Recent Advances in the Roles of Neutrophils in Toxoplasmosis

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Abstract

Neutrophils are now recognized as major components of the responses to *Toxoplasma gondii* by their contribution to the parasite elimination by a number of mechanisms. This review focuses on recent advances in the understanding of the mechanisms of migration, cytokine release and formation of extracellular traps by neutrophils during toxoplasmosis.

Neutrophil recruitment

Neutrophils participate in the control of *T. gondii*, but excessive recruitment of neutrophils may be harmful. The dynamic of this recruitment may vary according to the route of parasite infection. Biswas *et al*. have shown that intraperitoneal infection of C57BL/6 mice with cysts of the type 2 Me49 strain induced the recruitment of neutrophils from bone marrow to the blood [1]. Then, cerebral toxoplasmosis led to infiltration of neutrophils to the central nervous system, in close proximity to the parasites during the acute phase (days 10 to 14 after infection), but not during the chronic phase (from day 21 after infection). Two distinct subsets of neutrophils were identified in brain: a CD62-L^{low} subset producing high levels of IFN-γ and a CD62-L^{high} subset producing low levels of IFN-γ [1]. Depletion of neutrophils in the periphery by a treatment with an anti-Ly6G starting at day 10 post-infection led to a reduction of neutrophils in the brain of infected mice, associated to a significant increased parasite load and a significant decrease in the expression of genes coding for inflammatory and regulatory cytokines (IL-1β, IFN-γ, TNF and IL-10) [1]. In C57BL/6 mice deficient for IFN-α receptor, IFN-γ receptor or both receptors and infected with 15 cysts of the Me49 strain by the intraperitoneal route, the proportion of neutrophils was higher in the blood, the lungs, and in a lesser extent in the liver, than in wild type C57BL/6 infected mice [2]. In addition, in the absence of IFN-γ receptor, the parasite burden was increased in the three organs. On the contrary, no change in the cerebral parasite load was observed after the treatment of chronically infected C57BL/6 mice with a blocking anti-IL-10R antibody, leading to an increased recruitment of neutrophils in the brain [3]. However, the relationship between the recruitment of neutrophils and the parasite burden has not been investigated in these last two studies.

In a model of gut inflammation of C57BL/6 mice orally infected with the type 2 76K strain, neutrophil infiltration and levels of the chemokine CXCL1 were lower in protease-activated receptor $(PAR)_2^{-/-}$ mice compared to wild type mice [4]. Moreover, in the lack of a strong chemo-attracting activity of CXCL1, neutrophils were redistributed in the infected small intestine. Indeed, they were associated to epithelial layer erosion at the top of the villi in wild type mice, while they were localized in the middle or bottom part of the villi in $PAR_2^{-/-}$ mice [4].

Direct infection of eye vitreous of mice with tachyzoites of the type 1 RH strain induces recruitment of neutrophils. In a comparative study, Zhang *et al*. have shown that at day 8 post-infection, the infiltration of neutrophils was significantly lower in the eyes of BALB/c mice, known to be resistant to toxoplasmosis, than in the eyes of susceptible C57BL/6 mice. This could be related to the significantly lower ocular expression of TREM-1 (triggering receptor expressed on myeloid cells), a surface receptor of neutrophils playing a role in the regulation of their migration [5]. To understand whether neutrophils might play the role of vehicles of *T. gondii* into the retina, migration of human neutrophils infected with tachyzoites of the type 1 GT-1 strain was studied. Although non infected neutrophils migrate toward CXCL1, CXCL2 or CXCL8 chemokines, infected neutrophils did not [6]. In a co-culture experiment *in vitro*, retinal pigment epithelial cells (ARPE-19 cell line) infected with GT-1 tachyzoites secreted the cytokines GM-CSF, IL-6 and IL-18 that, in turn, activated neutrophils to produce elevated levels of reactive oxygen species (ROS) and to express higher levels of IL-1β and TNF-α transcripts [6]. This suggests that neutrophils are recruited in response to infection of cells of the retina, but don't participate in the spreading of the parasites in this organ. However, these results do not exclude that neutrophils carry the parasite in the human eye *in vivo*.

Neutralization of *T. gondii* by neutrophils

When primary human neutrophils isolated from blood were incubated with tachyzoites of the RH strain at a multiplicity of infection of 2, the percentage of infected neutrophils increased in a time-dependent manner (from 26 % after 30 min to 81 % after 24h) without effect on the cell viability [7]. The formation of neutrophil extracellular traps (NETs) was observed at 3 hours post-infection [7]. NETs are composed of a DNA backbone studded with histones and laced with antimicrobial peptides (Figure 1). These NET macromolecular structures can physically immobilized the parasites leading to their rapid death. *T. gondii* infection of farm animals is of economic importance and it is necessary to study the host-parasite relationships in such species. In an *in vitro* study on neutrophils of sheep and cattle, it has been shown that NETosis, the formation of NETs, increased with the time of incubation and the number of tachyzoites of the RH strain [8]. Although DNA, myeloperoxidase (MPO) and neutrophil elastase (NE) were detected in NETs of both species, MPO activity was higher for cattle neutrophils. Interestingly, NETs produced by sheep neutrophils could only immobilize the tachyzoites of *T. gondii*, while NETs produced by cattle cells were able to kill them [8]. The authors hypothesized that microbial peptides like defensins, present in cattle and not in sheep neutrophils, may have a lethal effect. As demonstrated with a DNase treatment, NETosis led to a decrease in the percentage of Vero cells infected with tachyzoites, and this was more marked with cattle than with sheep neutrophils [8]. Although equine neutrophils have specific responses, NETosis was observed with donkey neutrophils in response to *T. gondii* tachyzoites [9]. MPO, NE and ROS activities were increased, and the capability of the parasite to invade Vero cell was reduced in parallel [9]. In contrast to the results obtained with

86 neutrophils from intermediate host species, tachyzoites of the RH strain decreased the
87 viability of cat neutrophils after 3 hours of incubation [10]. At this time, their production of
88 ROS was increased and about 11 % of the neutrophils released NETs with a ratio trapped
89 parasites/neutrophils of 1.21 [10]. However, the roles of NETosis in the immune responses to
90 *T. gondii* in its definitive hosts, the felids, remain to be determined. Recently, *T. gondii* was
91 detected in marine mammals [11], reflecting increased pollution of the seas by oocysts
92 excreted by the definitive hosts [12]. *In vitro*, neutrophils isolated from blood of *Tursiops*
93 dolphin formed NETs that entrapped *T. gondii* tachyzoites in a typical mechanism of NETosis
94 dependent on the activity of histones and of NE and MPO enzymes [13]. From the three
95 existing different types of NETs, the aggregated (large and massive) and the spread (long and
96 fine) were the more frequent, while the diffuse (globular and compact) NETs were rare [13].
97 Since marine mammals have not evolved with the parasite *T. gondii*, NETosis might represent
98 an important mechanism of innate immune response to fight the infection.
99
100 **Concluding remarks**
101 The roles of neutrophils in the innate immune response to *T. gondii* infection are more studied
102 and it becomes clear that this cell type could be as important as macrophages and DCs in
103 many mechanisms of resistance against the protozoa. They are the first cells recruited to the
104 site of infection and they execute many anti-*Toxoplasma* immune functions as the production
105 of ROS and cytokines and the formation of NETs. After this early interaction of neutrophils
106 with *T. gondii* tachyzoites, they migrate to the draining lymph nodes, suggesting that they
107 certainly play a role in the establishment of the adaptive immune response through the
108 presentation of parasite antigens to lymphocytes. Although neutrophils might mature into DC-

like cells after infection with *T. gondii* as observed in other models [14], the roles of neutrophils as antigen presenting cells deserve to be studied. This translates into acquisition of major histocompatibility complex of class II and CD80/CD86 co-receptor molecules, T cell proliferation and differentiation, antigen presentation. In summary, neutrophils perform important functions to promote host defence against *T. gondii* and play a key role in early stage of toxoplasmosis.

Acknowledgments

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Conflicts of interest

The authors declare no conflict of interest.

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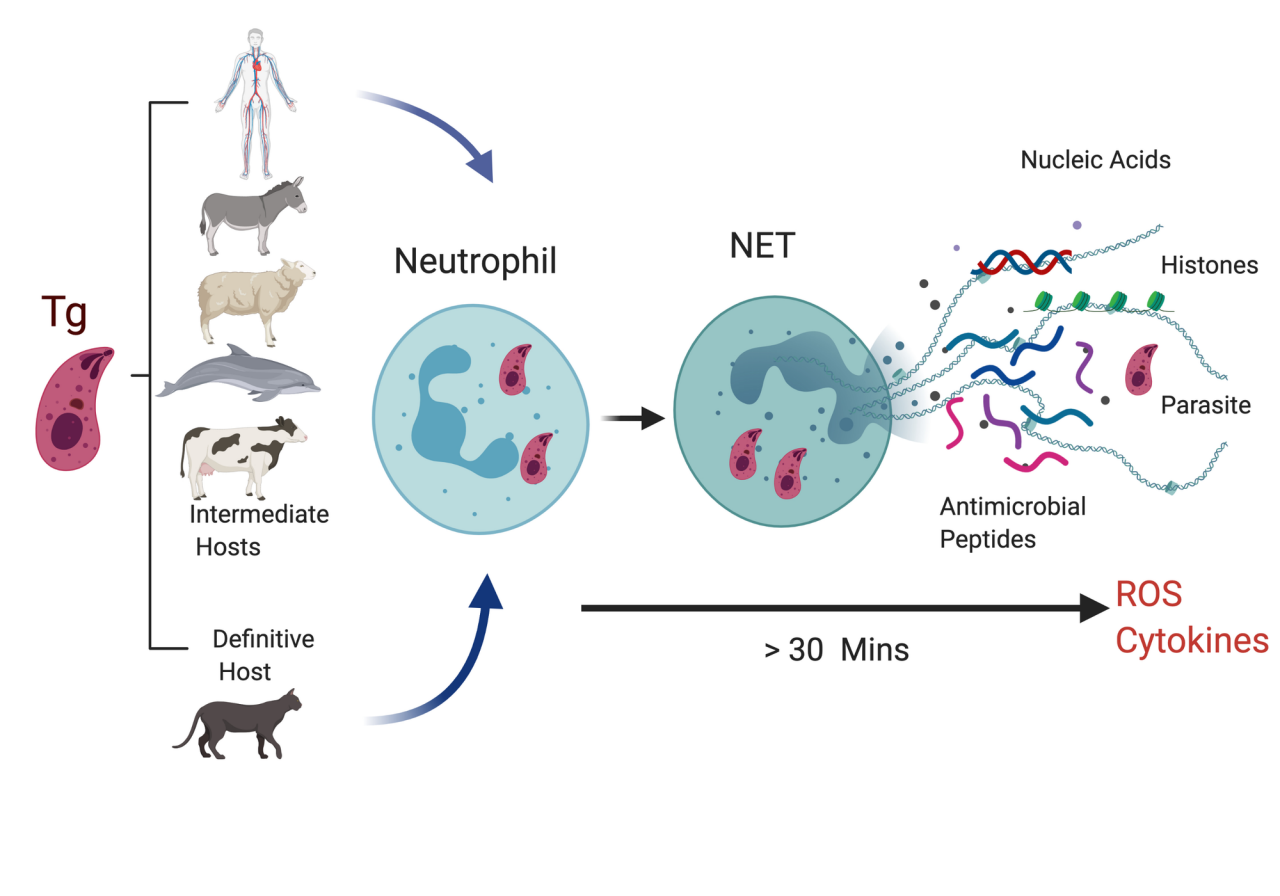
Figure 1. *T. gondii* triggers NETosis in neutrophils from different species. Tachyzoites of *T. gondii* (Tg) have been shown to infect neutrophils. Neutrophil extracellular traps (NETs) are constituted of chromatin, histones, enzymes and antimicrobial peptides in different proportions according to the origin of the neutrophils. The NETosis in response to *T. gondii* tachyzoites was recently demonstrated with neutrophils of intermediate (human, donkey, sheep, cattle and dolphin) and definitive (cat) hosts. NETosis increases with the time of contact with the parasites, and the first observations was made at 30 min of incubation. NETosis leads to the immobilization or the death of the tachyzoites, depending on the animal species. Neutrophils produce IFN-γ in infected brain and reactive oxygen species (ROS) in infected eyes of mice. Figure created with BioRender.


Tg
Intermediate Hosts
Definitive Host
Neutrophil
NET
Nucleic Acids
Histones
Parasite
Antimicrobial Peptides
> 30 Mins
ROS
Cytokines