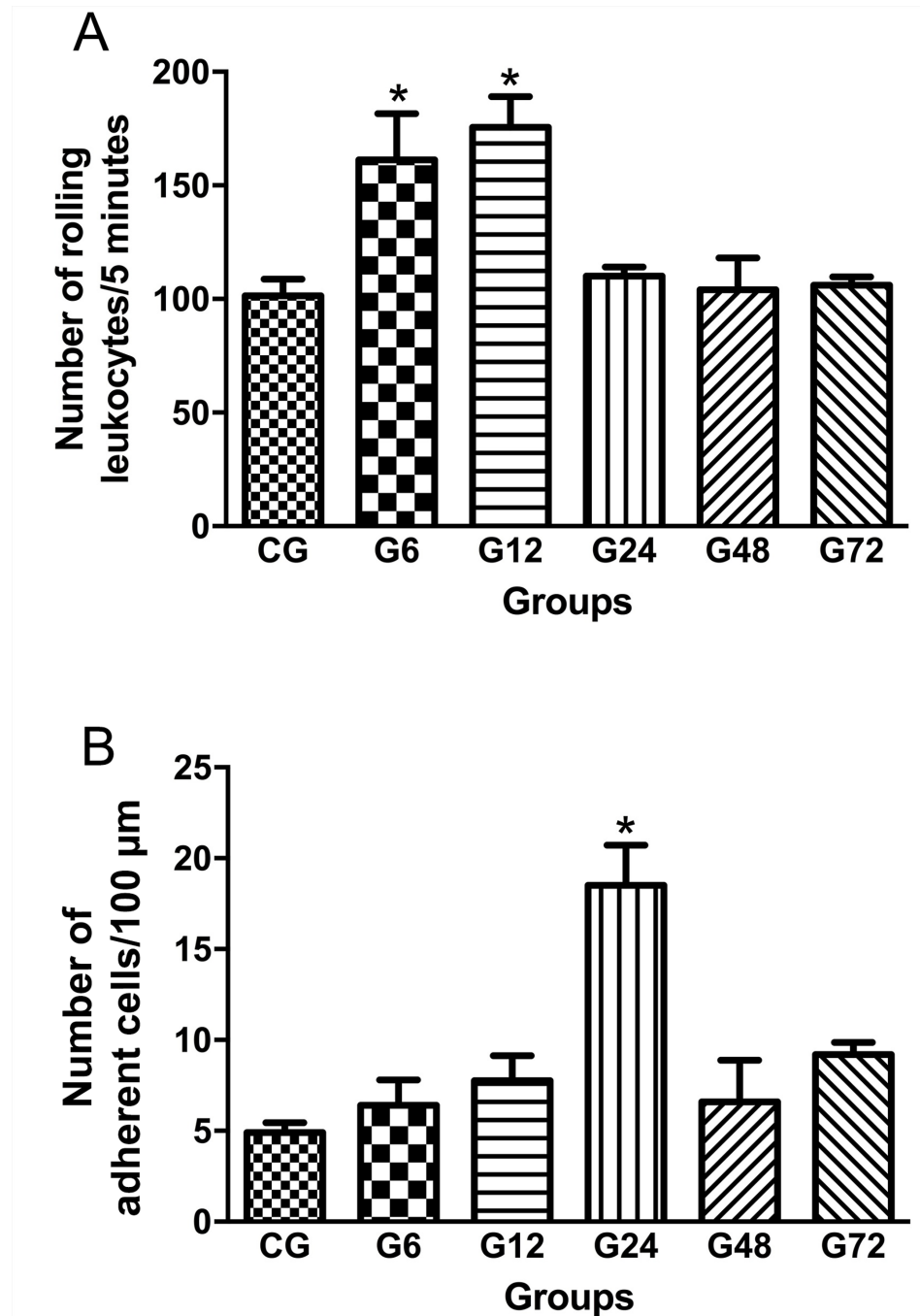


Fig 2. Number of rolling (A) and adherent leukocytes/100 μm venule length (B) in mesenteric microcirculation from distal ileum region of rats orally infected with *T. gondii* oocysts (ME49). Data are mean ± S.E.M., * $p < 0.05$, compared to the control group (ANOVA, Tukey's test). CG (Control group), G6, G12, G24 G48 and G72 (rats infected for 6, 12, 24, 48 or 72 hours with oocysts of ME 49 strain of *T. gondii*, respectively).

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susceptibility of the host to infection by *T. gondii*, however, the removal of the PMN does not interfere in later stages of the disease [27,28]. Therefore neutrophils are very important in early infection since they might influence the type of immune response and the control of tachyzoite replication [10,28,29].

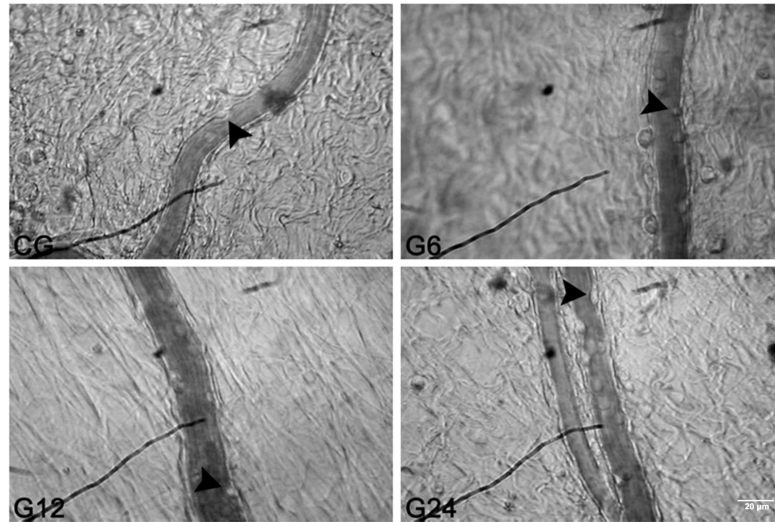


Fig 3. Representative photomicrographs of rat mesenteric microcirculation. CG (Control group), G6, G12 and G24 (infected for 6, 12 and 24 hours with oocysts of ME 49 strain of *T. gondii*). Increased number of rolling leukocytes was observed in the G6 and G12 and number of adherent in G24 in comparison with CG. Leukocytes (arrow). Final magnification: 3400x.

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We showed using qPCR that at 6 hours after oocysts inoculation the *T. gondii* DNA was present throughout the small intestine and remains in all segments for at least 72 hours, even in the ileum, which is farthest from the local of *T. gondii* inoculation. These results associated with the increase in the number of blood leukocytes led us to consider that the infectious forms of the parasite have already initiated the infection of the intestinal epithelial cells. In this context, the defense cells are mobilized from the blood to reach the site of infection. Other authors have shown that after eight hours, the parasite is internalized into phagocytic and non-phagocytic cells of the new host [30].

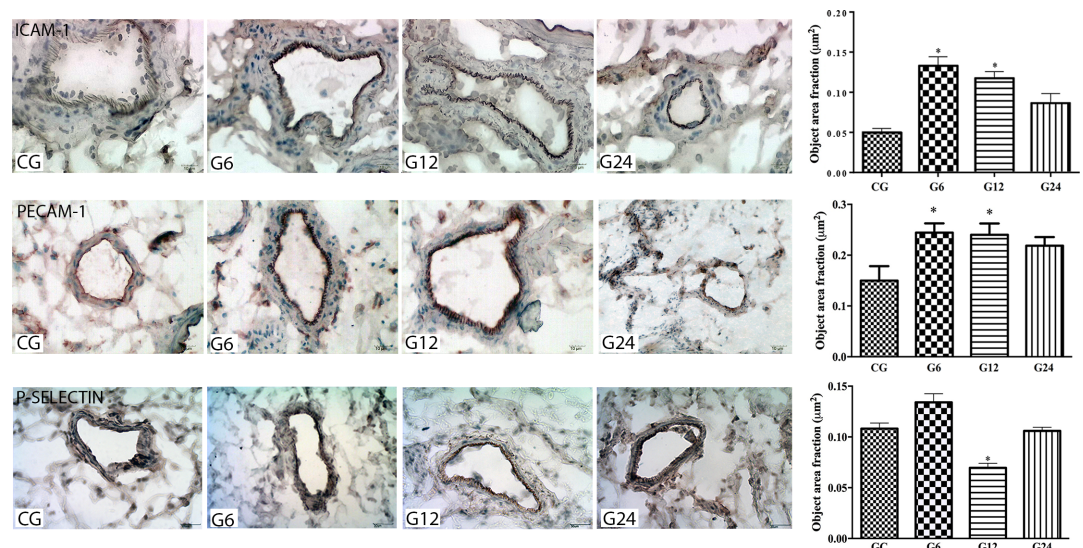


Fig 4. Representative photomicrograph and graphs for immunohistochemical evaluation of the expression of ICAM-1, PECAM-1 and P-selectin molecules on the mesenteric vascular endothelium of rats orally infected with *T. gondii* oocysts (ME49). Data are mean \pm S.E.M. * $p < 0.05$, compared to the control group (ANOVA, Tukey's test). CG (Control group), G6, G12, G24 G48 and G72 (rats infected for 6, 12, 24, 48 or 72 hours with oocysts of ME 49 strain of *T. gondii*, respectively).

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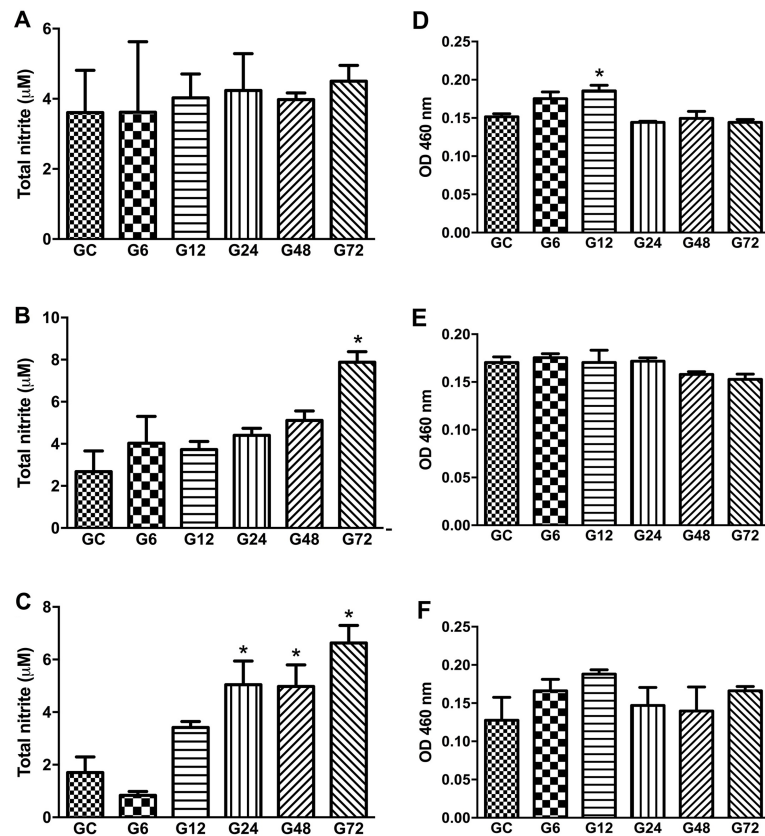


Fig 5. Myeloperoxidase activity in duodenum (A), jejunum (B) and ileum (C) and nitrite dosage as an indirect way to measure nitric oxide in the duodenum (D), jejunum (E) and ileum (F) at different times of early infection with oocysts of *T. gondii*. Data are mean \pm S.E.M., * $p < 0.05$, compared to the control group (ANOVA, Tukey's test). CG (Control group), G6, G12, G24, G48 and G72 (rats infected for 6, 12, 24, 48 or 72 hours with oocysts of ME 49 strain of *T. gondii*, respectively).

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We used intravital microscopy in order to evaluate the leukocytes behaviour in the mesenteric microcirculation and to study the leukocytes-endothelium interaction following the initial effects of oral *T. gondii* infection. Our results showed that there was an increased number of leukocytes rolling in the G6 and G12 groups and adherent cells in the G24 group. These hemodynamic changes happen at the beginning of inflammation, in which vascular endothelium enhances the expression of a series of membrane molecules to interact with circulating leukocytes, among them ICAM-1, PECAM-1 and P-selectin [31]. We also observed an increased expression of ICAM- and PECAM-1 also in G6 and G12 groups, showing the sequence of events preceding the leukocytes transmigration. This result has already been expected since the *T. gondii* adhesin (MIC 2) interacts with ICAM-1 to adhere and transigrate across cellular barriers [32], which could contribute to the spread of the parasite.

Unexpectedly, we also observed that P-selectin expression on mesenteric vessels was decreased in G12, although later the values were similar to the control group. Indeed, it was demonstrated that oral *T. gondii* infection induced the expression of P-selectin ligands on T cells without facilitating the cell recruitment into inflamed small intestine [33]. Our results suggest that the infection could be reducing P-selectin expression, since *T. gondii* can modulate

cytokines expression and other adhesion molecules such as those from the extracellular matrix [34–37]. While, in macrophages obtained from mice, the parasite is able to increase IL12 secretion [38–40] it can also inhibit the TNF α production [41] by disabling the NF κ B activation pathway, probably to avoid excessive proinflammatory cytokines production [42].

After the rolling process, these leukocytes firmly adhere to the vessel in an attempt to migrate to the site of infection to combat the causative agent of injury [13,43]. Previous studies from our group showed an increase in the number of intraepithelial lymphocytes after 48 hours of infection in rats [44].

Despite the fact that rats are considered a resistant model of toxoplasmosis, the changes observed indicate the beginning of an inflammatory process after infection. This is different from the experimental models using C57BL/6 that the infection by *T. gondii* causes excessive inflammation, increasing cytokine production and a large accumulation of leukocytes in the intestine [45]. The major histopathological changes occur in the distal segments of the small intestine after 72 hours of infection in mice [46]. Our experimental model develops a mild inflammation in the small intestine and does not present clinical signals of infection.

We suggest that after six hours of contact with infective forms, these have begun to cause injury to the intestine. At the site of inflammation, neutrophils try to combat the parasite through various pathways, including its enzymes. The MPO enzyme of neutrophils and other cells of myeloid origin is responsible for catalyzing the production of hypochlorous acid that kill the parasites. Studies have assigned the dosage of the MPO enzyme as an indirect indicator of neutrophil presence/activation in tissues [47,48]. In our study, the MPO activity increased only in the duodenum at 12 h of infection. The parasite possibly remains viable whereas microbicidal mechanisms of neutrophils are not activated as indicated by the non-increase of MPO activity found in this study. Hence, leukocytes that enter the small intestine are not able to eradicate the parasite.

T. gondii also has the ability to alter gene expression of the host cell, and may even avoid apoptosis by preventing the activation of the mitochondrial pathway of caspase [49] facilitating their replication within the cell [50]. Neutrophils are a reservoir for *T. gondii*, and it is believed that this fact helps the parasite to spread through the organism using their migration capability to reach other locations [10].

The nitric oxide synthesis by macrophages is an important mechanism microbicide for a variety of intracellular pathogens, including *T. gondii* [51] and possible neuroprotective action. There is an increased NO production by endothelial cells in the brain of mice after infection with ME-49 strain of *T. gondii* [52]. The findings about NO are consistent with later recruitment of mononuclear cells as observed by others [39]. We observed that the infected ileum responds more than the duodenum or jejunum probably due to the increased number of Peyer's Patches in this segment, which is part of mucosal-associated lymphoid tissue (MALT) containing T and B lymphocytes, dendritic cells and macrophages [53]. A previous study using mice showed that after infection there is an increase in CD4⁺ T cells, interferon- γ , tumor necrosis factor- α and inducible nitric oxide synthetase that mediate the development of ileus necrosis and subsequently lead to the death of the mice in 13 days [45]. However, rats might represent a compatible model of immunocompetent host, such as *T. gondii* infected immunocompetent humans. In this context, we questioned whether humans seropositive for *T. gondii* are more susceptible to inflammation in the intestine.

Our study was the first to demonstrate that the parasite parallel to the transposition of the intestinal barrier initiate the infection precociously (at 6 hours) leading to a systemic activation of innate immune response resulting in mild inflammation in a less susceptible experimental model of *T. gondii* infection.

Conclusion

Our results demonstrated that the response to *T. gondii* infection begins early even in immunocompetent hosts who do not develop clinical signs. At the same time, a sequence of systemic alterations detected by leukocyte mobilization in the peripheral blood and mesenteric microcirculation leads to a mild inflammatory response. Therefore, the parasite spreads through the body and resides in the intestine. It is important to conduct studies in an immunocompetent experimental model because the great majority of the human population seropositive to *T. gondii* antigens is also asymptomatic, but might trigger an inflammatory response similar to that seen in inflammatory bowel diseases.

Supporting information

S1 Fig. Experimental design. Schematic model exemplifying the experimental model used. (PDF)

S2 Fig. Intravital microscopy analysis. Schematic illustrating the step-wise protocol of the mesenteric microcirculation *in situ* in order to analyse the leukocyte endothelium interaction. (PDF)

S1 Table. Total and differential leukocyte count in rat peripheral blood. Total and differential leukocyte count (Cells mm⁻³) in rat peripheral blood. The rats of the infected groups received orally 5,000 sporulated oocysts of *T. gondii* (ME-49 strain, genotype II) that were resuspended in 1 mL sterile saline for 6 hours (G6), 12 hours (G12), 24 hours (G24), 48 hours (G48) and 72 hours (G72). The control group (CG) has received 1 mL sterile saline. (PDF)

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