Mitochondrial reactive oxygen species as major effectors of antimicrobial immunity

Elena Shekhova

Medical Research Council Centre for Medical Mycology at the University of Exeter, University of Exeter, Exeter, United Kingdom

*e.shekhoa@exeter.ac.uk

Reactive oxygen species play an important function in innate immune cells

Reactive oxygen species (ROS) are key weapons against pathogenic bacteria and fungi in the antimicrobial defense arsenal of host immunity. Innate immune cells, namely macrophages and neutrophils, release ROS as cytotoxic effectors that can irreversibly oxidize and thus damage cellular structures of the intruding pathogens. At the same time, ROS are important intracellular mediators that drive the appropriate antimicrobial responses and tune the inflammatory response. The best-recognized source of ROS in phagocytic cells is the NADPH oxidase (NOX) complex [1]. However, mitochondria also contribute to the enhanced ROS generation in these cells. This review focuses on the underappreciated but important roles of mitochondrial ROS (mitoROS) in antimicrobial immune defenses.

Mitochondria are one of two main sources of ROS in innate immune cells

ROS production in phagocytic cells is mainly mediated through the activity of the NOX complex. Upon pathogen recognition and engulfment, the NOX complex is formed within the phagosomal membranes, and it converts molecular oxygen into a highly reactive oxygen intermediate—superoxide [2]. Subsequently, other reactive intermediates can arise from NOX-derived superoxide depending on the pH levels, the presence of transitional metals, and other enzyme activities in activated phagocytes [3].

Remarkably, the levels of mitoROS rise when phagocytes encounter microbes [7]. Studies on murine macrophages point towards a specific mechanism responsible for the increased mitoROS in infected cells. Upon macrophage activation, mitochondrial conditions favor reverse electron transport in the ETC. The infection-associated increase in the activity of the mitochondrial complex II likely leads to over-reduction of coenzyme Q, which is one of the electron carriers in the ETC. Consequently, electrons from coenzyme Q travel to one of the active sites of complex I, where, in turn, oxygen accepts electrons and forms superoxide [8,9]. Superoxide in mitochondria can be further converted into other ROS such as hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) in a reaction mediated by mitochondrial superoxide dismutase (Sod) [5]. Evidently mitochondria contribute, along with NOX, to the increased production of ROS in immune cells.
cells during infection. Although mitochondrial generation of ROS in infected immune cells has been well documented both in vitro and in vivo, the exact underlying mechanisms that activate mitoROS production remain poorly defined.

**Increased mitoROS production is induced specifically in infected immune cells**

Sensing pathogens through pattern recognition receptors can trigger enhanced mitoROS production in immune cells. Once macrophages have recognized bacterial ligands via a subset of Toll-like receptors (TLRs) such as TLR1, TLR2, and TLR4, mitochondria are then recruited to the phagosomal membrane. The mammalian sterile 20-like kinases Mst1 and Mst2 are required for this juxtaposition of mitochondria and phagosome [10]. Meanwhile, the binding of tumor necrosis factor receptor-associated factor 6 (TRAF6) and a mitochondrial protein, evolutionarily conserved signaling intermediate in Toll pathways (ECSIT), promotes the increase in mitoROS production (Fig 1A) [11]. Interestingly, the TRAF6-ECSIT-dependent increase in mitoROS is required for oxidative killing of internalized *Salmonella typhimurium* by macrophages [11]. TLRs also influence the accumulation of mitoROS inside the phagosome via induction of mitochondria-derived vesicles. This happens when macrophages are challenged with *Staphylococcus aureus* [12]. In this scenario, endoplasmic reticulum (ER) stress induces the generation of mitochondrial vesicles containing Sod, which converts superoxide into H$_2$O$_2$ (Fig 1A) [12]. The functionality of TLR2/4/9 is required for these vesicles to accumulate inside the pathogen-containing phagosome, and this contributes to increased phagosomal concentrations of antibacterial H$_2$O$_2$.

TLR4 signaling is also linked to enhanced mitoROS generation, which in turn affects inflammation. This is observed in macrophages infected with *Escherichia coli*. Here, the increase in mitoROS is mediated by crosstalk between activated NOX and mitochondria (Fig 1B) [8]. Upon infection with live *E. coli*, NOX-derived ROS in macrophages react with the redox-sensitive Src-type tyrosine kinase, Fgr, which is activated in response to ROS exposure [8,13]. Fgr then increases the enzymatic activity of mitochondrial complex II, creating conditions for mitoROS production through reverse electron transport. The elevation of complex II activity, and thus the increase in mitoROS production, enhances accumulation of proinflammatory cytokine interleukin (IL)-1β, which in turn leads to the activation of the inflammatory program promoting bacterial killing [8]. This correlates well with the established notion that mitoROS positively regulate inflammasome formation and thus activate IL-1β [14].

Proinflammatory cytokines also increase mitoROS formation in infected immune cells. Cytokines such as interferon γ (IFNγ) and tumor necrosis factor (TNF) induce mitoROS production, which is essential for the elimination of *Listeria monocytogenes* and *Mycobacteria tuberculosis* [15–17]. However, the generation of mitoROS has to occur in a controlled manner to avoid host cell damage. Indeed, excessive TNF elevates levels of mitoROS and causes an overload of mitochondrial calcium in macrophages infected with *M. tuberculosis*. This ultimately results in the necrosis of macrophages and a release of bacteria to the extracellular environment, thereby exacerbating the infection [17].

Taken together, these findings show that mitoROS production can be induced specifically in infected immune cells. Triggering this system leads to the direct growth inhibition of the pathogen and the induction of inflammatory programs.

**Infection-associated redox reactions enable regulatory properties of ROS**

The direct antimicrobial function of ROS in immune cells is mainly accomplished by creating oxidative stress and damaging cellular components of invading pathogens. As described
above, signaling via mitoROS also contributes to antimicrobial immunity. However, little is known about the mechanisms behind the signaling properties of ROS, especially mitoROS, in infected immune cells. One possible mechanism is through the induction of oxidative modifications in regulatory proteins, which directly or indirectly mediate the synthesis of cytokines. For instance, in *L. monocytogenes*-infected macrophages, mitoROS are responsible for the formation of disulfide bridges in the NF-κB essential modulator (NEMO), which are required for dimerization of this protein (Fig 1B) [18]. Accordingly, the mitoROS-regulated formation of NEMO dimers induces a cascade of signaling reactions that subsequently leads to the secretion of proinflammatory cytokines, including IL-1β, TNFα, and IL-6 [18]. Therefore, mitoROS-mediated modifications of cellular regulators are involved in transforming macrophages into a proinflammatory state required to combat pathogens.
Induction of mitoROS in immune cells is required to resist infection

ROS modulate the antimicrobial functions of innate immune cells by inhibiting the growth of invading pathogens as well as regulating inflammatory responses. Disruption of ROS-mediated processes leads to the inability of the host to clear the pathogens. A pathology such as chronic granulomatous disease, which is associated with the reduced production of ROS through NOX, is characterized by increased susceptibility to bacterial and fungal infections [19,20]. Another human immunodeficiency syndrome, which occurs because of a mutation in the gene encoding Rac2, is characterized by impaired NOX-derived and mitoROS production by phagocytic cells and associated with severe bacterial infections [10,21]. The importance of mitoROS for the host to clear microbes has been also proven in mouse models of infection. Mice that are deficient in proteins responsible for the induction of mitoROS are highly susceptible to infections caused by *S. typhimurium* and *L. monocytogenes* [11,16]. Similar to NOX-derived ROS [22], mitoROS also impact the rate of cytokine production during infection and thus play an essential role in regulating inflammation in vivo [8,9].

Elevated levels of mitoROS may exhibit detrimental properties

Despite an essential role of mitoROS in antimicrobial responses of immune cells, mitoROS may also be responsible for damage of the host during infection. In particular, mitoROS may cause organ failure in several models of sepsis [23,24]. In corroboration, administration of specific inhibitors of mitoROS protect animals against organ damage in the lipopolysaccharide–peptidoglycan model of sepsis and lipopolysaccharide-induced endotoxemia [23,24] but do not exhibit a long-term beneficial effect in polymicrobial sepsis [25]. Moreover, mitoROS may contribute to exaggerated immune responses during viral infections such as infection with influenza A virus [26]. Accordingly, a pharmacological inhibitor of mitoROS, MitoTempo, can prevent lung inflammation and thus reduce mortality of mice infected with influenza A virus [26]. The harmful effects of mitoROS in these models might be associated with mitochondrial dysfunction, as well as impaired redox homeostasis. This possible correlation awaits further investigation.

Future perspective

Multiple studies have proven the important role of mitoROS for antimicrobial immunity. To translate this knowledge into therapeutic opportunities, further mechanistic insights into the mode of action of mitoROS are needed. ROS act by reacting with various molecules such as DNA, proteins, or lipids. Functions or localization of these redox-sensitive molecules might be altered because of exposure to ROS. For instance, oxidative protein modifications may activate or inhibit protein functions. Thus, to fully understand the impact of the increased mitoROS levels in infected immune cells, cellular targets of mitoROS such as redox-sensitive proteins need to be defined. Also, mitoROS may alter permeability of mitochondrial membranes and thus act through releasing mitochondrial components into other cellular compartments. Indeed, in activated immune cells, mitoROS production leads to membrane permeability transition (MPT) and a subsequent release of mitochondrial DNA into the cytosol, where it increases concentrations of IL-1β [27]. The mechanisms behind the activation of MPT by mitoROS in the context of infection require further investigation.

As discussed here, mitoROS are important against pathogenic bacteria, but their function against pathogenic fungi is unknown. Thus, more studies are required to explore whether mitoROS also play an important role in antifungal immunity. Finally, besides being major effectors of the innate immunity, mitoROS also contribute to the induction and regulation of adaptive immune responses. For example, mitoROS play an essential role in the processes of
antigen presentation by plasmacytoid dendritic cells [28]. Moreover, it is established that mitoROS levels affect T cell formation [29]. Currently, the exact molecular mechanisms by which mitoROS orchestrate adaptive immunity against pathogens remain largely unexplored.

Acknowledgments
I thank Professors Alistair Brown, Adilia Warris, and Gordon Brown for their useful suggestions.

References
1. Nathan C, Cunningham-Bussel A. Beyond oxidative stress: An immunologist’s guide to reactive oxygen species. Nature Reviews Immunology. 2013; 13(5): 349–361. https://doi.org/10.1038/nri3423 PMID: 23618831
2. Segal AW. How Neutrophils Kill Microbes. Annual Review of Immunology. 2005; 23: 197–223. https://doi.org/10.1146/annurev.immunol.23.021704.115653 PMID: 15771570
3. Piacenza L, Trujillo M, Raci R. Reactive species and pathogen antioxidant networks during phagocytosis. Journal of Experimental Medicine. 2019; 216(3): 501–516. https://doi.org/10.1084/jem.20181886 PMID: 30792185
4. Sena LA, Chandel NS. Physiological roles of mitochondrial reactive oxygen species. Molecular Cell. 2012; 48(2): 158–167. https://doi.org/10.1016/j.molcel.2012.09.025 PMID: 23102266
5. Murphy MP. How mitochondria produce reactive oxygen species. Biochemical Journal. 2009; 417(1): 1–13. https://doi.org/10.1042/BJ20081386 PMID: 19061483
6. Brand MD. The sites and topology of mitochondrial superoxide production. Experimental Gerontology. 2010; 45(7–8): 466–472. https://doi.org/10.1016/j.exger.2010.01.003 PMID: 20664600
7. Pinegin B, Vorobjeva N, Pashenkov M, Chernyakov B. The role of mitochondrial ROS in antibacterial immunity. Journal of Cellular Physiology. 2018; 233(5): 3745–3754. Epub 30 Aug 2017. https://doi.org/10.1002/jcp.26117 PMID: 28771715
8. Garaude J, Acín-Pérez R, Martínez-Cano S, Enamorado M, Ugolini M, Nistal-Vilalt E, et al. Mitochondrial respiratory-chain adaptations in macrophages contribute to antibacterial host defense. Nature Immunology. 2016; 17(9): 1037–1045. https://doi.org/10.1038/ni.3509 PMID: 27348412
9. Mills EL, Kelly B, Logan A, Costa ASH, Varma M, Bryant CE, et al. Succinate Dehydrogenase Supports Metabolic Reprogramming of Mitochondria to Drive Inflammatory Macrophages. Cell. 2016; 167(2): 457–470.e13. https://doi.org/10.1016/j.cell.2016.08.064 PMID: 27667687
10. West AP, Brodsky IE, Rahner C, Woo DK, Erdjument-Bromage H, Tempst P, et al. TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. Nature. 2011; 472(7344): 476–480. https://doi.org/10.1038/nature09973 PMID: 21525932
11. Roca FJ, Ramakrishnan L. TNF dually mediates resistance and susceptibility to mycobacteria via mitochondrial reactive oxygen species. Cell. 2013; 153(3): 521–534. https://doi.org/10.1016/j.cell.2013.03.022 PMID: 23582643
12. Sonoda J, Laganière J, Mehl IR, Barish GD, Chong LW, Li X, et al. Nuclear receptor ERRα and coactivator PGC-1α are effectors of IFN-γ-induced host defense. Genes Dev. 2007; 21(15): 1909–1920. https://doi.org/10.1101/gad.155307 PMID: 17671090
13. Roca FJ, Whitworth LJ, Redmond S, Jones AA, Ramakrishnan L. TNF Induces Pathogenic Programmed Macrophage Necrosis in Tuberculosis through a Mitochondrial-Lysosomal-Endoplasmic Reticulum Circuit. Cell. 2019; 178(6): 1344–1361.e11. https://doi.org/10.1016/j.cell.2019.08.004 PMID: 31474371
18. Herb M, Gluschko A, Wiegmann K, Farid A, Wolf A, Utermöhlen O, et al. Mitochondrial reactive oxygen species enable proinflammatory signaling through disulfide linkage of NEMO. Sci Signal. 2019; 12(568): eaar5926. https://doi.org/10.1126/scisignal.aar5926 PMID: 30755476

19. O’Neill S, Braut J, Stasia MJ, Knaus UG. Genetic disorders coupled to ROS deficiency. Redox Biology. 2015; 6: 135–156. https://doi.org/10.1016/j.redox.2015.07.009 PMID: 26210446

20. Warnatsch A, Ballou E. Oxidative responses and fungal infection biology. Seminars in Cell and Developmental Biology. 2018; 89: 34–46. https://doi.org/10.1016/j.semcdb.2018.03.004 PMID: 29522807

21. Gu Y, Jia B, Yang FC, D’Souza M, Harris CE, Derrow CW, et al. Biochemical and Biological Characterization of a Human Rac2 GTPase Mutant Associated with Phagocytic Immunodeficiency. J Biol Chem. 2001; 276(19):15929–15938. https://doi.org/10.1074/jbc.M010445200 PMID: 11278678

22. Warmatsch A, Tsourouktsoglou TD, Branzk N, Wang Q, Reincke S, Herbst S, et al. Reactive Oxygen Species Localization Programs Inflammation to Clear Microbes of Different Size. Immunity. 2017; 46(3): 421–432. https://doi.org/10.1016/j.immuni.2017.02.013 PMID: 28314592

23. Ramsey H, Wu MX. Mitochondrial anti-oxidant protects IEX-1 deficient mice from organ damage during endotoxemia. Int Immunopharmacol. 2014; 23(2): 658–663. https://doi.org/10.1016/j.intimp.2014.10.019 PMID: 25466275

24. Lowes DA, Thottakam BMV, Webster NR, Murphy MP, Galley HF. The mitochondria-targeted antioxidant MitoQ protects against organ damage in a lipopolysaccharide-peptidoglycan model of sepsis. Free Radic Biol Med. 2008; 45(11): 1559–1565. https://doi.org/10.1016/j.freeradbiomed.2008.09.003 PMID: 18845241

25. Rademann P, Weidinger A, Drechsler S, Meszaros A, Zipperle J, Jafarmadar M, et al. Mitochondria-targeted antioxidants SKQ1 and MitoTEMPO failed to exert a long-term beneficial effect in murine polymicrobial sepsis. Oxid Med Cell Longev. 2017; 2017: 6412682. https://doi.org/10.1155/2017/6412682 PMID: 29104729

26. To EE, Erlich JR, Liong F, Luong R, Liong S, Esaq F, et al. Mitochondrial Reactive Oxygen Species Contribute to Pathological Inflammation During Influenza A Virus Infection in Mice. Antioxid Redox Signal. 2020; 32(13): 929–942. Epub 12 Jul 2019. https://doi.org/10.1089/ars.2019.7727 PMID: 31190565

27. Nakahira K, Haspel JA, Rathinam VAK, Lee SJ, Dolinay T, Lam HC, et al. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol. 2011; 12(3): 222–230. Epub 12 Dec 2010. https://doi.org/10.1038/ni.1980 PMID: 21151103

28. Oberkampf M, Guillerey C, Mouriès J, Rosenbaum P, Fayolle C, Bobard A, et al. Mitochondrial reactive oxygen species regulate the induction of CD8+ T cells by plasmacytoid dendritic cells. Nat Commun. 2018; 9(1): 2241. https://doi.org/10.1038/s41467-018-04686-8 PMID: 29884262

29. Mehta MM, Weinberg SE, Chandel NS. Mitochondrial control of immunity: Beyond ATP. Nature Reviews Immunology. 2017; 17(10): 608–620. https://doi.org/10.1038/nri.2017.66 PMID: 28669986