# *Giardia intestinalis* trophozoites trigger NET formation in human PMN

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## Background

*Giardia intestinalis* is a zoonotic enteric protozoan parasite that causes giardiasis in humans, domestic animals and wildlife. This water- and food-borne disease causes > 280 million human diarrhoea cases per year, thereby affecting mainly infants and children. A typical host innate immune response against *G. intestinalis* includes the activation of polymorphonuclear neutrophils (PMN), which can release neutrophil extracellular traps (NETs). NETs are web-like structures composed of DNA, citrullinated histones, and antimicrobial proteins. It has recently been described that *G. intestinalis* trophozoites trigger the release of NETs in bovine PMN, however, related reports for human PMN are so far lacking. Despite the relevance and worldwide distribution of giardiasis, *G. intestinalis*-driven PMN innate immune responses remain poorly investigated, especially on the level of NET formation.

## Material and Methods

*G. intestinalis* trophozoites were axenically cultured in sterile TYI-S-33 complete medium at 37°C and 5% $CO_2$. Human PMN (hPMN) were isolated from healthy donors (*n = 3 - 4*) by immunonegative selection (Stemcell™). NETosis was induced by PMA stimulation or via exposure of human PMN to *G. intestinalis* trophozoites (1:3 ratio). NET release was illustrated by scanning electron microsocopy (SEM). Furthermore, typical characteristics of NETs were confirmed via immunofluorescence microscopy by detecting citrullinated histones, neutrophil elastase (NE) and DNA in NET structures. The hPMN reactive oxygen species (ROS) production was measured using a luminol-chemiluminescence-based method.

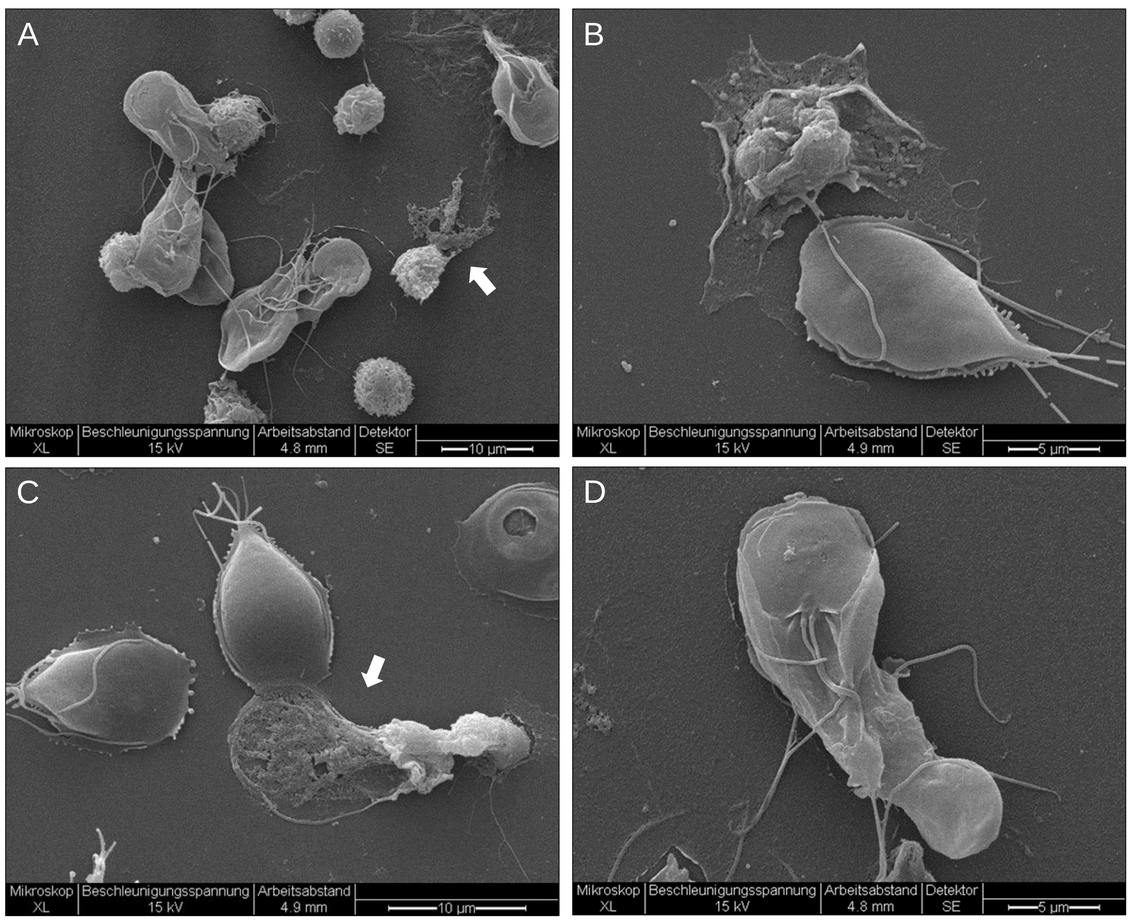


**Figure 2. Illustration of *G. intestinalis* trophozoite-triggered NETs in human PMN via scanning electron microscopy (SEM).** (A-C) Arrows indicate NETs released by hPMN. (D) Phagocytic activity of hPMN against trophozoites

## Results

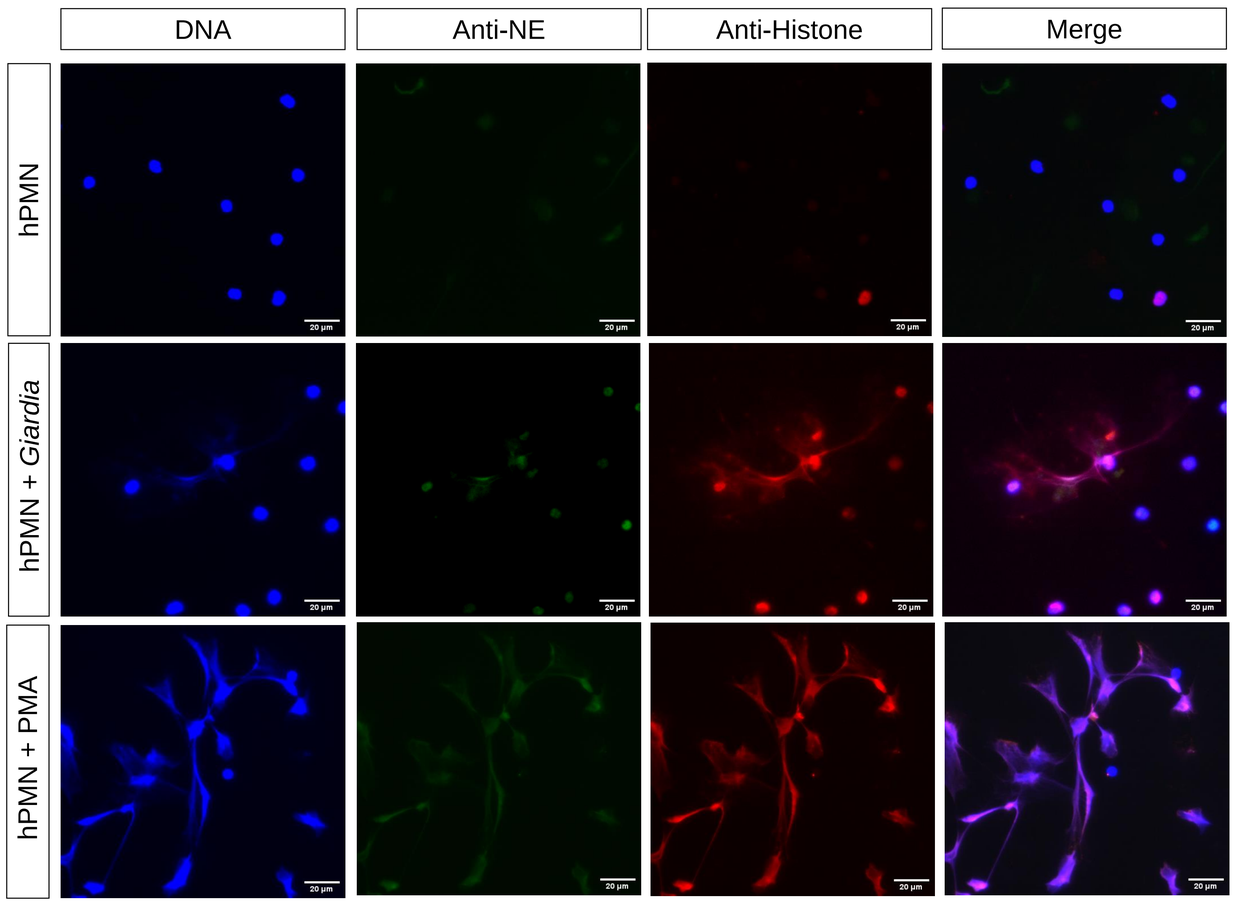


**Figure 1. *G. intestinalis* trophozoites activate human PMN resulting in NET release.** hPMN were cultured without stimuli (= negative control), in the presence of trophozoites or with PMA as positive control. Immunofluorescence microscopy shows extracellular NET structures by co-localization of DNA (blue), neutrophil elastase (NE; green) and citrullinated histones (red).

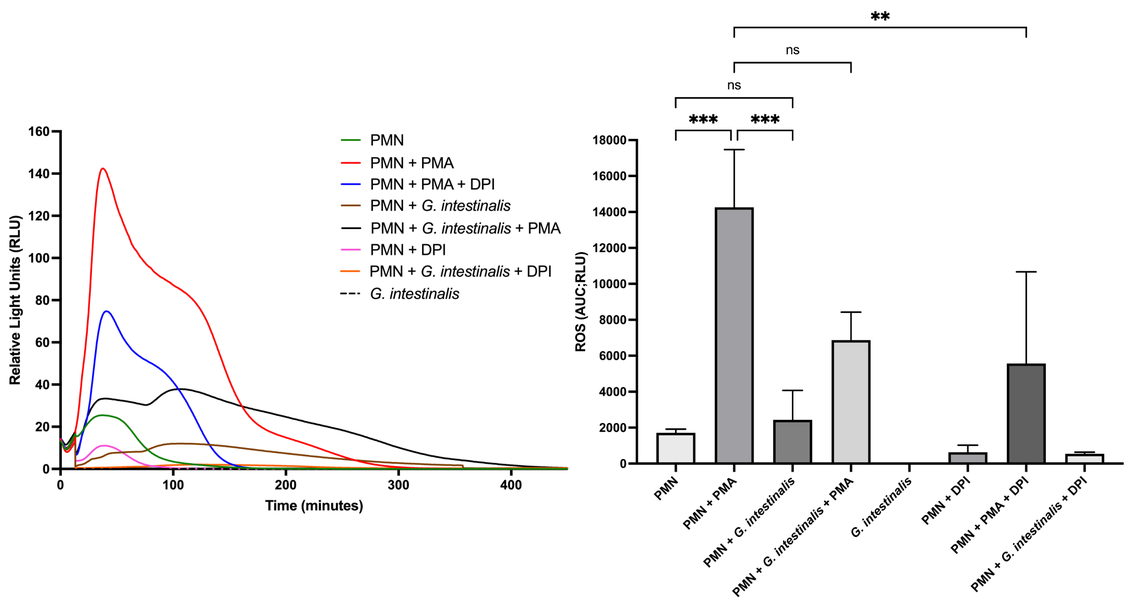


**Figure 3. *G. intestinalis* trophozoites fail to trigger ROS production in human PMN.** The bar graph shows the area under the curve (AUC) of total ROS production. No significative differences were observed in hPMN ROS production after parasite exposure when compared to unstimulated controls. Diphenyleneiodonium chloride (DPI) was used as a control to inhibit neutrophil NADPH oxidase-related ROS production.

## Conclusions

The current study presents a novel finding on early interactions between the enteropathogen *G. intestinalis* and hPMN by demonstrating that motile trophozoites are capable to stimulate NET release in hPMN. Despite contrasting with previous reports, the current data suggest that both, NET release and phagocytosis are used as effector mechanisms of human PMN in response to trophozoites. The lacking or reduced ROS production observed in hPMN confronted with *G. intestinalis* trophozoites may reflect an immune evasion strategy of this parasite and/or advanced antioxidant defense mechanisms. Further research is needed to better understand these mechanisms *in vivo*.