Oxidative stress and intracellular infections: more iron to the fire

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The immune system’s battle against pathogens includes the “respiratory burst,” a rapid release of ROS from leukocytes, thought to play a role in destroying the invading species. In this issue of the JCI, Paiva et al. demonstrate that oxidative stress actually enhances infection with the protozoan Trypanosoma cruzi, by a mechanism that may involve facilitating parasite access to iron. Their findings suggest a novel direction for the development of drugs against intracellular parasites.

The immune system of higher vertebrates is capable of rapidly recognizing pathogens, mounting an immediate innate response. The innate response is followed by adaptive immunity, the slower-developing process that is responsible for preventing reinfection by the same organism. An important component of the rapid innate immune response is production of ROS, highly reactive and toxic molecules produced by phagocytes and other cell types. ROS have been traditionally viewed as a “necessary evil” in the battle against pathogens, and their production is coupled to the antioxidant responses important for mitigating oxidative damage in cells and tissues. However, this is clearly not the whole story. In this issue of the JCI, Paiva et al. (1) demonstrate that antioxidant responses actually suppress infections with Trypanosoma cruzi, a protozoan parasite that seems to thrive in an oxidative environment. Earlier studies with viruses and bacterial pathogens suggested a similar scenario, but those observations were mostly attributed to a role of antioxidant pathways in innate immunity. Challenges established by parasitology and macrophages were exposed to CoPP, an activator of the transcription factor NRF2 that orchestrates antioxidant responses. NRF2 drives expression of heme oxygenase–1 (HO-1), an enzyme that shifts the cellular redox balance by degrading pro-oxidant heme (2). Surprisingly, activation of NRF2 and increased expression of HO-1 markedly reduced parasite burden in infected animals and in isolated macrophages. The process was independent of T lymphocyte–mediated immunity and did not seem to involve apoptotic clearance of infected cells or effectors known to be active against T. cruzi, such as NO, TNF, or type I IFN (1).Remarkably, NRF2 activation had the expected effect of inhibiting ROS generation in infected macrophages, but this did not affect the number of intracellular parasites found shortly after infection (1). This observation is consistent with the presence in T. cruzi and other trypanosomatid parasites of a unique and highly effective antioxidant machinery, the trypanothione-thiol system (3).

The unexpected role of high oxidative stress in promoting T. cruzi infection may have important implications for the pathology of Chagas disease, the chronic debilitating illness that affects millions of people infected by this parasite in Latin America. Although infective trypomastigote stages can invade practically every nucleated cell type, in vivo the parasites are frequently found replicating in skeletal and cardiac muscle (4). The most serious manifestation of chronic Chagas disease, cardiomyopathy, is responsible for significant mortality in infected patients and has been directly attributed to the persistence of T. cruzi within cardiomyocytes (5, 6). Interestingly, there is evidence that marked and sustained oxidative stress is established in cardiomyocytes following T. cruzi infection, due to parasite-induced disturbances in mitochondrial membrane potential and electron

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Iron is essential for several metabolic pathways, but its concentration inside cells has to be tightly regulated because it can catalyze the formation of dangerous free radicals. The mechanisms used by intracellular trypanosomatid protozoa (including the human parasites *T. cruzi* and *Leishmania*) to acquire this critical element in the challenging intracellular environment are only beginning to be elucidated (11). *T. cruzi* amastigotes take up iron-loaded transferrin when grown in vitro (12), but the physiological significance of this observation is unclear. Transferrin is restricted to the lumen of the endocytic pathway and thus is absent from the host cell cytosol, where intracellular amastigotes replicate. In *Leishmania*, a transferrin receptor–based mechanism for iron uptake was also initially postulated, but it was not confirmed by subsequent studies (13). Transferrin can reach the lysosome-like parasitophorous vacuoles where *Leishmania* resides in macrophages (14), but it appears to function mainly as a source of ferric iron (Fe³⁺) for the sequential action of two surface-associated parasite molecules: the Fe³⁺ reductase LFR1 (15) and the LIT1 transporter, which directly promotes ferrous iron (Fe²⁺) uptake (16). Intriguingly, the *T. cruzi* genome does not contain an obvious LIT1 ortholog, raising the possibility that this ferrous iron transporter represents a specific *Leishmania* adaptation to the low-iron environment of phagolysosomes. Mutations in the lysosomal iron efflux pump NRAMP1 confer susceptibility to *Leishmania* and other intravacuolar pathogens (17, 18), reinforcing the conclusion that *Leishmania* needs a high-affinity transporter such as LIT1 to compete effectively for iron within its parasitophorous vacuole (16).

**Ironing out the details**

So how does *T. cruzi* acquire iron during its replicative phase in the host cell cytosol? This question remains to be answered, but given their cytosolic location and apparent lack of a high-affinity Fe²⁺ transporter, these parasites may be particularly dependent on the intracellular labile iron pool, defined as a transitory pool of redox-active iron complexes (19). The findings of Paiva et al. support a scenario whereby parasite molecules: the Fe³⁺ reductase LFR1 (15) and the LIT1 transporter, which directly promotes ferrous iron (Fe²⁺) uptake (16). Intriguingly, the *T. cruzi* genome does not contain an obvious LIT1 ortholog, raising the possibility that this ferrous iron transporter represents a specific *Leishmania* adaptation to the low-iron environment of phagolysosomes. Mutations in the lysosomal iron efflux pump NRAMP1 confer susceptibility to *Leishmania* and other intravacuolar pathogens (17, 18), reinforcing the conclusion that *Leishmania* needs a high-affinity transporter such as LIT1 to compete effectively for iron within its parasitophorous vacuole (16).

**The challenge of finding iron inside cells**

Paiva et al. found that induction of antioxidant responses reduced *T. cruzi* burden in macrophages, but not in other cell types — suggesting that a macrophage-specific mechanism was at play (1). This is significant because macrophages play an important role in vivo as iron stores, which are mobilized and maintained through regulated expression of a macrophage-specific iron exporter, ferroportin (8). The antioxidant response regulator NRF2 upregulates expression of ferroportin and also of ferritin, the protein responsible for cytosolic storage of iron in a redox-inert form (9, 10). Increased levels of ferroportin and ferritin are expected to reduce the levels of iron available for intracellular pathogens, suggesting that this pathway could be the basis for the surprising effect of antioxidants in inhibiting *T. cruzi* infection.

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Conversely, ROS generation may help *T. cruzi* thrive in an environment of high oxidative stress by allowing superoxide-oxidized iron release from ferritin, and/or peroxide-mediated iron extraction from heme and/or iron-sulfur cluster proteins (ref. 19 and Figure 1). Importantly, in this scenario the stimulation of *T. cruzi* growth by oxidative stress would not be restricted to macrophages, since a persistent oxidative environment can be generated by the parasites in other cell types, such as cardiomyocytes (7). It will be very interesting to determine whether *Leishmania* parasites, which are closely related to *T. cruzi* but replicate in the endocytic compartment of macrophages, also fare better under oxidative stress. Such studies may provide an answer to the fascinating cell biological question of whether residence in the cytosol or within a membrane-bound compartment has a differential impact on the access of pathogens to iron, on the specific iron pools utilized, and on the consequences of oxidative stress.

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