JEM Journal of Experimental Medicine

REVIEW

Reactive species and pathogen antioxidant networks during phagocytosis

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The generation of phagosomal cytotoxic reactive species (i.e., free radicals and oxidants) by activated macrophages and neutrophils is a crucial process for the control of intracellular pathogens. The chemical nature of these species, the reactions they are involved in, and the subsequent effects are multifaceted and depend on several host- and pathogen-derived factors that influence their production rates and catabolism inside the phagosome. Pathogens rely on an intricate and synergistic antioxidant armamentarium that ensures their own survival by detoxifying reactive species. In this review, we discuss the generation, kinetics, and toxicity of reactive species generated in phagocytes, with a focus on the response of macrophages to internalized pathogens and concentrating on *Mycobacterium tuberculosis* and *Trypanosoma cruzi* as examples of bacterial and parasitic infection, respectively. The ability of pathogens to deal with host-derived reactive species largely depends on the competence of their antioxidant networks at the onset of invasion, which in turn can tilt the balance toward pathogen survival, proliferation, and virulence over redox-dependent control of infection.

Introduction

Activated macrophages and neutrophils play a key role in the innate immune response to infection by controlling pathogen proliferation and dissemination before antigen-specific adaptive immunity arises. As professional phagocytes, these cells use distinct mechanisms to recognize and engulf the invading agent. The resulting pathogen-containing phagosomal vacuole contains toxicants including reactive species (i.e., free radicals and oxidants), peroxidases, antimicrobial peptides, and hydrolases, which are delivered to the internalized pathogen. The chemical nature of the reactive species in the phagosome depends on the type of phagocytic cell and the presence of host-derived immune mediators at the onset of pathogen internalization. Thus, the term reactive species embodies a collection of free radicals and related oxidants with unique biochemical properties. In this review, we focus on redox processes involved in macrophage-mediated control of invading pathogens, with comparisons to processes in neutrophils where appropriate. We will address phagocyte generation of reactive species, including superoxide radicals ($O_2^{\bullet-}/HO_2^{\bullet}$), hydrogen peroxide ($H_2O_2$), hydroxyl radicals ($^{\bullet}OH$), nitric oxide ($^{\bullet}NO$), peroxynitrite ($ONOO^-/ONOOH$), and hypochlorous acid (HOCl), along with the armamentarium of pathogen-encoded detoxificants that combat host reactive species. Finally, we discuss how the interplay between host-derived reactive species and pathogen antioxidant networks influences the outcome of infection.

Reactive species generated by phagocytes

Superoxide radicals

Pathogen recognition and engulfment triggers the assembly and activation of macrophage NADPH oxidase, an enzyme complex comprising cytoplasmic regulatory components (p47phox and p67phox) and the membrane-bound $gp91^{phox}$-$gp22^{phox}$ heterodimeric catalytic subunit ($cytb_{558}$, or Nox2). Formation of NADPH oxidase leads to the univalent reduction of O_2 into $O_2^{\bullet-}$ radicals that directly damage the internalized pathogen (reviewed in DeCoursey, 2016). The reducing equivalents required for this reaction are delivered from NADPH, which is generated in the pentose phosphate pathway, allowing for $O_2^{\bullet-}$ generation that can last 10–90 min (Kamen et al., 2008; Alvarez et al., 2011; Tlili et al., 2011). As electrons flow from NADPH to O_2, protons move through voltage-gated proton channels, thereby sustaining NADPH oxidase activity (DeCoursey et al., 2001; DeCoursey, 2016). The phagocytic process in macrophages takes several minutes (Henry et al., 2004), and macrophages produce 10–20 times less $O_2^{\bullet-}$ than is produced by neutrophils. $O_2^{\bullet-}$ ($E^{\circ\prime}O_2/O_2^{\bullet-} = -0.33$ V) acts either as a univalent oxidant or a reductant (Reiss and Roos, 1978; Fridovich, 1995). The protonated form of $O_2^{\bullet-}$, perhydroxyl radical ($HO_2^{\bullet}$), is a weak acid ($pKa = 4.8$) and

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a more potent oxidant ($E^{\circ\prime}O_2^{\bullet-}$, $H^+/HO_2^{\bullet}$ = 1.0 V) compared with $O_2^{\bullet-}$, and its neutrality facilitates its diffusion across biological membranes. $HO_2^{\bullet}$ reacts rapidly with poly-unsaturated fatty acids (k ∼10^3 $M^{-1}s^{-1}$), initiating lipid peroxidation reactions (Bielski et al., 1983).

$O_2^{\bullet-}$ can dismutate to H_2O_2 and O_2 in a reaction that consumes hydrogen ions (H^+). At acidic pH, dismutation is rapid (k = 9.7 × 10^7 $M^{-1}s^{-1}$), but the rate constant of this reaction increases two orders of magnitude (k = 2 × 10^9 $M^{-1}s^{-1}$) in the presence of superoxide dismutases (SODs; Fridovich, 1995; Sheng et al., 2014). After pathogen engulfment, the pH of the macrophage phagosome decreases rapidly (10–30 min) to pH ≤5 due to the activity of V-ATPase, and this acidification is essential for pathogen killing. By contrast, the neutrophil phagosome is alkaline (pH ∼7.5–8.5) at early time points after pathogen uptake and drops to pH ∼6–6.5 after ∼1 h (Nordenfelt and Tapper, 2011). Thus, the relative $O_2^{\bullet-}/HO_2^{\bullet}$ concentration ratios and the relevance of their subsequent reactions vary with time as a function of the changing phagosomal pH. For instance, $O_2^{\bullet-}$ can oxidize iron–sulfur clusters (4Fe-4S; k = 10^6 to 10^7 $M^{-1}s^{-1}$) at the active site of dehydratases like aconitase (Gardner and Fridovich, 1991; Varghese et al., 2003) and fumarases (Liochev and Fridovich, 1992; Korshunov and Imlay, 2002), resulting in release of iron from the clusters and concomitant enzyme inactivation (Flint et al., 1993; Varghese et al., 2003). The consequences of the inactivation of these enzymes range from the disruption of the Krebs cycle to alterations of intermediary metabolism. As discussed later, $O_2^{\bullet-}$ reacts with $^{\bullet}NO$ in a diffusion-controlled manner, yielding peroxynitrite, a strong oxidant with potent antimicrobial activity (Zhu et al., 1992; Ferrer-Sueta et al., 2018).

Nitric oxide

Macrophages synthetize $^{\bullet}NO$ from L-arginine and O_2 in the presence of NADPH via a complex oxidoreductase reaction mediated by inducible nitric oxide synthase (iNOS). Immune- and pathogen-derived mediators, including TNF-α, IFN-γ, IL-1β, and bacterial LPS, activate iNOS and other related enzymes and transporters that together ensure potent (micromolar range) and sustained (hours) substrate availability for the production of $^{\bullet}NO$ (Nathan et al., 1983; Wu and Morris, 1998). iNOS has been localized to the cytosol, primary, and tertiary granules, as well as to the vicinity of macrophage and neutrophil phagosomes (Vodovotz et al., 1995; Evans et al., 1996). The majority of the work on iNOS has been done in murine macrophages due to the difficulties in obtaining and appropriately activating human macrophages in vitro (Fang, 2004; Nathan, 2006). Studies using activated human macrophages from patients with infections and/or inflammatory conditions demonstrated substantial person-to-person variability in $^{\bullet}NO$ output (Weinberg, 1998; Brito et al., 1999; Fang, 2004). Maximal iNOS protein expression in murine macrophages is achieved 4–5 h after stimulation with IFN-γ and LPS, with $^{\bullet}NO$ fluxes lasting for ∼24 h (Nathan and Hibbs, 1991; Alvarez et al., 2002, 2011).

$^{\bullet}NO$ is a relatively stable free radical and a poor one-electron oxidant ($E^{\circ\prime}$ $^{\bullet}NO,H^+/HNO$ = −0.8 V (Bartberger et al., 2002; Shafirovich and Lymar, 2002). The uncharged nature of $^{\bullet}NO$ allows diffusion to the phagosome, achieving micromolar concentrations. Although a weak oxidant, $^{\bullet}NO$ can react with biologically relevant radicals (e.g., $O_2^{\bullet-}$; $^{\bullet}NO_2$; thiyl, peroxyl, and tyrosyl radicals) with rate constants close to the diffusion-controlled limit (1–3 × 10^9 $M^{-1}s^{-1}$; Padmaja and Huie, 1993; Eiserich et al., 1995; Madej et al., 2008). $^{\bullet}NO$ can also react with and inhibit the heme-cytochrome C-terminal oxidase, initiating site-specific generation of $O_2^{\bullet-}$ and thus peroxynitrite, at the mitochondrial compartment, thereby impairing energy metabolism by the pathogen (Radi et al., 1994; Brown, 2001; Piacenza et al., 2009a; Estrada et al., 2018). Importantly, the cytostatic effects of $^{\bullet}NO$ on tumor cells and pathogens largely depend on the inhibition of ribonucleotide reductase (a crucial enzyme for DNA synthesis), achieved via reaction of $^{\bullet}NO$ with the catalytically essential and stable tyrosyl radical (Lepoivre et al., 1991; Nathan and Hibbs, 1991; Saleh et al., 2004).

Peroxynitrite

Peroxynitrite is produced by the diffusion-controlled reaction between $O_2^{\bullet-}$ and $^{\bullet}NO$ (k = 1 × 10^{10} $M^{-1}s^{-1}$; Beckman et al., 1990; Huie and Padmaja, 1993; Ferrer-Sueta et al., 2018), even in the presence of SODs (Radi, 2013a). Phagocytosis leads to activation of NADPH oxidase, and the resulting $O_2^{\bullet-}$ production in the presence of $^{\bullet}NO$ leads to peroxynitrite generation in the phagosome. Peroxynitrite is a strong oxidant and can promote one- or two-electron oxidations ($E^{\circ\prime}ONOO^-/^{\bullet}NO_2$ and $ONOO^-/NO_2^-$ = 1.4 and 1.2 V). Much of the cytotoxicity of $O_2^{\bullet-}$ and $^{\bullet}NO$ results from peroxynitrite formation, which impairs mitochondrial respiration and triggers cell death by oxidation- and nitration-mediated reactions (reviewed in Ferrer-Sueta et al., 2018). Peroxynitrite anion ($ONOO^-$) and its conjugated acid peroxynitrous acid (p*Ka* = 6.8) differ in stability, reactivity, and diffusion capacities; thus the biochemistry of peroxynitrite in biological systems is highly pH dependent (Denicola et al., 1998). $ONOO^-$ can react with carbon dioxide (CO_2 ∼1.2 mM, k = 5.8 × 10^4 $M^{-1}s^{-1}$) to yield a nitrosoperoxocarboxylate adduct ($ONOOCO_2^-$) that undergoes fast homolysis, yielding the potent oxidants $^{\bullet}NO_2$ and carbonate radical ($CO_3^{\bullet-}$; Denicola et al., 1996; Goldstein and Merényi, 2008). However, the small diffusion distances (<1 µm) required for reactive species to reach pathogens inside the phagosomes of macrophages ensure that $ONOO^-$ reaches the target before it can be consumed by its fast reaction with CO_2 (Alvarez et al., 2004). Nevertheless, the reaction of peroxynitrite with CO_2 becomes relevant inside the pathogen (Fig. 1 A). The $CO_3^{\bullet-}$ generated by this reaction can oxidize amino acid residues such as tyrosine to tyrosyl radical, with the latter rapidly combining with $^{\bullet}NO_2$ to generate protein-3-nitrotyrosine (Alvarez et al., 2007; Ferrer-Sueta et al., 2018), one of the molecular footprints of $^{\bullet}NO$-derived oxidants. This process leads to oxidative posttranslational protein modifications that can participate in the disruption of pathogen structure and metabolism and consequent killing (Ferrer-Sueta et al., 2018).

Peroxynitrite can also oxidize thiols (RSH) to their corresponding sulfenic acids (RSOH; Trujillo and Radi, 2002; Ferrer-Sueta et al., 2018) and can react with transition metals in proteins (e.g., Cu/Zn, Mn, and Fe-SODs), yielding the strongly

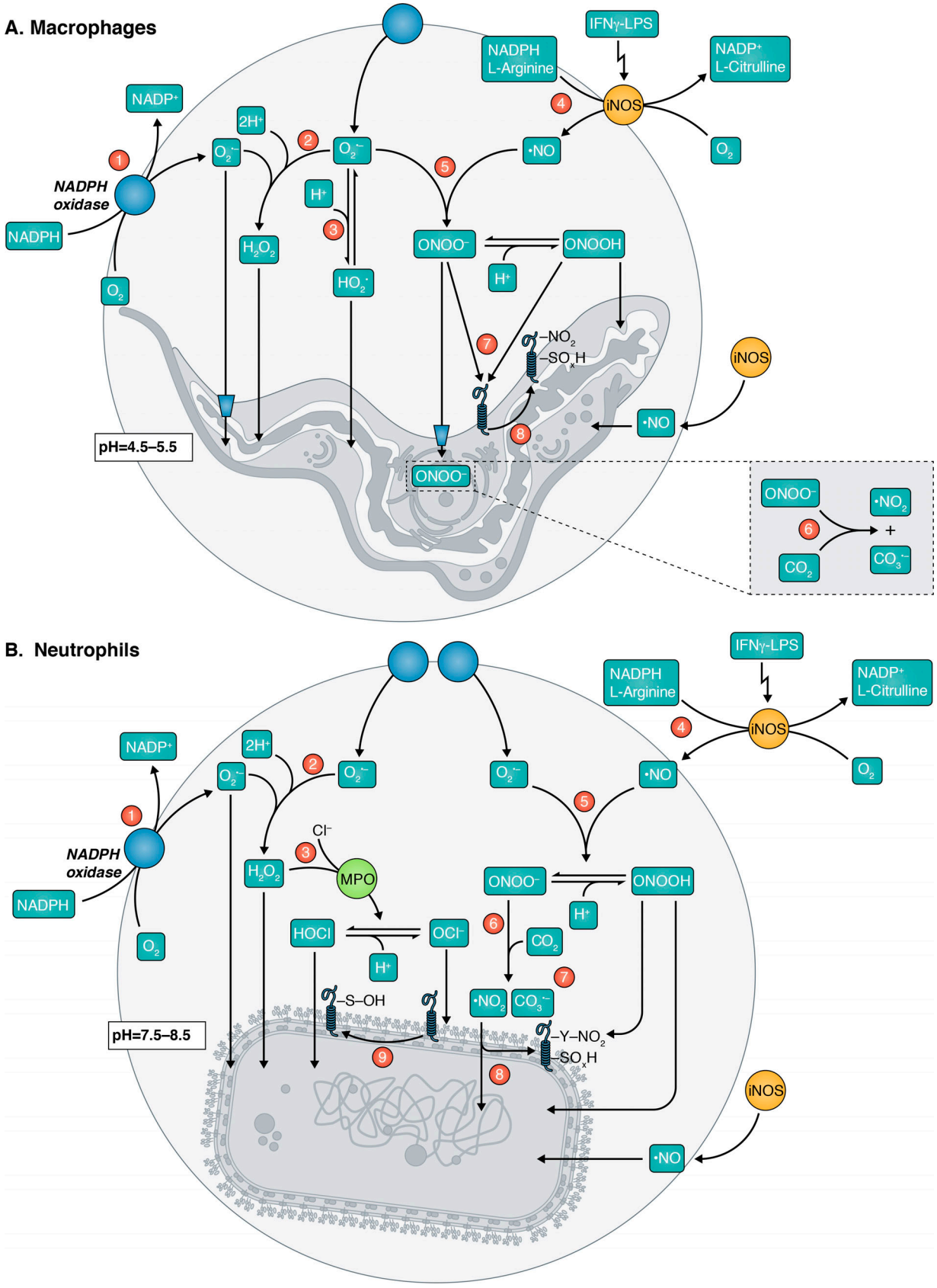

Figure 1. **Reactive species at the phagosome. (A)** In macrophages (phagosome pH ≤ 5), NADPH oxidase activation (blue sphere) leads to $O_2^{\bullet-}$ generation toward the phagocytic vacuole (1). $O_2^{\bullet-}$ dismutates to H_2O_2 (2) and/or protonates to generate $HO^{\bullet}_2$ radical (3). $O_2^{\bullet-}$ may reach the pathogen by means of anion channels, whereas both H_2O_2 and $HO_2^{\bullet}$ can diffuse across membranes. Immune-stimulated macrophages expressing iNOS (red sphere) produce •NO (4), which

diffuses to the phagosome while reacting fast with $O_2^{\bullet-}$ (5) to yield peroxynitrite anion ($ONOO^-$). $ONOO^-$ can protonate to peroxynitrous acid (ONOOH); it also permeates the parasite and reacts with CO_2 (6) to yield $^{\bullet}NO_2$ and $CO_3^{\bullet-}$ radicals (inset). Both $ONOO^-$ and ONOOH (7) promote the oxidation and nitration of membrane lipids and proteins (8). **(B)** In neutrophils (phagosome pH ≥ 7.5), MPO-derived HOCl is the dominant oxidant generated in this phagocyte (3) and promotes oxidation and chlorination reactions (9). Although the de novo production of $^{\bullet}NO$ (4) by human neutrophils has rarely been documented, $^{\bullet}NO$ may arise from exogenous sources existing in inflammatory foci and permeate the neutrophil plasma membrane. The other numbers in B denote the same processes described above for A.

oxidizing oxo-metal complex and $^{\bullet}NO_2$ that can lead to enzyme nitration and inactivation (Ferrer-Sueta and Radi, 2009). Peroxynitrite also reacts fast with Fe-S clusters, leading to Fe release and inactivation of proteins containing these clusters (Keyer and Imlay, 1997). In the phagosome, peroxynitrite reacts at the pathogen surface or diffuses into the pathogen cytosol, thereby promoting its toxic effects (Denicola et al., 1998). In addition, the secondary radicals arising from the reactions of peroxynitrite with CO_2 and transition metal centers ($^{\bullet}NO_2$, $CO_3^{\bullet-}$, oxo-metal complex and lipid-peroxyl-radicals) can promote protein tyrosine oxidation and nitration (Radi, 2013b). Peroxynitrite-dependent nitration of pathogen proteins in the phagosomal compartment has been observed in murine macrophages (Linares et al., 2001; Alvarez et al., 2011). The prolonged respiratory burst observed in immune-stimulated macrophages and the lack of significant myeloperoxidase (MPO) activity in these cells (Hampton et al., 1998; Alvarez et al., 2011) make the formation of peroxynitrite a critical pathway for host-derived pathogen cytotoxicity. The generation of peroxynitrite by human neutrophils and its role in pathogen killing was postulated based on early colocalization studies, which demonstrated the expression of iNOS and MPO in primary granules and the presence of nitrated *Staphylococcus aureus* and *Escherichia coli* in phagosomes (Evans et al., 1996; Wheeler et al., 1997).

Due to the experimental complications involved in obtaining highly purified neutrophil populations (Calzetti et al., 2017), together with the difficulties of in vitro immune stimulation of human phagocytes to generate iNOS expression, the extent of peroxynitrite generation in neutrophils remains elusive. It is important to note, however, that one study reported that only ∼20% of cytokine-stimulated neutrophils stained for iNOS, suggesting the existence of heterogeneous subpopulations of cells (Evans et al., 1996).

Hydrogen peroxide and hydroxyl radicals

H_2O_2 is generated either spontaneously or by SOD-dependent dismutation of $O_2^{\bullet-}$. It is a strong oxidant ($E^{\circ\prime}H_2O_2/H_2O$ = 1.77 V), but its reaction with most biomolecules is slow due to a high activation energy. Exceptions are Fe-S clusters present in dehydratases, heme-containing enzymes, and peroxidatic thiols (Winterbourn and Metodiewa, 1999; Trujillo et al., 2017). The p*Ka* of H_2O_2 is ∼11.6, and thus it will be mostly protonated at physiological pH. Both $O_2^{\bullet-}$ and H_2O_2 can release Fe from Fe-S clusters, and can generate $^{\bullet}OH$ via the Fenton reaction (Vasquez-Vivar et al., 2000). $^{\bullet}OH$ is the most reactive of the oxidants ($E^{\circ\prime}{}^{\bullet}OH/H_2O$ = 2.33 V) and reacts with myriad biomolecules. Conflicting evidence on the intra-phagosomal formation of $^{\bullet}OH$ in the absence of exogenously added metals has been reported (Britigan et al., 1986; Hampton et al., 1998). In view of its high reactivity, $^{\bullet}OH$ generated in the phagosome is unlikely to cause selective damage to internalized pathogens. The site-specific generation of $^{\bullet}OH$ has been proposed as the mechanism underlying DNA damage and toxicity resulting from release of intracellular Fe (which binds to negatively charged DNA; Dizdaroglu et al., 1991; Henle et al., 1999). A similar site-specific mechanism of $^{\bullet}OH$ generation was proposed for the *Bacillus subtilis* repressor factor PerR that becomes oxidatively inactivated secondary to the in situ metal-catalyzed histidine oxidation (Lee and Helmann, 2006).

Hypochlorous acid: A distinctive neutrophil-derived oxidant

HOCl is synthetized by the neutrophil MPO, a cationic heme-containing enzyme stored in the azurophilic granules and released into the phagosome and the extracellular media upon neutrophil activation. MPO reacts with H_2O_2 ($k = 2 \times 10^7\ M^{-1}s^{-1}$) and, in the presence of halide chloride, yields HOCl (p*Ka* = 7.4), a two-electron oxidant ($E^{\circ\prime}HOCl/Cl^-$ = 1.28 V) that can react with amines to yield the respective chloramines. Chloramines can diffuse into pathogens and react with enzyme thiolates with much higher selectivity than the more reactive HOCl; in this way, chloramines become longer lasting and more efficient HOCl-derived toxicants (Thomas et al., 1986; Winterbourn and Kettle, 2013). MPO can also oxidize tyrosine, ascorbic acid, and nitrite, among others, yielding their respective radicals. Amino acid–derived radicals react at diffusion-controlled rates with $O_2^{\bullet-}$ to form hydroperoxides with potential bactericidal activities (Winterbourn and Kettle, 2013). Preferred targets for HOCl are methionine and cysteine residues ($k \sim 10^7\ M^{-1}s^{-1}$), as well as tyrosines (Pattison and Davies, 2006; Winterbourn and Hampton, 2008). Tyrosine oxidation yields 3-chlorotyrosine, whereas methionine oxidation yields methionine sulfoxide (MetSO), which contributes to pathogen killing if not rapidly repaired (St John et al., 2001; Weissbach et al., 2002; Rosen et al., 2009).

Human and murine macrophages have very little or no MPO activity, and thus HOCl is not produced at significant levels by these cells in vitro (Hampton et al., 1998; Alvarez et al., 2011). By contrast, in vivo, macrophages engulf phagocytosis, thereby acquiring neutrophil MPO and other granule contents at the inflammatory foci (Silva et al., 1989; Silva, 2010).

Kinetics and toxicity of phagosomal reactive species in macrophages

The relevance of reactive species generated by phagocytes as a mechanism of the innate immune response to invading pathogens is illustrated in individuals with chronic granulomatosis disease, a disease characterized by recurrent bacterial and fungal infections (Fang, 2004; O'Neill et al., 2015). Mouse models of chronic granulomatosis disease have been described that

replicate the human disease (Jackson et al., 1995; Pollock et al., 1995). For example, mice lacking iNOS are more susceptible to various infections including *Mycobacterium tuberculosis* (*Mtb*), *Trypanosoma cruzi*, *Leishmania* spp., *Salmonella typhimurium*, and *Pseudomonas aeruginosa* (Hölscher et al., 1998; Nathan and Shiloh, 2000; Yu et al., 2000), although deficiencies in iNOS have not been described in humans. Mice lacking both NADPH oxidase and iNOS are highly susceptible to spontaneous infections arising from native flora and rapidly succumb to infection with virulent or even avirulent *S. typhimurium*, suggesting compensatory activities of NADPH oxidase and iNOS in the control of infection (Shiloh et al., 1999).

The nature of reactive species generated in the phagosome depends on the immune activation status, tissue localization, and oxygen concentration, and the composition of these reactive species is highly heterogeneous between individual phagosomes (Albrett et al., 2018; Fig. 1). The majority of studies of phagosomal reactive species have been performed at 21% O_2 (~200 µM). Tissue O_2 concentrations range from 15% (~150 µM) in the alveoli to 4% (~40 µM) in muscle, and thus phagocytes may respond differently in these different anatomical sites (Carreau et al., 2011). For neutrophil NADPH oxidase, the K_M (Michaelis constant) for O_2 is ~2–3% (20–30 µM); thus at 2–5% O_2, the enzyme function is at 45–70% of its maximum activity (Nisimoto et al., 2014). iNOS is more sensitive to O_2 concentration, and thus the K_M for O_2 is higher (~11% O_2, 110 µM; McCormick et al., 2000). Importantly, the stabilization of hypoxic inducible transcription factor (HIF-1) in response to infection and/or hypoxic conditions leads to an increase in the phagocyte's glycolytic capacity, glucose transport, pentose phosphate pathway activity, and iNOS expression, ensuring optimal O_2 utilization (Palazon et al., 2014).

Peroxynitrite is the most potent oxidant produced by immune-stimulated murine macrophages and is highly cytotoxic to many pathogens, including *E. coli* (Jiang and Hurst, 1997), *S. typhimurium* (De Groote et al., 1997), *Mtb* (St John et al., 2001), *P. aeruginosa* (Alegria et al., 2017), and *T. cruzi* (Gazzinelli et al., 1992; Denicola et al., 1993; Alvarez et al., 2011; Fig. 1 A). Due to the high reactivity and short biological half-life of peroxynitrite (~1–20 ms) and the radicals it generates ($^{\bullet}NO_2$, $CO_3^{\bullet-}$; in the µs range), unambiguous measurements of intraphagosomal peroxynitrite concentrations have been challenging. Optimal stimulation of murine macrophages leads to the extracellular formation of ~0.1–0.2 nmol peroxynitrite $min^{-1}/10^6$ cells, which translates into a phagosomal peroxynitrite production of 0.1–0.4 mM s^{-1}, in the small *T. cruzi*–containing vacuole (3–5 fl; Alvarez et al., 2002). A recent study assessed peroxynitrite generation in response to phagocytosed *T. cruzi*, using a fluorescein-based boronate-derived probe that takes advantage of the fast reaction of peroxynitrite with boronic-esters (Zielonka et al., 2012; Rios et al., 2016; Prolo et al., 2018). The study showed that the amount of peroxynitrite needed to kill *T. cruzi* (LD_{100}) was ~0.6 fmol/parasite; thus the amount of peroxynitrite generated during the time that the pathogen remains in the macrophage phagosome (90 min) is sufficient to kill the parasite. This conclusion was further supported by the complete disruption of parasite ultrastructure, along with the detection of protein tyrosine nitration and oxidation, inside the phagosome of immune-stimulated macrophages (Alvarez et al., 2004, 2011). The LD_{100} for peroxynitrite is much smaller than that reported for H_2O_2-dependent *T. cruzi* killing (50–100 fmol/parasite; Nathan et al., 1979; Tanaka et al., 1982), in agreement with the higher microbicidal activity of peroxynitrite.

Several pathogens, such *S. typhimurium* and *Mtb*, are protected against phagosomal $O_2^{\bullet-}$ and/or limit peroxynitrite generation by encoding periplasmic and/or membrane-bound SOD, demonstrating the importance of reactive species and the synergic activity of NADPH oxidase and iNOS in pathogen killing (De Groote et al., 1997; Piddington et al., 2001). Following oxidative burst, NADPH oxidase becomes deactivated, whereas $^{\bullet}NO$ generation is maintained for several hours (~24 h; Alvarez et al., 2002). In this situation, $^{\bullet}NO$ reactions inside the pathogen become central to host defense. The toxicity of $^{\bullet}NO$ to intracellular pathogens depends on its ability to diffuse and reach parasite targets, leading to energy imbalance, inhibition of DNA synthesis, S-nitrosothiol formation, protein oxidation, and ultimately pathogen death (Green et al., 1991; Fang, 1997; Vallance and Charles, 1998; St John et al., 2001; Piacenza et al., 2013).

A pivotal role for the IFN-γ/IL-12/iNOS axis as an immune effector mechanism against *T. cruzi* and *Leishmania* spp. infection was shown using mice lacking IFN-γ receptor and/or iNOS. These mice show enhanced susceptibility to infection and an inability to control parasite proliferation (Hölscher et al., 1998; Michailowsky et al., 2001). Moreover, H_2O_2 production was increased in iNOS-deficient macrophages, which are highly susceptible to *T. cruzi* infection, probably due to the lack of macrophage and/or intra-parasite peroxynitrite generation in the absence of $^{\bullet}NO$ (Hölscher et al., 1998; Shiloh et al., 1999). For comparative purposes, the formation of oxidants in the neutrophil phagosome is shown in Fig. 1 B; the $^{\bullet}NO$ pathway does not seem to be as relevant as in macrophages, and the formation of HOCl becomes a dominant process in neutrophil oxidative responses (reviewed in Hurst, 2012; Winterbourn and Kettle, 2013; Nauseef, 2014).

Pathogen virulence and antioxidant networks

Virulence is difficult to define and is one of a number of possible outcomes of host–pathogen interactions (Casadevall and Pirofski, 2001). For the purposes of this review, virulence is defined as the capacity of a pathogen to cause disease, while maintaining survival and rapid proliferation in the mammalian host. Different pathogenic unicellular microorganisms use specific enzymatic and nonenzymatic antioxidant strategies, and each pathogen has its own particular systems; nonetheless, the overall redox processes often share general characteristics, such as the presence of $O_2^{\bullet-}$, H_2O_2, and peroxynitrite, and the necessity for low-molecular-weight (LMW) thiols and thiol-based enzymatic systems, which supply reducing equivalents for peroxide catabolism via connections with intermediary metabolism (Staerck et al., 2017). Alternative mechanisms exist, such as the synthesis of melanin by certain fungi and bacteria that scavenges reactive species, inhibits phagocytosis, and increases virulence (reviewed in Nosanchuk and Casadevall, 2006).

We focus here on *Mtb* and *T. cruzi* as examples of intracellular bacteria and protozoa parasites, respectively, that preferentially invade macrophages. Both rely on a sophisticated network of enzymatic and nonenzymatic antioxidant defenses, allowing the pathogen to persist for years in the infected host. Intracellular redox homeostasis in the pathogen depends on the redox status of the LMW thiols that participate in myriad redox, antioxidant enzyme, and drug detoxification reactions. The reducing equivalents for these redox cascades are delivered from the pentose phosphate pathway and other sources such as NADP-linked dehydrogenases (including mitochondrial and cytosolic malic enzyme, isocitrate dehydrogenase, and transhydrogenases), ensuring reducing power in almost all host microenvironments (Leroux et al., 2011; Allmann et al., 2013). The pathogen's armamentarium of antioxidant detoxifying defenses includes SODs, peroxidases (i.e., heme dependent, thiol dependent), truncated hemoglobins (•NO dioxygenases [NODs]), and diverse LMW reductants that work synergistically to combat host-derived oxidants.

Mtb

Mtb is the etiological agent of tuberculosis (TB), which is undergoing a resurgence throughout the world, being responsible for nearly 2 million deaths in 2016. The emergence of multidrug–resistant isolates further complicates treatment, underscoring the urgency of developing new therapeutic strategies (Chakraborty and Rhee, 2015; World Health Organization, 2017). Aerosol transmission is the predominant route of infection of *Mtb*, and inhaled bacilli are engulfed by alveolar macrophages. *Mtb* has developed different survival strategies, which makes it the world's most successful pathogen (Hingley-Wilson et al., 2003; Gengenbacher and Kaufmann, 2012). In an attempt to control bacterial dissemination, infected macrophages release chemokines that recruit other inflammatory cells into the airways, ultimately resulting into the formation of granulomas. Inside the macrophage phagosome, *Mtb* is exposed to different reactive species (Chan et al., 1992; Russell, 2001; Vandal et al., 2009; Guirado et al., 2013), and *Mtb* survival in this hostile environment depends on several bacterial antioxidant systems (Fig. 2 A).

***The mycothiol (MSH)-dependent antioxidant network of* Mtb.** In most eukaryotes and bacteria, glutathione (GSH) is the main LMW thiol responsible for maintaining redox homeostasis in the cell. *Mtb* lacks GSH and instead relies on MSH (1-D-*myo*-inosityl-2-N-acetylcysteinyl-amido-2-deoxy-α-D-glucopyranoside) as its main LMW thiol (Newton and Fahey, 2002). MSH biosynthesis is complex and proceeds through a five-step pathway with L-inositol-1-phosphate and UDP-N-acetylglucosamine as the initial substrates (Newton et al., 2008). *Mtb* strains with decreased MSH content are hyper-susceptible to oxidants, acidic conditions, and drugs (Buchmeier et al., 2003, 2006; Nilewar and Kathiravan, 2014). *Mtb* can also synthetize ergothioneine (2-mercaptohistidine trimethylbetaine; Genghof and Vandamme, 1964) and gamma-glutamyl cysteine (Sao Emani et al., 2018), and the interplay between these three thiols ensures optimal protection of the bacteria against reactive species (Ta et al., 2011).

The flavoenzyme mycothiol disulfide reductase (MshR) is responsible for maintaining MSH in its reduced state and is essential for *Mtb* survival (Holsclaw et al., 2011). Upon oxidant exposure, several proteins form mixed disulfides with MSH, and these disulfides are then reduced by the glutaredoxin-like protein mycoredoxin-1 (Mrx-1; Van Laer et al., 2012; Hugo et al., 2014). Mrx-1 is in turn reduced by MSH/MshR using the reducing equivalents provided by NADPH (Van Laer et al., 2012). A novel MSH-dependent reductase (Rv2466c) involved in the activation of the pro-drug TP052, a thieno-pyrimidine derivative able to kill *Mtb*, was found to be essential for bacterial survival during exposure to H_2O_2 (Newton et al., 2011; Albesa-Jové et al., 2014; Rosado et al., 2017). Recently, it was shown that Rv2466c functions as a noncanonical nitroreductase that relies on MSH for the reduction of nitro-containing compounds (Negri et al., 2018). Under oxidative stress conditions, *Mtb* increases (via sigma H, E, and B) the expression of thioredoxin reductase/thioredoxin together with enzymes involved in MSH synthesis (Raman et al., 2001; Newton and Fahey, 2008). Moreover, ergothioneine biosynthesis increases under acidic conditions (such as those found in macrophage phagosome). WhiB3, a 4Fe-4S containing protein, regulates *Mtb* gene expression, sensing changes in the MSH redox status (Mehta et al., 2016; Saini et al., 2016). Interestingly, *Mtb* strains lacking whiB3 or sigma H have attenuated virulence in animal models (Kaushal et al., 2002; Mehta et al., 2016), underscoring the importance of the regulatory systems involved in redox homeostasis for bacterial pathogenesis.

Sulfur metabolic pathways (required for the synthesis of cysteine, MSH, and ergothioneine) have also been shown to be essential for *Mtb* virulence and survival and are up-regulated under oxidative stress conditions (Paritala and Carroll, 2013). Inhibitors that target these redox biosynthetic pathways are currently under investigation (Paritala and Carroll, 2013). Recently, the novo L-arginine biosynthetic pathway was shown to be essential for *Mtb* growth and virulence, as one of the early adaptive responses to oxidative stress (Tiwari et al., 2018). Disruption of the pathway leads to LMW thiol depletion, increase in oxidative stress, and DNA damage with rapid sterilization of *Mtb* (Tiwari et al., 2018).

Other* Mtb *antioxidant enzymes. One of the major secretory proteins found in clinical human isolates from patients with TB is Fe-SOD, suggesting that this protein has a major role in *Mtb* pathogenesis (Deshpande et al., 1993). Two types of SODs are found in *Mtb*, SODA and SODC. SODA is Fe dependent and is abundantly released from virulent *Mtb* strains (Braunstein et al., 2003). SODC is Cu dependent and is associated with the outer bacterial membrane. Both SODs protect *Mtb* from the action of macrophage-derived $O_2^{\bullet -}$ (Piddington et al., 2001; D'Orazio et al., 2009). Fe-SODA was reported to be essential for *Mtb* growth, and bacteria with reduced Fe-SODA content are largely attenuated (Edwards et al., 2001). Both Fe-SODA and Cu-SODC can inhibit immune functions as well as promote macrophage apoptosis, contributing to bacterial survival by mechanisms independent of $O_2^{\bullet -}$ dismutation (Liao et al., 2013).

The genome of *Mtb* encodes several heme- and nonheme-dependent peroxidases (Cole, 1998). Catalase-peroxidase (KatG)

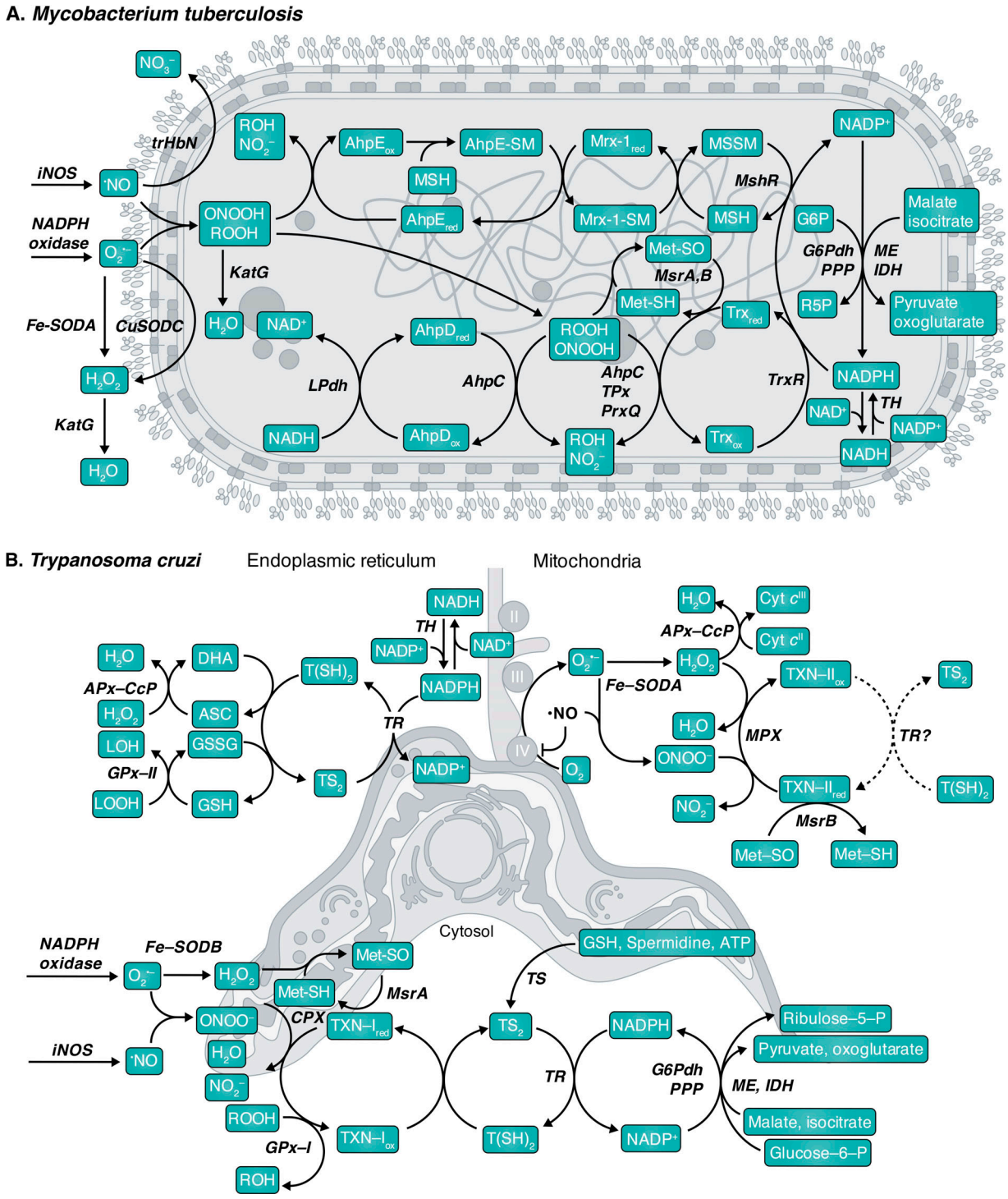

Figure 2. **Pathogen antioxidant networks: The examples of *Mtb* and *T. cruzi*. (A)** *Mtb*. NADPH oxidase-derived ($O_2^{\bullet-}$) and iNOS-derived ($^{\bullet}NO$) radicals can react with specific enzymes (Fe- or Cu-containing SODs to form H_2O_2; trHbN to form nitrate) or recombine to form peroxynitrite. H_2O_2, peroxynitritous acid (ONOOH), and organic hydroperoxides (ROOH) are reduced particularly by KatG and peroxiredoxins (Prx). Most *Mtb* Prx (AhpC, TPx, PrxQB) can use Trx as reducing substrate. AhpC can also be reduced by AhpD, an adaptor protein that links antioxidant with metabolic enzymes and NADH. NADPH—derived from the pentose phosphate pathway (PPP), isocitrate dehydrogenase (IDH), malic enzyme (ME), and H^+-transhydrogenases (TH)—is the final electron donor for TrxR and MshR. TrxR reduces Trx B and C (collectively indicated herein as Trx's). The one-Cys Prx AhpE is reduced by Mrx-1 either directly (data not shown) or through the formation of a mixed disulfide with MSH, which is reduced by Mrx-1 in a monothiolic mechanism that leads to mycothiolated Mrx-1 (Mrx-1-SM) and is resolved by MSH/MshR/NADPH. MsrA and B reduce S– and R–Met-SO, respectively, and accept electrons from the Trx/TrxR/NADPH system. **(B)** *T. cruzi*. Enzymatic and nonenzymatic redox-active molecules use reducing equivalents from NADPH—derived from the PPP, IDH, ME, and TH—are funneled through the $T(SH)_2$, GSH, ascorbate (ASC), and/or TXN-I/II redox systems. H_2O_2 is metabolized by APxCcP at the endoplasmic reticulum, mitochondria, and plasma membrane using ASC/Cyt c^{II} as the electron donors. Dehidroascorbate (DHA) is reduced by a direct reaction with $T(SH)_2$. Organic hydroperoxides (ROOH) are substrates for GPX-II that uses GSH. $T(SH)_2$ reduces oxidized GSH (GSSG), while TR reduces TS_2. Mitochondrial $O_2^{\bullet-}$ formation by the electron transport chain

(mainly at complex III) is detoxified by Fe-SODA with H_2O_2 generation. •NO can reach the mitochondria and inhibit respiration at complex IV (CIV; with an enhanced $O_2^{\bullet-}$ generation). Fe-SODA outcompetes for $O_2^{\bullet-}$ inhibiting $ONOO^-$ formation. Mitochondrial peroxiredoxin (MPX) decomposes H_2O_2 and/or $ONOO^-$, probably using reduced TXN-II and $T(SH)_2$ as the reducing substrate (dashed arrows). Met-SO is repaired by the action of MsrB at the expense of TXN. In the cytosol, cytosolic peroxiredoxin (CPX) detoxifies ROOH. $T(SH)_2$ is synthesized from two molecules of GSH and one spermidine in a reaction catalyzed by the enzyme trypanothione synthetase (TS). Met-SO is repaired by the presence of MsrA and B in the different compartments.

is a heme-containing peroxidase that acts either as a catalase or peroxidase, using a broad range of oxidizing and reducing substrates (Johnsson et al., 1997; Wengenack et al., 1999). KatG is required for the activation of the first-line anti-*Mtb* pro-drug isoniazid, and inactivating mutations cause isoniazid resistance (Zhang et al., 1992; Vilchèze and Jacobs, 2014). Isoniazid activation involves its one-electron oxidation by KatG compound I, generating an isoniazid-NAD adduct that inhibits enoyl-acyl carrier protein reductase, thereby impairing mycolic acid biosynthesis and damaging the structure of the bacterial wall (Vilchèze and Jacobs, 2014). *Mtb* strains lacking KatG are attenuated in wild-type and $iNOS^{-/-}$ mice, but they replicate and persist in mice lacking gp91*phox*, indicating that a major contribution of KatG to pathogenesis is via reduction of NADPH oxidase–derived hydroperoxides (Ng et al., 2004).

Mtb alkyl-hydroperoxide reductase-C (AhpC) is a typical two-cysteine peroxiredoxin that catalyzes the reduction of H_2O_2, organic hydroperoxides (ROOH), and peroxynitrite (Bryk et al., 2000; Hillas et al., 2000). AhpC is reduced by TrxC (and TrxR) using NADPH (Jaeger et al., 2004; Wong et al., 2017); it can also be reduced by NADH and by the Trx-like protein AhpD (Bryk et al., 2002; Maksymiuk et al., 2015). Clinical isolates of isoniazid-resistant *Mtb* strains frequently compensate for the loss of KatG by increasing the expression of AhpC (Sherman et al., 1996; Datta et al., 2016). *Mtb* strains harboring inactive mutants of components of the AhpC complex are susceptible to killing by murine macrophages expressing acidified nitrite and iNOS (Shi and Ehrt, 2006; Maksymiuk et al., 2015), underscoring the importance of this enzyme for detoxification of reactive nitrogen species by *Mtb*. Moreover, the persistence of *Mycobacterium bovis* bacillus Calmette-Guerin in infected macrophages decreases when bacterial AhpD is inactivated (Farivar et al., 2008). *Mtb* strains lacking AhpC show increased susceptibility to ROOH (Springer et al., 2001) and peroxynitrite, and decreased survival in macrophages (Master et al., 2002).

Mtb expresses a thiol peroxidase (TPx), which is more efficient than AhpC in peroxynitrite reduction and also reduces H_2O_2 and ROOH using TrxB/C as reducing substrates (Jaeger et al., 2004). *Mtb* lacking TPx is hypersensitive to H_2O_2 and •NO; these mutants fail to grow and survive in murine macrophages and show attenuated virulence in mouse models of TB (Hu and Coates, 2009). *Mtb* also encodes an alkyl hydroperoxide reductase-E (AhpE), a different one-cysteine peroxiredoxin belonging to a subfamily unique to bacteria. AhpE is very efficient in reducing peroxynitrite and fatty acid hydroperoxides (Hugo et al., 2009; Reyes et al., 2011), itself being reduced either by Mrx-1 directly or after formation of a mixed disulfide with MSH (Hugo et al., 2014). The residues involved in the interactions of AhpE with Mrx-1 have been recently described, serving as a starting point for structure-guide drug design (Kumar et al., 2017).

The other two peroxiredoxins expressed in *Mtb* are members of the PrxQ subfamily. PrxQB is essential for *Mtb* growth in cholesterol-rich media (Griffin et al., 2011; Lee et al., 2013; Wipperman et al., 2014) and catalyzes the reduction of peroxynitrite, fatty acid hydroperoxides, and H_2O_2 at the expense of TrxB/C (Reyes et al., 2016). Finally, *Mtb* protection against host-derived •NO relies on the presence of the truncated hemoglobin N (trHbN) that associates with the bacterial membrane via a preA helix that is found only in pathogenic bacteria (Rhéault et al., 2015). trHbN protects aerobic respiration from •NO inhibition and prevents its own irreversible oxidation and nitration by rapidly metabolizing •NO to an innocuous nitrate via the NOD reaction. trHbN catalyzes the NOD reaction with a second-order rate constant, $k_{NOD} = 7.45 \times 10^8\ M^{-1}s^{-1}$, corresponding to a rate approaching the diffusion limit, making it the most efficient NOD described thus far (Couture et al., 1999). *Mtb* trHbN is highly expressed during macrophage infection and maintains its NOD activity even under low oxygen concentrations (Pawaria et al., 2008). A recent study elucidated the mechanism underlying the highly efficient •NO-scavenging ability of *Mtb* trHbN (Carabet et al., 2017).

T. cruzi

T. cruzi belongs to the kinetoplastid family (which includes *Leishmania* spp. and other *Trypanosoma* spp.), a primitive eukaryote parasite responsible for American trypanosomiasis or Chagas disease, which infects an estimated 7 million people worldwide, with another 28 million people at risk of infection. The disease is transmitted via insect vectors and is spreading as a result of both human and vector migration (Pérez-Molina and Molina, 2018). While the acute phase of Chagas disease is usually asymptomatic, *T. cruzi* establishes chronic infection, characterized by organ damage that mainly, but not exclusive, affects the heart (myocarditis) and the intestine (megacolon). The pathogenesis of chronic Chagas disease myocarditis is complex and involves parasite immune evasion strategies, genetically determined defects in host immune homeostasis, and auto-reactive phenomena characterized by the presence of autoantibodies. *T. cruzi* genetic material can be found in damaged tissue during chronic infection, indicating an active role of parasite persistence in pathology (Rassi et al., 2010; Báez et al., 2011). Indeed, despite the generation of a robust immune response, the host fails to eliminate parasites from tissues, enabling the pathogen to persist chronically for years.

***The trypanothione-dependent antioxidant network of* T. cruzi.** *T. cruzi* relies on an unusual redox metabolism, as its major LMW thiol is a derivative of GSH and spermidine: N^1, N^8-bis(glutathionyl)-spermidine or trypanothione ($T(SH)_2/TS_2$; reduced and oxidized trypanothione, respectively; Fairlamb et al., 1985). This thiol is synthetized by the action of monoglutathionyl

spermidine synthetase and trypanothione synthetase (TS; Fig. 2 B). In *T. cruzi*, both conjugation reactions are performed by TS (Oza et al., 2002). Although *E. coli* is able to synthesize monoglutathionyl spermidine (Bollinger et al., 1995), a complete biosynthetic pathway for $T(SH)_2$ is exclusive to kinetoplastids.

$T(SH)_2$ is more reactive than GSH at physiological pH due to its lower p*Ka* (~7.4 and 8.3, respectively). TS_2 is reduced by the flavoenzyme trypanothione reductase (TR), with reducing equivalents provided by NADPH. In all cases $T(SH)_2$ delivers reducing equivalents to intermediary molecules, such as GSSG, dehydroascorbate), or the dithiol protein tryparedoxin (TXN); in their reduced state, these molecules can transfer electrons to the parasite peroxidases, reductases, glyoxalases, and transferases (Manta et al., 2013). TXN represents a distinct molecular class within the thioredoxin superfamily of oxidoreductases, differing from host cells, in which similar redox reactions are mediated by thioredoxins. *T. cruzi* contains two TXNs; one is a cytosolic protein (TXN-I) and the other a transmembrane protein (TXN-II) that is anchored to the surface of endoplasmic reticulum and mitochondria with the redox domain oriented toward the cytoplasm (Arias et al., 2015). An ATP-dependent transporter for both TS_2 and $T(SH)_2$ has been described in *Leishmania major* and may also exist in *T. cruzi* (Perea et al., 2018). Trypanothione and TXN metabolism in kinetoplastids are considered attractive target pathways for drug development, but efforts to inhibit TR activity have met with little success. In *Trypanosoma brucei*, TR activity needs to be reduced >90% to render parasites avirulent (Krieger et al., 2000).

T. cruzi *antioxidant enzymes.* Kinetoplastids express only Fe-SODs for $O_2^{\bullet-}$ detoxification. In *T. cruzi*, four Fe-SODs have been described and are located in distinct subcellular compartments. Fe-SODA and SODC reside in the mitochondrial matrix, Fe-SODB1 in the cytosol, and Fe-SODB2 in the glycosome, a uniqueparasite cellular organelle in which several glycolytic enzymes are located (Temperton et al., 1996; Ismail et al., 1997). While some authors have reported the presence of an extracellular Fe-SOD due to the presence of *T. cruzi* SOD-specific antibodies in patients with Chagas disease, the exact nature and mechanism of release of this Fe-SOD remain elusive (Mateo et al., 2010).

Despite the high structural homology among *T. cruzi* Fe-SODs, they have disparate susceptibilities to peroxynitrite inactivation. Cytosolic Fe-SODB is extremely resistant to oxidant inactivation as compared with its mitochondrial counterpart (Martinez et al., 2014). *T. cruzi* Fe-SODs readily eliminate $O_2^{\bullet-}$ and may contribute to *T. cruzi* intracellular survival in the vertebrate host by various immune evasion mechanisms. These mechanisms include protection from the directly cytotoxic effects of $O_2^{\bullet-}/HO_2^{\bullet}$ on parasite mitochondria in the macrophage phagosome, inhibition of $ONOO^-$ formation in $^{\bullet}NO$-challenged parasites, and modulation of redox-signaling processes that trigger parasite programmed cell death (Piacenza et al., 2007, 2013; Estrada et al., 2018). Indeed, peroxynitrite generation was demonstrated in the presence of $^{\bullet}NO$ and, importantly, parasites with increased mitochondrial Fe-SODA content detoxified $O_2^{\bullet-}$ before peroxynitrite formation (Piacenza et al., 2009a; Estrada et al., 2018). These observations are of central importance during the chronic stage of Chagas disease, in which $^{\bullet}NO$ is produced by different cellular sources, leading to control of pathogen proliferation by the host. Efforts are being made to unravel the structural characteristics of Fe-SOD in an effort to design specific inhibitors (Bachega et al., 2009).

T. cruzi peroxiredoxins participate in parasite virulence by means of efficient H_2O_2 and peroxynitrite detoxification in the phagosome (Alvarez et al., 2011). In *T. cruzi*, increased expression of both mitochondrial and cytosolic peroxiredoxins is associated with enhanced virulence in mouse models of Chagas disease, resulting in high parasitemia and severe tissue damage (Piacenza et al., 2009b; Zago et al., 2016). Recently, holdase activity has been demonstrated for *Leishmania* peroxiredoxins and shown to be involved in parasite virulence (Castro et al., 2011). Whether this pathway is also active in *T. cruzi* infection is still under investigation.

To date, only one heme-containing peroxidase was described in *T. cruzi*, *Tc*APxCcP, a hybrid type-A peroxidase that uses both ascorbate and cytochrome c as reducing substrates for H_2O_2 detoxification (Wilkinson et al., 2002). *Tc*APxCcP is a membrane-bound peroxidase located at the endoplasmic reticulum and mitochondria in all parasite stages and at the plasma membrane in the infective stages of the *T. cruzi* life cycle (Hugo et al., 2017). Parasites overexpressing *Tc*APxCcP are more resistant to H_2O_2 toxicity, even more so than those overexpressing cytosolic and/or mitochondrial peroxiredoxin (Piacenza et al., 2008). Although the enzyme is not essential for the establishment of infection (Taylor et al., 2015), parasites with higher *Tc*APxCcP content show increased virulence in the vertebrate host, underscoring its role in pathogenicity (Hugo et al., 2017).

Finally, *T. cruzi* contains two GSH-dependent–like peroxidases (GPXs) that lack selenium at the active site. All trypanosomatid GPXs have a phospholipid hydroperoxidase-type structure and can metabolize fatty acids and phospholipid hydroperoxides, but none have activity with H_2O_2 (Wilkinson and Kelly, 2003). GPX-I is located at the cytosol and glycosome whereas GPX-II localizes to the endoplasmic reticulum in the noninfective epimastigote. GPX-I has a higher affinity for TXN-I, with rates of hydroperoxide reduction 8–15-fold higher than with GSH (Wilkinson and Kelly, 2003). Overexpression of GPX-I and -II confers resistance to exogenous H_2O_2, although this oxidant cannot be directly metabolized by either peroxidase, suggesting that increased concentrations of these enzymes confers protection by detoxifying secondary products of lipid peroxidation (Wilkinson and Kelly, 2003). The relevance of the *T. cruzi* GPXs in the infective stage of the parasite is still to be determined. Overall, *T. cruzi*'s armamentarium of antioxidants acts as a virulent factor by detoxifying reactive species at the phagosomal compartment (Fig. 2 B). However, potentially conflicting observations were reported using gp91-*phox*$^{-/-}$ macrophages, which failed to allow proliferation of some *T. cruzi* strains (Paiva et al., 2012; Santiago et al., 2012). This observation might be explained by the requirement for a mild oxidizing environment for efficient iron mobilization, which enhances intracellular parasite growth (Andrews, 2012). However, in spite of this observation, pioneering experiments using murine macrophages defective in NADPH oxidase activity (Tanaka et al., 1982) or from

silica-treated mice (in which phagocyte respiratory burst is exhausted; Kierszenbaum et al., 1974; Trischmann et al., 1978) showed that these cells are highly susceptible to *T. cruzi* infection. Future studies should clearly define the role of redox signaling in parasite iron metabolism and proliferation and the "switch" to conditions leading to oxidative killing.

Pathogen repair systems for oxidized biomolecules

Another virulence strategy relies on pathogen-encoded repair systems that limit potentially harmful oxidation of proteins and DNA. Methionine oxidation is mediated by different reactive species including H_2O_2, peroxynitrite, HOCl, and metal catalyzed oxidation systems, giving rise to the epimers methionine-(*S*) and methionine-(*R*)-sulfoxide (Met-SO; Weissbach et al., 2002). Enzymatic mechanisms for methionine oxidation have also been described (Lim et al., 2011; Cao et al., 2018; Wu et al., 2018). Methionine sulfoxide reductases (Msr) had been described in a wide range of pathogenic species, and these enzymes reduce Met-SO using the reducing equivalents form the Trx/TrxR and NADPH (Boschi-Muller et al., 2005). Two different enzymes, MsrA and MsrB, catalyze the reduction of the diastereoisomers of oxidized methionine. The activity of MsrA is restricted to Met(*S*)-SO residues in proteins, whereas MsrB reduces Met(*R*)-SO. Some pathogenic bacteria contain a fusion protein of MsrA and B (MsrAB), which has higher catalytic efficiency than the individual enzymes (Han et al., 2016). Organisms lacking Msr's are more susceptible to oxidative stress, have a shortened life span and, for bacterial pathogens, have reduced virulence (Moskovitz et al., 1997; St John et al., 2001; Zhao et al., 2010). *Mtb* expresses both MsrA and B, but their contribution to oxidative stress is limited. *Mtb* lacking MsrA or MsrB alone does not show increased susceptibility to HOCl or acidified nitrite, whereas strains lacking both enzymes do (Lee et al., 2009). *Mtb* MsrA also reduces the methylsulfinylfuranosyl groups in lipoarabinomannan, a glycoprotein formed from the interaction of two-electron oxidants with components of the bacterial cell wall (Stalford et al., 2009).

Trypanosomes contains both MsrA and MsrB; MsrA is located at the cytosol and MsrB at the parasite mitochondria. The activity of both enzymes depends on reducing equivalents delivered from the $T(SH)_2$-TXN-NADPH system. In *T. brucei*, only MsrA seems to participate in H_2O_2 resistance, as no change to oxidant sensitivity was observed when expression of MsrB was reduced (Guerrero et al., 2017). The contribution of Msr in *T. cruzi* virulence is still not well established and requires further study.

Another important system for *T. cruzi* virulence involves the sanitization of oxidized bases in DNA. Guanine is highly susceptible to oxidation, and its most common oxidation product is 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxoG), which is potentially mutagenic due to its structural similarity with thymine (Neeley and Essigmann, 2006). All organisms contain 8-oxoG repair systems, which catalyze the removal of 8-oxoG from the cellular pool (MuT and MTH1 in bacteria and eukaryotes, respectively) and/or eliminate 8-oxoG from DNA (MutM/MutY and OGG1/MUTYH for bacteria and eukaryotes, respectively; Michaels et al., 1992; Barnes and Lindahl, 2004). The *Mtb* genome contains a high guanine and cytosine (GC) content, suggesting high susceptibility to oxidative stress, but it also encodes highly efficient DNA repair systems, which could constitute candidates for drug targeting (Dos Vultos et al., 2009; Reiche et al., 2017). Trypanosomes also contain efficient DNA repair systems (Passos-Silva et al., 2010), and overexpression of some components of these systems leads to benznidazol resistance, indicating that one of the principal targets of benznidazol is the nucleotide pool (Rajão et al., 2014). The extensive incorporation of oxidized nucleotides as a result of benznidazol treatment normally leads to potentially lethal double-stranded DNA breaks in the pathogen genome.

Conclusions and perspectives

While the past few decades have seen considerable progress in our understanding of the biology of reactive species in the phagosomal compartment, a precise understanding of the interplay, effects, and metabolism of these species remains challenging. On one hand, pathogens have a large variety of antioxidant components, levels, and responses that serve to safeguard microbial viability and proliferation. On the other hand, phagocytic cells have multifaceted oxidative mechanisms which, acting independently or synergistically in a stimuli-dependent and time-dependent manner, assist in the control of pathogen dissemination.

An intriguing question, for which there are virtually no data available for macrophages, is how microbial substrates, proteins, or enzymes may be secreted or extruded to potentially modulate the oxidant tone in the phagosome. In the case of trypanosomes, active transport of reduced/oxidized thiols has been recently reported and may serve to pump thiols to the phagosome (Perea et al., 2018). Similarly, ergothioneine is secreted by *Mtb* (Sao Emani et al., 2013). Both observations contribute to the speculation that high reductant concentrations may be achieved in the phagosomal lumen, thereby attenuating the oxidative assault and constituting a first antioxidant "shield."

The antioxidant responses of pathogenic microorganisms secondary to the release of reactive species at the phagosome remain an unknown and fascinating field. Pathogens may also regulate the extent of oxidant production by modulating the host immune response (i.e., delay in the maturation of the phagocytic vacuole, inhibition of NADPH oxidase assembly, and inhibition of granule fusion, among others). Several other factors influence the magnitude of the oxidative assault, including tissue O_2 concentration and pro-inflammatory mediators (e.g., cytokines) that prime macrophages for an increase in oxidant capabilities. In addition, low levels of host-derived redox mediators such as H_2O_2 and peroxynitrite can trigger up-regulation of pathogen antioxidant defenses. Importantly, the ability of virulent versus avirulent strains of microbes to cope with reactive species greatly differs. The importance of phagocyte-mediated oxidative killing of pathogens is well documented in humans, considering the poor outcome of infection in individuals with deficiencies in different components of the host oxidant response. Conversely, pathogens with robust antioxidant systems result in more virulent phenotypes. Distinctive characteristics of the pathogen

antioxidant networks in relation to their mammalian host cell counterparts offer potential for the development of pharmacological therapies to interfere with the redox-based detoxification systems of infective organisms.

Acknowledgments

This work was supported by grants from the National Institutes of Health (1R01AI095173 to R. Radi), Espacio Interdisciplinario (Centros 2015 to R. Radi), and Comisión Sectorial de Investigación Científica (CSIC I+D 2016 to L. Piacenza and M. Trujillo and CSIC Grupos 2014 to R. Radi). Additional support was obtained through Fundación Manuel Pérez via Ley de Fundaciones to L. Piacenza (Biriden Scientific Instruments) and R. Radi (Ridaline Scientific Instruments) and from Programa de Desarrollo de Ciencias Básicas to all the authors.

The authors declare no competing financial interests.

The general outline of the manuscript was conceived by R. Radi. L. Piacenza was primarily responsible for manuscript writing and consolidation of the first draft; M. Trujillo directly contributed to the sections related to pathogen antioxidant networks. The original artwork was created by L. Piacenza. Revision of intermediate versions and final editing and analysis of the work were provided by R. Radi. All three authors worked jointly throughout the process and revised and approved the final version.

Submitted: 13 November 2018
Revised: 4 January 2019
Accepted: 4 February 2019

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