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# Oxidative stress and intracellular infections: more iron to the fire

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**Commentary**

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.

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shadow diet-induced obesity in genetically identical mice. *PLoS Genet*. 2006;2(5):e81.
11. Lagathu C, et al. Dact1, a nutritionally regulated preadipocyte gene, controls adipogenesis by coordinating the Wnt/beta-catenin signaling network. *Diabetes*. 2009;58(3):609–619.
12. Nielsen R, et al. Genome-wide profiling of PPARgamma:RXR and RNA polymerase II occupancy reveals temporal activation of distinct metabolic pathways and changes in RXR dimer composition during adipogenesis. *Genes Dev*. 2008; 22(21):2953–2967.
13. Siersbaek R, et al. Extensive chromatin remodelling and establishment of transcription factor 'hotspots' during early adipogenesis. *EMBO J*. 2011; 30(8):1459–1472.
14. Quwailid MM, et al. A gene-driven ENU-based approach to generating an allelic series in any gene. *Mamm Genome*. 2004;15(8):585–591.
15. Satoh W, Gotoh T, Tsunematsu Y, Aizawa S, Shimono A. Sfrp1 and Sfrp2 regulate anteroposterior axis elongation and somite segmentation during mouse embryogenesis. *Development*. 2006; 133(6):989–999.
16. Okamura M, et al. COUP-TFII acts downstream of Wnt/beta-catenin signal to silence PPARgamma gene expression and repress adipogenesis. *Proc Natl Acad Sci U S A*. 2009;106(14):5819–5824.
17. Solinas G, et al. JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. *Cell Metab*. 2007;6(5):386–397.
18. Li Y, Rankin SA, Sinner D, Kenny AP, Krieg PA, Zorn AM. Sfrp5 coordinates foregut specification and morphogenesis by antagonizing both canonical and noncanonical Wnt11 signaling. *Genes Dev*. 2008;22(21):3050–3063.
19. Scott MS, Barton GJ. Probabilistic prediction and ranking of human protein-protein interactions. *BMC Bioinformatics*. 2007;8:239.
20. McDowall MD, Scott MS, Barton GJ. PIPs: human protein-protein interaction prediction database. *Nucleic Acids Res*. 2009;37(database issue):D651–D656.
21. Grumolato L, et al. Canonical and noncanonical Wnts use a common mechanism to activate completely unrelated coreceptors. *Genes Dev*. 2010; 24(22):2517–2530.
22. Bovolenta P, Esteve P, Ruiz JM, Cisneros E, Lopez-Rios J. Beyond Wnt inhibition: new functions of secreted Frizzled-related proteins in development and disease. *J Cell Sci*. 2008;121(pt 6):737–746.

# 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 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 to established dogma are usually slow to emerge, but this detailed *T. cruzi* study raises the intriguing possibility that the deleterious effects of antioxidants on intracellular pathogens may occur by a mechanism fundamentally different from classical innate or adaptive immune responses.

## The role of oxidative stress

This insight came from experiments wherein mice and mouse-derived 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 prooxidant 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 was still able to reduce the very high *T. cruzi* load that is observed in iNOS-deficient mice. 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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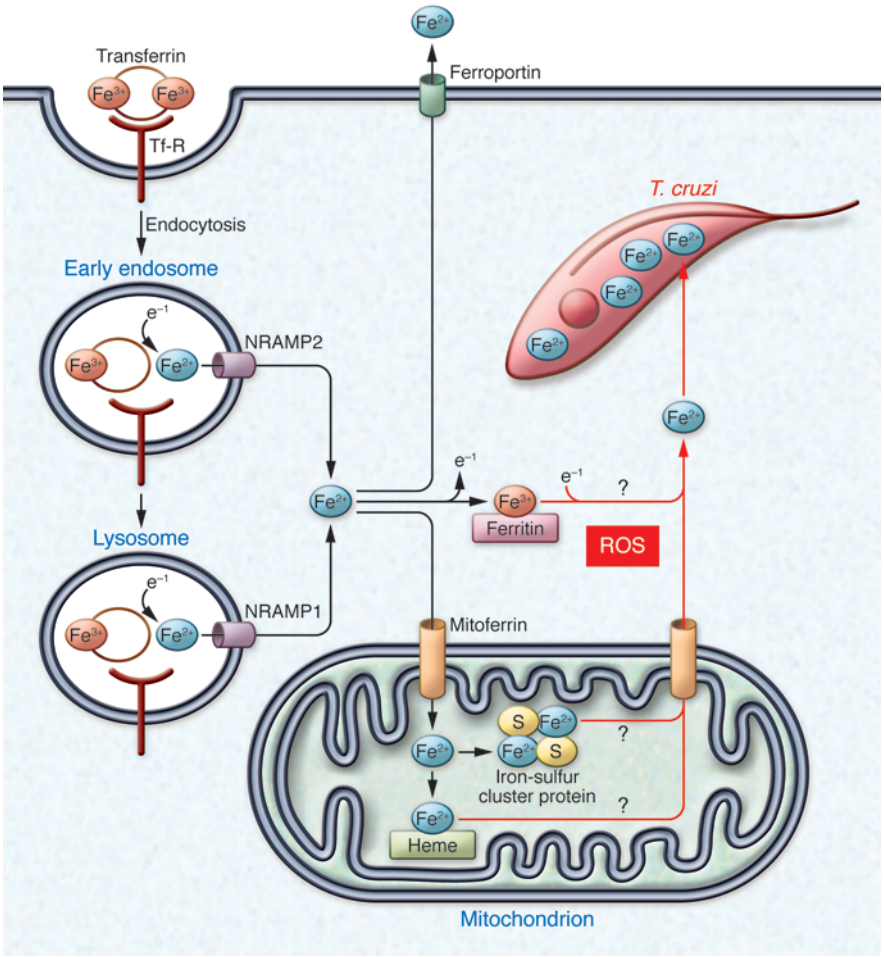


**Figure 1**
Increase in iron availability: an unexpected benefit of ROS for intracellular pathogens. Ferric iron ($Fe^{3+}$) enters mammalian cells complexed to transferrin, and after reduction to the ferrous form ($Fe^{2+}$), it is translocated to the cytosol by endosome transporters. $Fe^{2+}$ is rapidly utilized for metabolic pathways such as heme and iron-sulfur cluster protein biosynthesis or oxidized to $Fe^{3+}$ and stored as a complex with ferritin. This scenario makes access to available iron very challenging for intracellular pathogens. Paiva et al. show that oxidative stress in macrophages promotes the intracellular growth of *T. cruzi*, by a mechanism that may involve releasing $Fe^{2+}$ from ferritin, or from heme and/or iron-sulfur cluster proteins. Tf-R indicate transferrin receptor.

transport. The recurring tissue injuries that are generated in this environment were proposed to contribute to the progression of Chagas disease pathology (7). The findings of Paiva et al. now suggest that the persistent oxidative environment generated in cardiomyocytes may not only explain the heart pathology, but may also be self-perpetuating by directly promoting *T. cruzi* replication (1).

## 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.

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 ($Fe^{3+}$) for the sequential action of two surface-associated parasite molecules: the $Fe^{3+}$ reductase LFR1 (15) and the LIT1 transporter, which directly promotes ferrous iron ($Fe^{2+}$) 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 $Fe^{2+}$ 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 growth in macrophages is inhibited by depletion of the labile iron pool, as a consequence of NRF- and HO-1–mediated upregulation of ferroportin and ferritin, which cause efflux of iron and its cytosolic sequestration in a redox-inactive form.

Conversely, ROS generation may help *T. cruzi* thrive in an environment of high oxidative stress by allowing superoxide-mediated 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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1. Paiva CN, et al. Oxidative stress fuels *Trypanosoma cruzi* infection in mice. *J Clin Invest*. 2012; 122(7):2531–2542.
2. Poss KD, Tonegawa S. Reduced stress defense in heme oxygenase 1-deficient cells. *Proc Natl Acad Sci U S A*. 1997;94(20):10925–10930.
3. Muller S, Liebau E, Walter RD, Krauth-Siegel RL. Thiol-based redox metabolism of protozoan parasites. *Trends Parasitol*. 2003;19(7):320–328.
4. Andrade LO, Andrews NW. The Trypanosoma cruzi-host-cell interplay: location, invasion, retention. *Nat Rev Microbiol*. 2005;3(10):819–823.
5. Vago AR, et al. Kinetoplast DNA signatures of Trypanosoma cruzi strains obtained directly from infected tissues. *Am J Pathol*. 1996;149(6):2153–2159.
6. Tarleton RL, Zhang L, Downs MO. "Autoimmune rejection" of neonatal heart transplants in experimental Chagas disease is a parasite-specific response to infected host tissue. *Proc Natl Acad Sci U S A*. 1997;94(8):3932–3937.
7. Gupta S, Dhiman M, Wen JJ, Garg NJ. ROS signalling of inflammatory cytokines during Trypanosoma cruzi infection. *Adv Parasitol*. 2011;76:153–170.
8. Kaplan J. Mechanisms of cellular iron acquisition: another iron in the fire. *Cell*. 2002;111(5):603–606.
9. Marro S, et al. Heme controls ferroportin1 (FPN1) transcription involving Bach1, Nrf2 and a MARE/ARE sequence motif at position -7007 of the FPN1 promoter. *Haematologica*. 2010;95(8):1261–1268.
10. Hintze KJ, Theil EC. DNA and mRNA elements with complementary responses to hemin, antioxidant inducers, and iron control ferritin-L expression. *Proc Natl Acad Sci U S A*. 2005;102(42):15048–15052.
11. Taylor MC, Kelly JM. Iron metabolism in trypanosomatids, and its crucial role in infection. *Parasitology*. 2010;137(6):899–917.
12. Lima MF, Villalta F. Trypanosoma cruzi receptors for human transferrin and their role. *Mol Biochem Parasitol*. 1990;38(2):245–252.
13. Wilson ME, Britigan BE. Iron acquisition by parasitic protozoa. *Parasitology Today*. 1998;14(9):348–353.
14. Borges VM, Vannier-Santos MA, de Souza W. Subverted transferrin trafficking in Leishmania-infected macrophages. *Parasitol Res*. 1998;84(10):811–822.
15. Flannery AR, Huynh C, Mittra B, Mortara RA, Andrews NW. LFR1 ferric iron reductase of Leishmania amazonensis is essential for the generation of infective parasite forms. *J Biol Chem*. 2011; 286(26):23266–23279.
16. Huynh C, Sacks DL, Andrews NW. A Leishmania amazonensis ZIP family iron transporter is essential for parasite replication within macrophage phagolysosomes. *J Exp Med*. 2006;203(10):2363–2375.
17. Blackwell JM, et al. SLC11A1 (formerly NRAMP1) and disease resistance. *Cell Microbiol*. 2001;3(12):773–784.
18. Marquis JF, Gros P. Intracellular Leishmania: your iron or mine? *Trends Microbiol*. 2007;15(3):93–95.
19. Kakhlon O, Cabantchik ZI. The labile iron pool: characterization, measurement, and participation in cellular processes(1). *Free Radic Biol Med*. 2002;33(8):1037–1046.

# Fibroblast growth factor 23: friend or foe in uremia?

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**Uremia is a complex metabolic state marked by derangement of many signaling molecules and metabolic intermediates; of these, the massively increased levels of FGF23 are among the most striking. It has remained unclear whether FGF23 is directly implicated in the pathogenesis of chronic kidney disease (CKD) and its complications, a consequence of other dysregulated pathways, or perhaps an adaptive — and thus desirable — response. In this issue of the *JCI*, Shalhoub et al. describe the chronic effects of antibody-mediated FGF23 neutralization in a CKD mouse model, shedding new light on this complicated story and moving us one step closer to understanding the role of FGF23 in CKD.**

Chronic kidney disease (CKD) is a global health problem that has a grave impact on morbidity and mortality rates. An extensive treatise published by the National Kidney Foundation in 1998 pointed out the sobering reality that a dialysis patient in his or her twenties has the same risk of dying as an 80-year-old person not on dialysis (1). Rather regrettably, the situation has not improved dramatically in the interceding years despite numerous efforts to understand the pathophysiology and improve treatment of CKD. Renal replacement therapy and organ transplant are undoubtedly life-saving implementations, but these options are reserved for end-stage renal failure and harbor their own limitations; dialysis is restricted to clearance of a limited group of molecules and therefore not a true "renal replacement," and organ transplant is complicated by the requirement for universal suppression of the immune system. How can one devise additional strategies to significantly improve both life span and quality of life of patients with CKD?

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