

Short notes

Toxoplasma gondii – an amazing parasite. A comment to the article of Koshy A.A. et al. „*Toxoplasma* co-opts host cells it does not invade".

Marcin M. Grzybowski, Henryka Długońska

Department of Immunoparasitology, Faculty of Biology and Environmental Protection, University of Łódź, Banacha 12/16, 90-237 Łódź, Poland

Corresponding author: Marcin M. Grzybowski; E-mail: marcin@biol.uni.lodz.pl

ABSTRACT. Ubiquitous parasite of humans and endothermic animals *Toxoplasma gondii* (type Apicomplexa), identified by Nicolle and Manceaux over 100 years ago, is still an object of numerous extensive studies bringing very interesting and often even surprising observations as that announced in the title [1].

Key words: *T. gondii*, rhoptries, host-parasite interaction

T. gondii is able to penetrate actively most nucleated cells of the host. The invasion is an active, rapid and multistep process accompanied by the sequential discharge of three secretory apical organelles: micronemes, rhoptries and dense granules. Rhoptries, the most unusual secretory organelles among eukaryotic organisms [2], release into the host cell numerous vesicle-like bodies („evacuoles", short for empty vacuoles), which participate in the biogenesis of the parasitophorous vacuole (PV) [3] – a safe intracellular compartment where the parasite grows. The first stage of the invasion involves a strong attachment of the parasite to the host cell surface. Surprisingly, an *in vitro* study of *T. gondii* invasion process for a representative strain RH (clonal lineage I) revealed that only one in four attachment events is succesful and leads to PV formation, while 75% of initially bound parasites detach from the host cells (Fig.1) [4]. One of the addressed questions was: what happens during the short contact?

As *T. gondii* easily undergoes a variety of genetic manipulations in the laboratory, it serves as a model parasite to study biology of the Apicomplexa [5]. For instance, the *in vitro* experiments using transgenic parasites with expression of the protein Cre (a conservative site-specific recombinase) fused to the toxofilin (a rhoptry-derived, actin-binding protein) revealed that those engineered parasites efficiently introduced the fusion protein to the Cre-reporter host cells but, surprisingly, some of them were found to be free from parasites [6]. To confirm the observed phenomenon the authors changed the reporter system to a less sensitive one and used β-lactamase-toxofilin (fusion protein) and primary human foreskin fibroblasts as host cells, but again, they were able to detect the presence of fusion protein in many cells which did not contain a parasite (injected-uninfected cells, I-U) [1]. The next question was, whether the amount of injected rhoptry proteins is sufficient to alter significantly the host cell physiology. Based on the previous findings [7,8] that rhoptry kinase ROP16 causes rapid (within 1 min) phosphorylation and nuclear translocation of STAT6 (Signal Transducer and Activator of Transcription 6), the authors applied it as an activation marker. Again, about 6% of cells that showed activation of STAT6 contained no parasite. Further *in vivo* studies proved that the observations made *in vitro* are not experimental artefacts. Very sophisticated *in vivo* experiments showed convincingly that in the brain the cells of I-U type outnumber the infected cells and at least some of them are not simple a result of cell division.

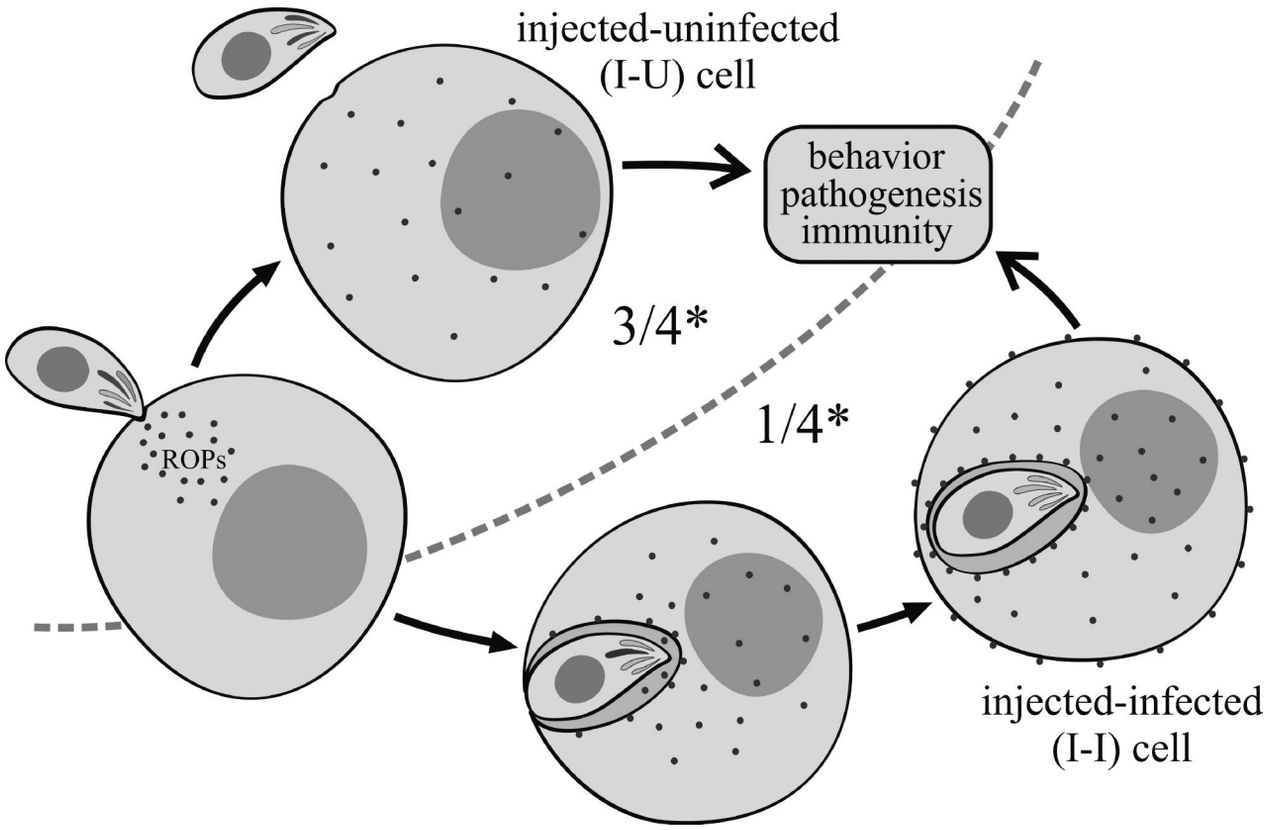

Fig.1. Attachment of *T. gondii* to a host cell leads or does not lead to the infection; * the proportion of the „I-U" to „I-I" host cells

High frequency of I-U cells, especially in the brain, may help explain the significant behavioral changes in *T. gondii*-infected hosts caused by a relatively low level of parasite cysts [9]. Besides, injection of rhoptry proteins into immune cells (e.g. macrophages) and activation of STAT3/6, could result in a decrease in IL-12 synthesis and alter the Th1/Th2 cytokine profile. Then, the I-U cells could present processed injected antigens and become targets for specific cytotoxic T lymphocytes or antibodies, although previous working models of MHC-restricted presentation of *T. gondii* antigens rather exclude this possibility [10]. The actual and global influence of rhoptry proteins injection on the host biology is not explained and surely needs further research. The *T. gondii*-driven processes: injection and infection are quite different. The latter is associated with the formation of a moving junction when the C-terminal region of rhoptry neck protein RON2 binds to parasite's adhesion molecule AMA1. This connection is not required for rhoptry protein injection [11]. It is also not known, if *T. gondii* pre-selects host cells as appropriate for each of these both processes, and how long the injected proteins influence host cell physiology. Many detailed problems linked to rhoptry protein injection and non-productive invasion are still open.

The phenomenon of „injection of rhoptry-derived *T. gondii* effector proteins without infection" would possibly initiate studying other important apicomplexan parasites (including *Plasmodium*) to answer a question whether they use a homologous mechanism to manipulate the host. The global success of *T. gondii* suggets that the parasite balances efficiently between evasion and activation of the host immune system and the newly described, above mentioned, phenomenon supplements a battery of different mechanisms in which the parasite alters the host's physiology.

References

[1] Koshy A. A., Dietrich H. K., Christan D. A., Melehani J. H., Shastri A. J., Hunter C. A., Boothroyd J. C. 2012. *Toxoplasma* co-opts host cells it does not invade. *PloS Pathogens* 8: e1002825.

[2] Dubremetz J. F. 2007. Rhoptries are major players in *Toxoplasma gondii* invasion and host cell interaction. *Cellular Microbiology* 9: 841-848.

[3] Håkansson S., Charron A. J., Sibley L. D. 2001. *Toxoplasma* evacuoles: a two-step process of secretion

and fusion forms the parasitophorous vacuole. *The EMBO Journal* 20: 3132-3144.

[4] Kafsack B. F., Carruthers V. B., Pineda F. J. 2007. Kinetic modeling of *Toxoplasma gondii* invasion. *Journal of Theoretical Biology* 249: 817-825.

[5] Kim K., Weiss L. M. 2004. *Toxoplasma gondii*: the model apicomplexan. *International Journal for Parasitology* 34: 423-432.

[6] Koshy A. A., Fouts A. E., Lodoen M. B., Alkan O., Blau H. M., Boothroyd J. C. 2010. *Toxoplasma* secreting Cre recombinase for analysis of the host-parasite interactions. *Nature Methods* 7: 307-309.

[7] Saeij J. P., Coller S., Boyle J. P., Jerome M. E., White M. W., Boothroyd J. C. 2007. *Toxoplasma* co-opts host gene expression by injection of a polymorphic kinase homologue. *Nature* 445: 324-327.

[8] Ong Y. C., Reese M. L., Boothroyd J. C. 2010. *Toxoplasma* rhoptry protein 16 (ROP16) subverts host function by direct tyrosine phoshorylation of STAT6. *The Journal of Biological Chemistry* 10: 28731-28740.

[9] Gatkowska J., Wieczorek M., Dziadek B., Dzitko K., Dlugonska H. 2012. Behavioral changes in mice caused by *Toxoplasma gondii* invasion of brain. *Parasitology Research* 111: 53-58.

[10] Goldszmid R. S., Sher A. 2010. Processing and presentation of antigens derived from intracellular protozoan parasites. *Current Opinion in Immunology* 22: 118-123.

[11] Tyler J. S., Boothroyd J. C. 2011. The C-terminus of *Toxoplasma* RON2 provides the crucial link between AMA-1 and the host-associated invasion complex. *PLoS Pathogens* 7: e1001282.

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