**T. gondii inveSTING in a latent future**

DOI 10.1074/jbc.H119.011296

Ramya Nandakumar and Søren R. Paludan
From the Department of Biomedicine, Aarhus University, Aarhus C DK-8000, Denmark

Edited by Chris Whitfield

**Toxoplasma gondii** is an obligate protozoan parasite that naturally infects all mammals, where it alters the host environment to establish chronic infection. Wang and colleagues uncover a new role for the **T. gondii** protein GRA15 in inducing an anti-parasite response via the interferon stimulator STING. This parasite-driven host defense limits *Toxoplasma* replication while maintaining host survival, creating an ideal niche for the establishment of latency.

An estimated one-third of the global population is infected with *Toxoplasma gondii*. Although infection is often asymptomatic in healthy individuals, there is no vaccine, and the available treatment options are poorly tolerated, meaning that toxoplasmosis in immunosuppressed individuals can be potentially fatal. Better knowledge of molecular host-parasite interactions could shed light on potential therapeutic targets against **T. gondii** and perhaps also other apicomplexan parasites, such as *Plasmodium* species. Although much has been learned about **T. gondii** infection, such as the identity of T cells that confer protective immunity (1) and some of the pathways involved in their detection, many pieces of this puzzle remain unsolved. For example, in the widely used mouse model for **T. gondii** infection, immune responses are driven by the Toll-like receptors TLR11 and TLR12, which recognize the parasite protein profilin (2, 3). However, the human TLR11 is nonfunctional and the human genome does not encode TLR12, leaving the immune sensor for **T. gondii** in humans a mystery. Wang et al. (4) now bring us one step closer to a complete picture in their discovery of protein 15 (GRA15), which is secreted into the cellular cytosol and is a known regulator of NF-κB, which is upstream from interferon β (7). Interestingly, previous research has not identified a role for the proteins that detect foreign DNA, including the DNA sensor cGMP-AMP synthase (cGAS),2 in sensing **T. gondii**.

Suspecting that cGAS might play a role in eliciting an anti- **T. gondii** response, Wang et al. decided to examine cGAS-deficient mice infected with **T. gondii**. The authors observed that the loss of cGAS resulted in significant animal deaths compared with WT mice. Whether this response is generated against parasite DNA or **T. gondii**-induced leakage of host DNA into the cytosol is currently unclear. To test what was happening downstream of cGAS, Wang et al. additionally examined whether deletions of STING, which is activated by the secondary messenger synthesized by cGAS, impacted mouse biology. Indeed, the authors showed that the STING signaling axis is indispensable in controlling **T. gondii** infection in mice. Specifically, spleens of STING<sup>−/−</sup> mice exhibited decreased levels of cytokine *Il12a*, the interferon *Ifnb1*, and the interferon-stimulated gene *Isg15* compared with WT mice. Gross disruptions of these important immune signaling pathways in STING<sup>−/−</sup> mice resulted in higher splenic parasite burden. As a result, STING<sup>−/−</sup> mice exhibited significant weight loss and fared poorly compared with WT mice. Given the striking effect of STING deletion, the authors suspected that there might be a more direct route to impact STING function than through cGAS.

To track down this other pathway, Wang et al. started by investigating the GRA proteins that are known determinants of **T. gondii** pathogenesis. They discovered that only GRA15 was able to enhance cGAS/STING-mediated induction of a reporter gene, suggesting that this molecule might be a missing piece in the puzzle. Next, the authors used a CRISPR/Cas9 approach to generate a GRA15 mutant of **T. gondii**. Mice infected with these GRA15<sup>−/−</sup> parasites fared poorly compared with mice infected with WT parasites. In line with this finding, spleens of mice infected with GRA15<sup>−/−</sup> **T. gondii** parasites exhibited higher parasite count and lower expression of *Il12a*, *Isg15*, and the chemokine *Cxcl10* as compared with when infected with WT parasite, confirming that GRA15 is essential in enhancing **T. gondii**-stimulated innate immune response.

Wang et al. then undertook a series of experiments to learn more about the specific mechanism by which GRA15 stimulates the STING pathway. First, they observed that GRA15 localizes to the ER, rather than remaining free in the cytosol.

---

The authors declare that they have no conflicts of interest with the contents of this article.

1 To whom correspondence should be addressed. Tel.: 45-86196128; Fax: 45-86196128; E-mail: srp@biomed.au.dk.

2 The abbreviations used are: cGAS, cGMP-AMP synthase; TNF, tumor necrosis factor; TRAF, TNF receptor–associated factor.
They then generated truncation mutants of GRA15 and were able to uncover two transmembrane domains in the first 100 amino acids. Interestingly, cellular co-localization studies on GRA15 truncation mutants revealed a role for the second of two transmembrane domains in docking to the ER. Once there, GRA15 promotes ubiquitination of STING at position lysine 337. This initial ubiquitination resulted in its further polyubiquitination and oligomerization, facilitating activation of its downstream targets IRF3 and type I interferon.

Finally, the authors sought to determine how exactly GRA15 was causing STING modifications. A pulldown assay using FLAG-GRA15–transfected HEK cells uncovered interactions of GRA15 with two members of the TNF receptor–associated factor (TRAF) family, TRAF2 and TRAF6. Furthermore, GRA15 was capable of recruiting TRAF2 and TRAF6 to the ER, and deletion of TRAF2 and TRAF6 prevented GRA15-mediated interferon-β promoter activation. However, this was also seen in cells devoid of TRAF3 or TRAF5, thus leaving open the question on the specificity of the individual TRAFs in STING activation.

Several pieces of the puzzle have now fallen into place, allowing Wang et al. to propose a general mechanism by which the effector protein GRA15 can activate interferon responses (Fig. 1). However, many other pieces remain to be understood. This includes how GRA15 is able to activate TRAF proteins to activate STING and how GRA15 modulates both the TRAF/STING and NF-κB pathways. Second, it remains to be seen how human and murine systems differ in their anti-T. gondii responses and whether GRA15 will play a similar role in human infections. In addition to host considerations, there are different clonal lineages (type I and type II) of T. gondii that lead to disparate phenotypes; the basis for these distinct outcomes is still elusive. Finally, will GRA15’s mechanism of inducing the immune system provide any new insights relevant to potential treatment strategies? Overall, the new insights from Wang et al. provide a fascinating picture as to how T. gondii will go to great lengths for establishment of latency.

References

1. Suzuki, Y., Orellana, M. A., Schreiber, R. D., and Remington, J. S. (1988) Interferon-γ: the major mediator of resistance against Toxoplasma gondii. Science 240, 516–518 CrossRef Medline

2. Koblansky, A. A., Jankovic, D., Oh, H., Hiény, S., Sungnak, W., Mathur, R., Hayden, M. S., Akira, S., Sher, A., and Ghosh, S. (2013) Recognition of profilin by Toll-like receptor 12 is critical for host resistance to Toxoplasma gondii. Immunity 38, 119–130 CrossRef Medline

3. Yarovinsky, F., Zhang, D., Andersen, J. F., Bannenberg, G. L., Serhan, C. N., Hayden, M. S., Hiény, S., Sutterwala, F. S., Flavell, R. A., Ghosh, S., and Sher, A. (2005) TLR11 activation of dendritic cells by a protozoan profilin-like protein. Science 308, 1626–1629 CrossRef Medline

4. Wang, P., Li, S., Zhao, Y., Zhang, B., Li, Y., Liu, S., Du, H., Cao, L., Ou, M., Ye, X., Li, P., Gao, X., Wang, P., Jing, C., Shao, F., Yang, G., and You, F. (2019) The GRA15 protein from Toxoplasma gondii enhances host defense responses by activating the interferon stimulator STING. J. Biol. Chem. 294, 16494–16508 CrossRef Medline

5. Rosowski, E. E., Nguyen, Q. P., Camejo, A., Spooner, E., and Saeij, J. P. (2014) Toxoplasma gondii inhibits IFN-γ- and IFN-β-induced host cell STAT1 transcriptional activity by increasing the association of STAT1 with DNA. Infect. Immun. 82, 706–719 CrossRef Medline

6. He, H., Brenier-Pinchart, M. P., Brau, L., Kraut, A., Touquet, B., Couté, Y., Tardieux, I., Hakimi, M. A., and Bougdour, A. (2018) Characterization of a Toxoplasma effector uncovers an alternative GSK3β-catenin-regulatory pathway of inflammation. Elife 7, e39887 CrossRef Medline

7. Rosowski, E. E., Lu, D., Julien, L., Rodda, L., Gaiser, R. A., Jensen, K. D., and Saeij, J. P. (2011) Strain-specific activation of the NF-κB pathway by GRA15, a novel Toxoplasma gondii dense granule protein. J. Exp. Med. 208, 195–212 CrossRef Medline