



A fine balance between life and death: modulation of BCL-2 family members by *Toxoplasma gondii*

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A commentary on

The differential effect of *Toxoplasma gondii* infection on the stability of BCL2-family members involves multiple activities by Carmen, J. C., and Sinai, A. P. (2011). *Front. Microbiol.* 2:1. doi: 10.3389/fmicb.2011.00001

Apoptosis has been shown to serve as a mechanism by which cells infected with viruses, bacteria, or intracellular parasites can be eliminated without eliciting an unwanted inflammatory response. Not surprisingly intracellular pathogens have evolved mechanisms by which to inhibit apoptosis, not only avoiding the elimination of the host cell but also extending its life span. Inhibition of apoptosis by intracellular pathogens is accomplished by affecting the fine balance between pro- and anti-apoptotic factors in the infected cell. One mechanism utilized by various microbes is regulation of the pro- and anti-apoptotic proteins of the BCL-2 family (Carmen and Sinai, 2007; Faherty and Maurelli, 2008; Galluzzi et al., 2008). The BCL-2 family includes the pro-apoptotic proteins BCL-2-associated X protein (BAX) and the BCL-2 antagonist/killer-1 (BAK), which directly participate in the release of Cytochrome C from mitochondria (Youle and Strasser, 2008). Other members of the BCL-2 family (e.g., BAD, BID, BIM) participate in the direct or indirect activation of BAX or BAK, thus having pro-apoptotic effects, while others (e.g., BCL-2 and BCL-XL) inhibit these pore forming proteins, thus acting as anti-apoptotic agents (Youle and Strasser, 2008).

In a recent *Frontiers in Cellular and Infection Microbiology* research article, Carmen and Sinai (2011) show that *Toxoplasma gondii*, an important pathogenic parasite of humans and other animals, affects the stability of several BCL-2 family proteins via a diversity of proteolytic activities. In order to ensure its survival and

successful propagation within an infected host, *T. gondii* inhibits apoptosis in infected cells. Anti-apoptotic events associated with *T. gondii* infection include activation of the nuclear factor- κ B (NF- κ B) inhibition of phosphoinositide 3-kinase (PI3-K), Cytochrome C release from the mitochondrion, and direct inhibition of the apoptosome activity (reviewed in Carmen and Sinai, 2007; Laliberte and Carruthers, 2008). Obstruction of Cytochrome C release in *T. gondii* infected cells has been reported to correlate with increase of BCL-2 transcript level inhibition of mitochondrial targeting and activation of BAX (Hippe et al., 2009), as well as selective degradation of the pro-apoptotic proteins BAX, BAD, and BID (Goebel et al., 2001; Molestina et al., 2003). Degradation of pro-apoptotic members of the BCL-2 family is also observed with other pathogens such as *Chlamydia trachomatis* (Dong et al., 2005).

To better define the requirements for the *T. gondii* dependent degradation of BCL-2 family proteins, Carmen and Sinai (2011) have now analyzed the levels of BCL-2 family proteins at varying multiplicities of infection (MOI) and in the presence of inhibitors for distinct classes of proteases. While these experiments intrinsically exhibit a great degree of variability, ratio-metric analysis combined with rigorous statistical analysis has allowed the authors to uncover trends that reveal the complexity of the effect of *T. gondii* infection on the stability of BCL-2 family members. Their analysis was focused on anti-apoptotic BCL-2, and the pro-apoptotic proteins BAX, BAD, and BID. While the transcript level of BCL-2 increases upon infection (Molestina et al., 2003), there is no difference in the levels of BCL-2 protein between uninfected and heavily infected cells. Thus, it is plausible that *T. gondii* is overcoming an increase in BCL-2 turnover in response to infection by up-regulating its transcription. By contrast, while *T. gondii* infection increases

transcript levels of pro-apoptotic genes BAD and BID (Molestina et al., 2003), their protein products are reduced at high MOIs. Parasite dependent protein degradation is also observed for BAX. By also performing these experiments in mutant human cells, the authors find that none of the effects on the stability of BCL-2 proteins is dependent NF- κ B. This suggests that *T. gondii* can regulate the life span of the host cell by both NF- κ B dependent and independent mechanism to block apoptosis.

By analyzing stability trends with increasing MOIs in the presence of specific inhibitors it was determined that the *T. gondii* dependent degradation of BAD and BID depends on the activity of the proteasome. This suggests a role for ubiquitination in the parasite dependent turn over of these proteins. The degradation of these two pro-apoptotic factors upon parasite infection was also disrupted by addition of inhibitors of either trypsin-like or chymotrypsin-like proteases, but was not affected by a cysteine protease inhibitor. On the other hand the parasite dependent degradation of BAX was resistant to all inhibitors tested indicating that a type of proteolytic activity different from cysteine, trypsin-like, or chymotrypsin-like protease is responsible for its infection-dependent turnover. Intriguingly, in the presence of cysteine protease inhibitor, BAX actually became more sensitive to parasite dependent degradation. This suggests that BAX is partly protected from parasite dependent degradation by the action of a cysteine protease. An interesting aspect of this study is that by looking at the effect of the inhibitors in uninfected cells the authors can draw some conclusions about how normal levels of BCL-2 family members are maintained. Thus, it appears that steady state levels of BAD and BAX are maintained by cysteine protease driven turnover, while the steady state level of BID is regulated by trypsin-like proteolytic activity.

An important question that remains unresolved is whether the *T. gondii* dependent turnover of the anti-apoptotic members of the BCL-2 family is due to parasite or host derived proteins. Evidence that parasite proteins are delivered into the host cell (Laliberte and Carruthers, 2008) opens the possibility that *T. gondii* secretes proteins that directly target host factors for selective degradation. Nonetheless, it is most likely that a parasite protein mediates activation of intrinsic cellular pathways for degradation. Regardless, a better understanding of how *T. gondii* infection leads to the degradation of these important regulators of apoptosis is likely to provide new insights into the normal dynamics of BCL-2 family proteins.

REFERENCES

- Carmen, J. C., and Sinai, A. P. (2007). Suicide prevention: disruption of apoptotic pathways by protozoan parasites. *Mol. Microbiol.* 64, 904–916.
- Carmen, J. C., and Sinai, A. P. (2011). The differential effect of *Toxoplasma gondii* infection on the stability of BCL2-family members involves multiple activities. *Front. Microbiol.* 2:1. doi: 10.3389/fmicb.2011.00001
- Dong, F., Pirbhaj, M., Xiao, Y., Zhong, Y., Wu, Y., and Zhong, G. (2005). Degradation of the proapoptotic proteins Bik, Puma, and Bim with Bcl-2 domain 3 homology in *Chlamydia trachomatis*-infected cells. *Infect. Immun.* 73, 1861–1864.
- Faherty, C. S., and Maurelli, A. T. (2008). Staying alive: bacterial inhibition of apoptosis during infection. *Trends Microbiol.* 16, 173–180.
- Galluzzi, L., Brenner, C., Morselli, E., Touat, Z., and Kroemer, G. (2008). Viral control of mitochondrial apoptosis. *PLoS Pathog.* 4, e1000018. doi: 10.1371/journal.ppat.1000018
- Goebel, S., Gross, U., and Luder, C. G. (2001). Inhibition of host cell apoptosis by *Toxoplasma gondii* is accompanied by reduced activation of the caspase cascade and alterations of poly(ADP-ribose) polymerase expression. *J. Cell Sci.* 114, 3495–3505.
- Hippe, D., Weber, A., Zhou, L., Chang, D. C., Hacker, G., and Luder, C. G. (2009). *Toxoplasma gondii* infection confers resistance against BimS-induced apoptosis by preventing the activation and mitochondrial targeting of pro-apoptotic BAX. *J. Cell Sci.* 122, 3511–3521.
- Laliberte, J., and Carruthers, V. B. (2008). Host cell manipulation by the human pathogen *Toxoplasma gondii*. *Cell. Mol. Life Sci.* 65, 1900–1915.
- Molestina, R. E., Payne, T. M., Coppens, I., and Sinai, A. P. (2003). Activation of NF-kappaB by *Toxoplasma gondii* correlates with increased expression of anti-apoptotic genes and localization of phosphorylated IkkappaB to the parasitophorous vacuole membrane. *J. Cell Sci.* 116, 4359–4371.
- Youle, R. J., and Strasser, A. (2008). The BCL-2 protein family: opposing activities that mediate cell death. *Nat. Rev. Mol. Cell Biol.* 9, 47–59.

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