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INSIGHTS

Die another way: Ferroptosis drives tuberculosis pathology

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In this issue of *JEM*, Amaral et al. (https://doi.org/10.1084/jem.20181776) provide the first evidence that ferroptosis, a newly described form of regulated cell death, is detrimental for the host during a *Mycobacterium tuberculosis* infection. This finding has important implications for the development of host-directed therapies for tuberculosis.

Ferroptosis is a regulated form of cell death that exhibits features of both apoptosis and necrosis (Dixon, 2017). This form of cell death was first characterized in 2012 by Dixon et al., who studied the toxic effects of various molecules on Ras mutant tumor cells, namely the RAS-selective lethal compounds (Dixon et al., 2012). RAS-selective lethal compound–triggered cell death was found to involve either iron overload–driven dysregulated ROS-dependent lipid peroxidation and/or a lack of oxidized lipid removal by the glutathione-dependent antioxidant enzyme glutathione peroxidase 4 (GPX4). This promotes plasma membrane destabilization and subsequent so-called "ferroptosis" (Cao and Dixon, 2016). Several studies have evaluated the regulatory pathways controlling ferroptosis and the role of ferroptosis in various physiological and pathological settings, including cancer, diabetes, neurodegenerative diseases (e.g., Huntington's, Alzheimer's, and Parkinson's diseases), kidney and liver failures, as well as, very recently, the maintenance of *Pseudomonas aeruginosa* biofilms in vitro (Cao and Dixon, 2016; Dar et al., 2018). Yet, the immune role of ferroptosis during microbial infection in vivo has remained unknown so far.

In this pioneering study, Amaral et al. hypothesized that ferroptosis might play a major role during *Mycobacterium tuberculosis* infections for two reasons. First, iron is an essential component for successful infection by the tuberculosis (TB) bacillus; second, in vivo *M. tuberculosis* infections induce the appearance of necrotic lesions of uncharacterized origin (Rodriguez, 2006; Belton et al., 2016). Consequently, Amaral et al. (2019) found that macrophage infection by *M. tuberculosis* promotes labile iron accumulation as well as lipid peroxidation, which drives cell necrosis. In addition, the authors identified that macrophages dying of necrosis have high oxidized lipids contents and low expression of the GPX4 enzyme, which is involved in lipid peroxide removal. Conversely, using the ferroptosis inhibitor ferrostatin-1, which inhibits lipid peroxidation, or the iron chelator pyridoxal isonicotinoyl hydrazone, the authors showed that macrophage necrosis during *M. tuberculosis* infection was inhibited. Together, these results encouraged the authors to characterize *M. tuberculosis*–induced macrophage necrosis as ferroptosis. Regarding the aforementioned pathways by which ferroptosis can occur in cells, the authors' findings nicely fit with the importance of both iron overload and lipid peroxidation as drivers of ferroptotic cell death. Macrophages are a well-known cell target for *M. tuberculosis* (McClean and Tobin, 2016), yet whether other phagocytic and nonphagocytic cells can also undergo ferroptosis upon *M. tuberculosis* infection will warrant further work. Given that neutrophils can undergo NETosis upon ROS accumulation (Braian et al., 2013; Francis et al., 2014), it is tempting to speculate that both ferroptosis and NETosis might have some common regulators, including lipid peroxidation and/or the GPX4 enzyme. Regarding this latter possibility, a recent study revealed that GPX4-inhibited lipid peroxidation also repressed caspase-1– and caspase-11–driven macrophage pyroptosis, another form of regulated necrosis (Kang et al., 2018). Another burning question relies on the molecular and cellular mechanisms by which *M. tuberculosis* triggered macrophage ferroptosis. The findings by Amaral et al. (2019) about the decreased levels of GPX4 and iron accumulation suggest a virulence mechanism driven by *M. tuberculosis*. Whether one or several microbial factors influence either *Gpx4*/GPX4 expression/activity levels and/or iron accumulation will constitute future exciting research avenues. Regarding the host regulation of iron metabolism, a recent work described that ferritin deficiency led to iron overload, hence promoting *M. tuberculosis* growth, dissemination, and death of infected mice (Reddy et al., 2018). Importantly, ferritin degradation through the lysosomal and autophagic pathways (ferritinophagy) has been characterized as a strong ferroptosis trigger (Mancias et al., 2014; Tang et al., 2018). Indeed, while ferritin allows iron storage in vesicles, its degradation induces labile iron release and accumulation into the host cell cytosol, a process required for ferroptosis induction. Should ferritinophagy be a cellular process by which *M. tuberculosis* promotes macrophage

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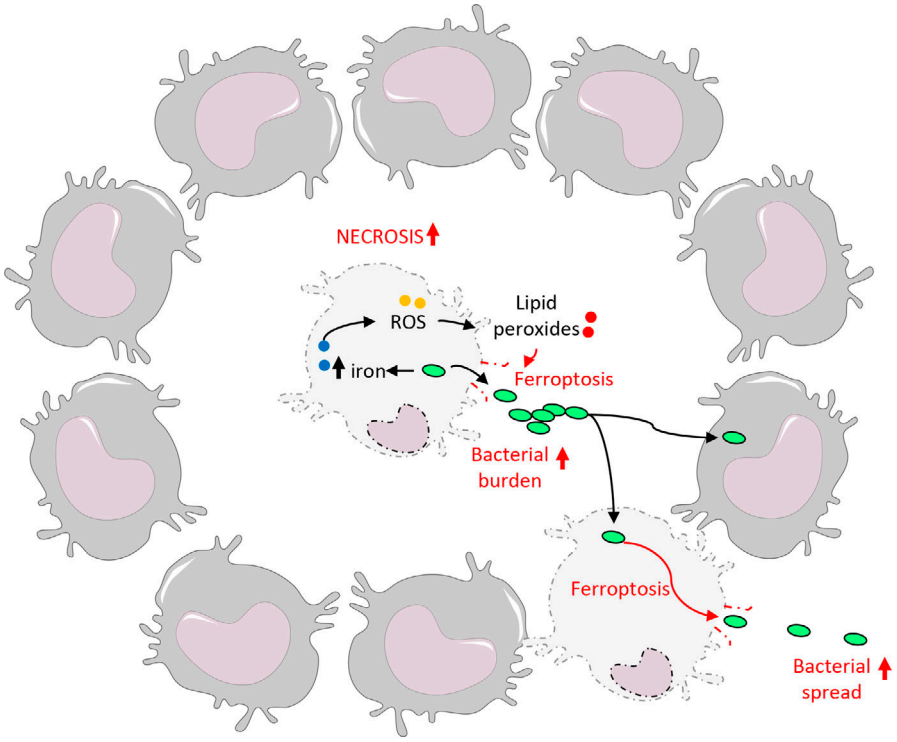
ferroptosis, it would be of great interest in future researches.

To further evaluate whether ferroptosis occurs in vivo and plays a part in immunity to *M. tuberculosis* or TB pathology, Amaral et al. (2019) used a mouse model of *M. tuberculosis* infection. Remarkably, they found that macrophages specifically expressed decreased levels of *Gpx4*, which correlated with an accumulation of lipid peroxides. Importantly, in vivo lipid peroxidation accumulation was inhibited by ferrostatin-1, confirming that this process occurred in a ferroptosis-dependent manner. Furthermore, ferrostatin-1 also suppressed *M. tuberculosis*–induced tissue necrosis after 28 d of infection. Strikingly, ferrostatin-1 also led to a strong decrease in lung bacterial burden, showing the central role of ferroptosis on *M. tuberculosis* growth. In addition, ferrostatin-1 reduced spleen bacterial levels, which suggests that ferroptosis might also promote *M. tuberculosis* dissemination.

In vivo, the severity of TB pathology has been correlated with the appearance of hypoxic and necrotic granulomas, which are thought to allow *M. tuberculosis* extracellular proliferation and spread (Belton et al., 2016). Eukaryotic cells express ancient and conserved response mechanisms to adapt to hypoxia. Specifically, the hypoxia-inducible factors (HIF) 1 and 2 are heterodimeric (subunits α and β) sensors of oxygen levels (Speer et al., 2013; Belton et al., 2016). In normoxic conditions, prolyl hydroxylase domain enzymes (PHDs) hydroxylate the α subunit of HIF proteins, which drives their degradation by the proteasome. Hypoxia decreases the activity of PHDs, which stabilizes HIF complexes (subunits α and β association) and the subsequent transcription of specific genes, including oxygen-detoxifying enzymes (Speer et al., 2013). In particular, a recent study demonstrated that inhibition of PHD enzymes sensitizes cells to oxygen-induced ferroptosis in a HIF2-dependent manner (Siddiq et al., 2009). Thus, in the context of the hypoxic TB granulomas, PHD–HIF complexes might play a key part in modulating ferroptosis, which remains to be studied. Interestingly, a mouse model of virulent *Mycobacterium avium* infection revealed that HIF1α deficiency in macrophages accelerated necrotic lesions in the granuloma (Cardoso et al., 2015).

Beyond TB, the study by Amaral et al. (2019) paves the way to the identification of additional microbial pathogens and compounds able to induce or modulate ferroptosis. Strikingly, a recently published study showed that the opportunistic bacterium *P. aeruginosa* also triggers ferroptosis in bronchial epithelial cells in vitro, although the clinical importance of this phenomenon remains to be evaluated (Dar et al., 2018). Importantly, this process requires a secreted lipoxygenase (pLoxA) expressed by *P. aeruginosa* during biofilm development. The pLoxA lipoxygenase uses host cell–derived arachidonic acid to generate 15-hydroperoxy-arachidonic/adrenic phosphatidylethanolamines (15-HOO-AA-PE), which promote ferroptosis. This elegant study suggested that several microbial species might use different means to modulate ferroptosis. Both studies by Amaral et al. (2019) and Dar et al. (2018) point toward a detrimental role for ferroptosis during infection. However, ferroptosis is an ancient and phylogenetically conserved form of cell death (Dixon et al., 2012; Cao and Dixon, 2016; Dixon, 2017). Whether it plays a beneficial role in other physiological or pathological settings remains to be explored.



Ferroptosis drives tuberculosis pathology. Upon macrophage infection by *M. tuberculosis*, increased levels of labile iron promote ROS-dependent lipid peroxidation. Accumulation of lipid peroxides induces plasma membrane destabilization, leading to ferroptosis-mediated cell death. Ferroptosis drives tissue necrosis and allows *M. tuberculosis* to thrive and spread, probably due to iron availability and the lack of an efficient macrophage antimicrobial immune response.

The findings by Amaral et al. (2019) were obtained in mice; whether they apply to human TB will need to be considered. Previous clinical studies showed lipid peroxides accumulate in the blood of TB patients. The use of ferroptosis inhibitors might constitute novel host-directed therapy to reduce lipid peroxide accumulation and possibly TB pathology in patients, as suggested by a study exploring the effect of vitamin E, which has anti-ferroptosis effects (Hinman et al., 2018), on TB outcome (Seyedrezazadeh et al., 2008).

For a long time, it has been thought that necrosis might only be due to an unregulated effect of cell overstimulation (Galluzzi et al., 2018). The discovery of genetically regulated necrosis such as gasdermin-driven pyroptosis or necroptosis has made novel paradigms emerge. With the rise of ferroptosis, a lipid peroxidation-dependent form of necrosis, a novel scientific barrier was breached (Dixon et al., 2012). Finally, the study by Amaral et al. (2019) reveals a yet unappreciated role for ferroptosis in *M.*

tuberculosis infection and TB pathology. This work opens new and promising avenues for the development of novel therapeutics against TB and possibly other infectious diseases by targeting ferroptosis and its regulatory components.

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