TITLE: *Coxiella burnetii* effector MceF employs the host protein GPX4 to protect mitochondria against oxidative stress-induced cell death pathways

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ABSTRACT*: Coxiella burnetii* is the causative agent of Q fever in humans. The bacterium is highly adapted to infect alveolar macrophages and subvert their functions. Its virulence relies on the translocation of effector proteins into the host cytoplasm through the Dot/Icm type 4 secretion system (T4SS). Collectively, these effectors facilitate the formation of a spacious vacuole that supports bacterial replication. Similarly, *Legionella pneumophila* also relies on a Dot/Icm T4SS, and previous studies have demonstrated that *L. pneumophila* can translocate *C. burnetii* effectors through this T4SS. Therefore, we have used *L. pneumophila* to individually express *C. burnetii* effectors and monitor their impact on pathways normally activated during *L. pneumophila* infection. After screening 70 effectors in *L. pneumophila*, we identified one effector that was modulating cell death pathways, cytokine production, and facilitating intracellular replication in BMDMs. The ectopic expression of this effector in epithelial cells showed its mitochondrial inner membrane localization. Expression of the Mitochondrial *Coxiella* effector protein F (MceF) in epithelial cells protected membrane integrity and enhanced mitochondrial functions. Proteomic analysis demonstrated that MceF increases the abundance of antioxidant proteins and we successfully affinity-purified Glutathione Peroxidase 4 (GPX4) from cells expressing MceF. In accordance, mitochondria isolated from epithelial cells expressing MceF showed increased mitochondrial localization of GPX4, and BMDM GPX4 CRISPR-cas9 KO cells lost the MceF protective effect during *L. pneumophila* infection. Importantly, overexpression of MceF during THP-1 infection protected against rotenone-induced cell death and decreased activation of caspases 1 and 3. Finally, we genetically engineered a clean deletion of the MceF encoding gene, which led to a more virulent phenotype than the WT in *Galleria mellonella*. Thus, we identified a unique bacterial effector that relocates host GPX4, which contributes to protect mitochondria against oxidative damage, and therefore, enhances oxidative phosphorylation capacity. Simultaneously, GPX4 relocation protects host cells against apoptosis, pyroptosis, and for the first time, ferroptosis. This study accounts for understanding how *C. burnetii* modulates leukocyte biology and provides insights for future targeting GPX4 to counteract bacterial infections or to modulate inflammatory processes during human diseases.

KEYWORDS: *Coxiella*. T4SS-effectors. Mitochondria. Cell death. GPX4.
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