

Oxidative and Nitrosative Stress on Phagocytes' Function: from Effective Defense to Immunity Evasion Mechanisms

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Abstract Although oxygen, nitrogen, and chlorine reactive species have been associated with disease pathogenesis, their partial absence is very harmful to the body's innate immune defense. Lacking of adequate release of free radicals from activated phagocytes is related to impaired ability on fungi, bacteria, and protozoa killing. We constructed an updated conceptual landmark regarding the paramount role of free radicals in phagocyte defense systems (phagocyte oxidase, myeloperoxidase, and nitric oxide/peroxynitrite system) on natural immunity. Diverse fungal, bacterial and protozoal pathogens evade the phagocytes' oxidative/nitrosative burst through antioxidant genes, enzymes and proteins. The most important evasion mechanisms were also described and discussed. These interconnected systems were reviewed and discussed on the basis of knowledge from relevant research groups around the globe. Phagocyte-derived free radicals are essential to destroy important human pathogens during the course of innate immunity.

Keywords Phagocyte oxidase · Macrophage · Myeloperoxidase · Nitric oxide · Peroxynitrite · Malaria · *Candida* spp. · *Mycobacterium tuberculosis* · *Trypanosoma cruzi*

Introduction

Cell survival depends on a lot genetic, biochemical and physiologic factors. One important subject regarding cell and tissue protection is oxidative balance, e.g., the adequate equilibrium between production of reactive oxygen, nitrogen and chlorine species (by mitochondria, peroxisome, endoplasmic reticulum and other cell organelles) and their removal by the antioxidant defense systems. When this equilibrium is broken by overproduction of the reactive species, especially the reactive oxygen species (ROS), and/or decreased antioxidant concentrations (by failed synthesis or diminished nutritional intake and bioavailability) cell and tissues suffer from the so-called oxidative stress (Vladimirov and Proskurnina 2009). For example, *Cryptococcus neoformans* induced lung inflammation, pneumonia, oxidative stress and lipid peroxidation in an animal experimental model (Hall et al. 2010).

Free Radicals, Reactive Oxygen and Nitrogen Species (ROS/RNS)

A free radical (FR) is any molecule that has one or more incomplete orbitals. Thus, a FR can gain electrons, oxidizing another atom/molecule or lose them reducing an element. Some ROS are FR [superoxide anion (O_2^-), hydroxyl radical ($\cdot OH$), nitric oxide ($NO\cdot$), peroxynitrite ($ONOO^-$) although others are not [hydrogen peroxide (H_2O_2), singlet oxygen (1O_2)]. ROS/FR and reactive nitrogen species promote redox reactions in molecules, virtually damaging all cell domains. For example, hydrogen peroxide is capable of triggering the activation of nuclear and mitochondrial DNA-based antimicrobial extracellular traps that can kill microorganisms and

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activate innate immunity (von K  ckritz-Blickwede and Nizet 2009). The same hydrogen peroxide is essential to destroy trophozoites of *Entamoeba histolytica* (Nandi et al. 2010). Interestingly, an opposite pathway *Streptococcus pyogenes* group A can trigger host cell death by Rac1-dependent ROS formation (Aikawa et al. 2010). ROS can react directly with lipids, proteins, enzymes, DNA and cell and tissue carbohydrates, modifying them and inducing pathophysiologic disorders (Ferrari et al. 2009). It should be emphasized that reactive species are essential in innate response to pathogens and this review addressed recent research regarding the multiple roles of ROS and RNS on phagocyte function against infectious and parasitic diseases, including immune evasion mechanisms.

The Importance of Phagocyte Oxidase in Microbial Killing

In professional phagocytes, such as macrophages and neutrophils, the plasma membrane contains the nicotinamide adenine dinucleotide (NADPH)-oxidase enzyme which converts four electrons and 4O_2 into 4O_2^- radicals, a mechanism called “oxidative burst”. Half of these superoxide anions react with H^+ yielding $2\text{H}_2\text{O}_2$ both able to kill intracellular pathogens (Escorza and Salinas 2009; Fran  a-Botelho et al. 2006; Hii and Ferrante 2007).

Therefore, in phagocytes NADPH-oxidase is characterized as a phagocyte-oxidase with an iron-containing protein (HEME protein) and it represents an important defense mechanism in microbial and parasite killing by professional phagocytes (Minakami and Sumimoto 2006; Sumimoto et al. 1996). Its better activity in eosinophils occurs at pH 7.5 (Morgan et al. 2005). The efficient killing of microbes depends on the ability of the cytochrome b558, an active site of the enzyme, to bind and form a complex with flavin adenine dinucleotide which in turn triggers intensive superoxide anions loading and cytotoxic effects (Hashida et al. 2004). Arachidonic acid binding to S100 proteins activates NADPH-oxidase triggering the respiratory burst defense against pathogens (Hii and Ferrante 2007).

Impaired capacity of NADPH-oxidase to generate superoxide in neutrophils has been associated with chronic granulomatous diseases, which comprise at least genetic defects impairing one of the five subunits of the phagocyte NADPH-oxidase rendering the host susceptible to recurrent fungi and bacterial infections, polycythemia vera, and diabetic periodontal infections (Holland 2010). NADPH-oxidase deficient mice had massive and disseminate infections characterized by abscesses and granulomas in soft tissues (Gozalo et al. 2010). A 2-month-old girl with NADPH-oxidase deficiency had chronic granulomatous lung aspergillosis with pneumonial presentation which was

successfully treated with voriconazole (Lee et al. 2010). In this regard, NADPH-oxidase is a critical defense that restores the normal lung defense functions against pathogens (Segal et al. 2010). In fact, NADPH-oxidase and subsequent ROS formation is critical for antiviral defense (Soucy-Faulkner et al. 2010). Defective phagocyte-oxidase generating-free radicals enable survival and evasion of *Salmonella* spp. from macrophage killing (V  zquez-Torres and Fang 2001; V  zquez-Torres et al. 2001). However, it should be emphasized that NADPH-oxidase activity is up-regulated during encephalomyocarditis B virus microglial infection which was associated with worsening of neuronal oxidative stress (Ano et al. 2010). Other researchers have also been found that *Chlamydia trachomatis* rapidly suppressed NADPH-oxidase providing the survival of pathogens in infected epithelial cells (Boncompain et al. 2010).

Myeloperoxidase and NO-Induced Cysteine Disruption: Another Free Radical Killing System

In addition to the phagocyte membrane NADPH-oxidase system, which generates large amounts of hydrogen peroxide, activated neutrophils and macrophages contains myeloperoxidase (MPO), a heme-enzyme that kills bacteria and fungi by hypochlorous acid (HOCl) that also reacts with superoxide anion yielding the toxic hydroxyl radical ($\cdot\text{OH}$) and Cl^- (Vladimirov and Proskurnina 2009).

Hypochlorous acid can react with iron II (Fe^{2+}) to form $\cdot\text{OH}$; Fe^{2+} reacts with superoxide forming hydrogen peroxide (H_2O_2), both highly toxic products capable of killing intraphagosomal pathogens (Nappi and Vass 2002).

Besides hypochlorous acid, $\cdot\text{OH}$, $^1\text{O}_2$ and ozone (O_3) represents other important products of the potent bactericidal and fungicidal MPO system (Aratani et al. 2006; Klebanoff 2005). MPO-null mice are susceptible to infection but overexpression of inducible nitric oxide synthase (iNOS) with massive NO production restored defenses against *Escherichia coli* experimental lung infection and decreased mice mortality (Brovkovich et al. 2008). The chemical interaction between MPO and NO affords nitrosation of proteins and other membrane and cytosolic biomolecules (Lakshmi et al. 2005). This mechanism is responsible for destruction of viruses, fungi, bacteria and parasites (Colasanti et al. 2002). The most important group of proteins affected by nitrosation is the cysteine containing proteins (cysteine proteases, cystein-rich aspartases or “caspases”, and capsid viral proteins) (Colasanti et al. 2002; Torre et al. 2002) a promise in target-cell based immunotherapies against malaria and viruses (Cao et al. 2003; Izuhara et al. 2008; Rosenthal et al. 2002; Sharma et al. 2004; Venturini et al. 2000).

Higher and sustained synthesis of IgG, IgM, and the C₃ complement opsonin were required in triggering of the oxidative burst and the subsequent phagocyte killing of different *Salmonella* spp. strains (Gondwe et al. 2010).

In experimental models with *Salmonella typhimurium*, *Candida albicans* and *Leishmania donovani* a combined deficiency of both phagocyte oxidase and NO synthesis have compromised the phagocytic activity enhancing the infection rates (Balishi et al. 2005; Mastroeni et al. 2000; Murray et al. 2006).

NO Killing by Phagocytes in Candida and Malaria: When the Excess Is Harmful

Nitric oxide synthases (NOS) are widely distributed throughout the mammalian tissues. Macrophage (mNOS), endothelial (eNOS), neuronal (nNOS), inducible (iNOS) and mitochondrial (mtNOS) isoforms (Ferrari et al. 2009) are prompted to convert arginine in the NO radical (NO[•]), which was discovered at the end of the 80s. Nitric oxide quickly reacts with superoxide yielding peroxynitrite (ONOO[•]).

Acting as a highly toxic nitrogen-derived radical, peroxynitrite is involved in inflammation, pain, cardiovascular diseases, neurodegeneration, cancer, stroke, and necrotizing enterocolitis among other pathologic conditions (Chokshi et al. 2008; De Palma et al. 2008; Ferrari et al. 2009; Pacher et al. 2007; Szabó et al. 2007).

Very interestingly is the fine tune negative feed-back mechanism displayed by the reactive oxygen and nitrogen products. The excessive amount of NO released by mitochondria represses NADPH-oxidase activity protecting the cell against superoxide (Selemidis et al. 2007) and peroxynitrite overload.

Adequate activity of iNOS and other NOS isoforms affords effective protection against infection. During the course of macrophage phagocytosis, NO and oxygen radical species are essential to kill *C. albicans* (Balishi et al. 2005; Rodríguez-Galán et al. 2002). But hindering the oxidative-burst of macrophages is an active evasion mechanism of *C. albicans* (Wellington et al. 2009). In the same manner, some NO reductase enzymes (NorvW) from *S. typhimurium*, and NorB from *Neisseria meningitidis*, and flavohemoglobins (Hmp) from *Salmonella enterica*, *S. typhimurium*, and *E. coli* are able to catalyze the reaction of NO and oxygen converting the first in the inert nitrate avoiding and escaping from peroxynitrite cytotoxicity (Laver et al. 2010; McLean et al. 2010).

S-nitroso-thiols are important for bacterial destruction. In this respect, *E. coli*, *S. enterica* and *N. meningitidis* are able to deplete NO macrophage stores inhibiting S-nitroso-thiol synthesis and intracellular killing (Laver et al. 2010).

In experimental models with *Plasmodium berghei* or *P. chabaudi* NO releasing by professional phagocytes is positive associated with parasite killing (Bastos et al. 2002; Garnica et al. 2003). Following the same trend, NO generation during macrophage phagocytosis is necessary to control human malaria, especially caused by *Plasmodium falciparum*, but also by other malarial parasites (Balmer et al. 2000; De Souza et al. 2008; Nahrevanian 2006; Sobolewski et al. 2005).

During the course of an infection, the enhanced mitochondrial energy metabolism generates NO which, in turn, can help to destroy pathogenic bacteria, fungi and protozoa. Lacking of NO synthesis from leucocyte phagocytes is associated with increased risk of infection by *Porphyromonas gingivalis* (Gyurko et al. 2003), *Trypanosoma cruzi* (Hölscher et al. 1998; Talvani et al. 2002), and *P. falciparum* (Boutlis et al. 2003). Still considering malaria, weaker NO production by macrophages was associated with a worst outcome of cerebral malaria (Gramaglia et al. 2006; Lopansri et al. 2003), indicating that NO is essential in parasite killing, confirming previous reports (Balmer et al. 2000; Nahrevanian 2006; Nahrevanian et al. 2008). However, excessive amounts of NO and peroxynitrite can aggravate infections, explaining why NO overload could also be toxic, especially to the brain (Ferrari et al. 2009; Nahrevanian 2006; Nahrevanian et al. 2008). In fact, NO decreases expression of intercellular and vascular cell adhesion molecules inhibiting *P. falciparum* adhesion to endothelium of brain vessels, but the parasite induces release of IgE and subsequent NO overload which aggravates cerebral malaria (Mazie and Idrissa-Boubou 1999). Then, NO is essential in pathogen killing but some patients which produce excessive NO levels are susceptible to

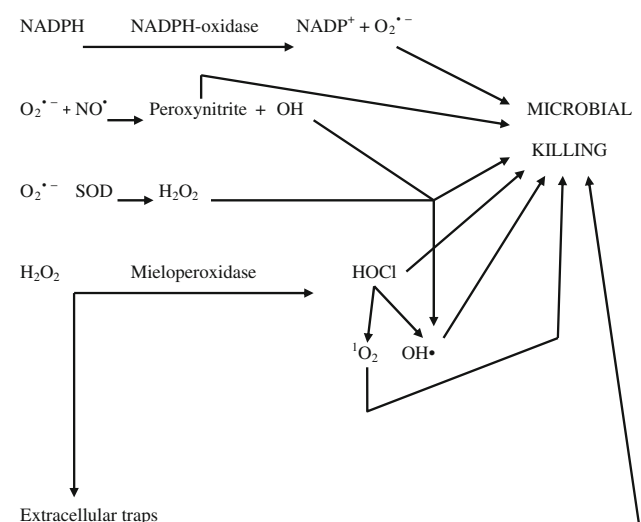


Fig. 1 Cytotoxic effectors from phagocytes' oxidative/nitrosative burst enzyme systems

Table 1 Pathogen's evasion mechanisms against phagocytes' oxidative burst

System	Mechanism	Pathogen	References
ahpc encoding peroxiredoxins	Conversion of peroxynitrite to nitrate	<i>E. coli</i> , <i>E. faecalis</i> , <i>H. pylori</i> , <i>Legionella pneumophila</i> , <i>Salmonella</i> sp., <i>Mycobacteria</i> , <i>Staphylococcus</i> sp., <i>Streptococcus</i> sp., <i>Treponema pallidum</i> , and <i>Trypanosoma cruzi</i>	Bryk et al. 2000; Chuang et al. 2006; LeBlanc et al. 2006 Cosgrove et al. 2007; Knapp and Swartz 2007; La Carbona et al. 2007; Lechardeur et al. 2010; Parsonage et al. 2010; Piacenza et al. 2008; Alvarez et al. 2011
Ascorbate peroxidase	Scavenging H ₂ O ₂	<i>L. major</i> and some pathogenic bacteria	Pal et al. 2010
cymr	Inactivation of oxidants	<i>S. aureus</i>	Soutourina et al. 2010
Depletion of S-nitroso-thiols	Depletion of nitric oxide precursor	<i>E. coli</i> , <i>N. meningitidis</i> , <i>S. enterica</i> , <i>Plasmodium falciparum</i> , <i>Trypanosoma cruzi</i>	Laver et al. 2010; Gutierrez et al. 2009 De Souza et al. 2008 Nahrevanian 2006; Sobolewski et al. 2005; Balmer et al. 2000
Kat (catalase)	Scavenging H ₂ O ₂	<i>Mycobacterium tuberculosis</i> and many other pathogens	Ng et al. 2004; Cosgrove et al. 2007
NAD-1	Suppression of oxidative burst and phagocytosis	<i>Mycobacterium tuberculosis</i>	Velmurugan et al. 2007; Miller et al. 2010
Nitric oxide reductases	Converting NO ⁻ to innocuous nitrate	<i>C. albicans</i> , <i>E. coli</i> , <i>Salmonella</i> sp. and <i>Neisseria</i> sp.	Cardinale and Clark 2005; McLean et al. 2010; Laver et al. 2010; Barth et al. 2009; Wellington et al. 2009
Phagocyte oxidase inhibitors	Suppression or dysruption of the enzyme	<i>Coxiella burnetii</i> and other bacteria	McCaffrey et al. 2010; Hill and Samuel 2011
Superoxide dismutase (SODA and SODCI)	Scavenging O ₂ ⁻	<i>Salmonella</i> sp., <i>Entamoeba histolytica</i> and other pathogens	Craig and Slauch 2009; Hinchey et al. 2007; Kim et al. 2010; França-Botelho et al. 2010
Thioredoxin/thioredoxin-interacting protein	Free radical induced apoptosis of the host cells	To be discovered!	Chen et al. 2010; Saxena et al. 2010

inflammation, necrosis and other adverse effects. The same concept is applied to pathogen virulence. Higher virulent strains of *Entamoeba* spp. were associated with elevated levels of oxidative stress (França-Botelho et al. 2010). In diabetic rats excessive amounts of superoxide anion impaired phagocyte defense which was reversed by insulin treatment (França et al. 2009). Figure 1 summarizes the roles of the cytotoxic oxidative/nitrosative effectors against bacterial, fungal, and protozoal pathogens.

Pathogens Antioxidant Systems: Scaping from Phagocytes Killing

Comparing eight pathogenic strains of *Candida* spp., it has been observed that *C. albicans* and *C. glabrata* were more resistant to oxidative stress which provides a further advantage in pathogenesis (Abegg et al. 2010).

Francisella tularensis produces factors that inhibits or disrupts NADPH-oxidase activity increasing bacterial colonization and virulence (McCaffrey et al. 2010). Following a similar approach, *Coxiella burnetii* synthesizes an acid phosphatase with potent inhibitory effects on free radical release from neutrophil NADPH-oxidase (Hill and Samuel 2011).

The induction of apoptosis as well as an increased membrane repair activity, and the triggering of oxidative burst on macrophages cytosol are important mechanisms to decrease tuberculosis infection (Divangahi et al. 2009; Gan et al. 2008). However, the *nuoG* gene of *Mycobacterium tuberculosis* encodes a NAD-1 subunit which is involved in reduction of macrophage oxidative burst and apoptosis of engulfed bacteria increasing infection resistance (Miller et al. 2010; Velmurugan et al. 2007).

Superoxide dismutase A also performs an important role in scavenging of superoxide radicals, decreasing oxidative

response which confers infection resistance (Hinchey et al. 2007). This mechanism is present in *S. enterica* serovar *Thyphimurium* which encodes a protease resistant SODCI enzyme that metabolizes and inactivates the superoxide anions into the phagosome (Kim et al. 2010).

Another evasion mechanism is represented by the enzyme ascorbate peroxidase which detoxifies H_2O_2 an essential step for *Leishmania major* intramacrophage survival (Pal et al. 2010). *Mycobacterium tuberculosis* and other pathogens evade from macrophage oxidative burst by catalase enzymes (Kat A and Kat G) which deactivate H_2O_2 to inert oxygen atom and water (Cosgrove et al. 2007; Ng et al. 2004).

The cymr, a cysteine containing protein, consists of sulfhydryl groups which inactivate and scavenge oxidants, increases survival of *Staphylococcus aureus* and decreases bacterial virulence resulting in more prolonged infection in the host (Soutourina et al. 2010). Other studies strongly confirmed that neutrophil-derived free radicals are essential in *S. aureus* killing, since hypoxic conditions increased bacterial survival (McGovern et al. 2011).

After phagocytosis and lysozyme-triggered *S. aureus* cell wall disruption are important factors that induce activation of the inflammasome protein complex with a concomitant secretion of the IL-1 β cytokine (Shimada et al. 2010). Thioredoxin is coupled to the thioredoxin-interacting protein (TXNIP) forming a stable complex. When inflammasome is activated it triggers the disruption of the TXNIP from thioredoxin by a free-radical associated mechanism which culminates with IL-1 β secretion, glucose intolerance, and insulin resistance (Zhou et al. 2010). TXNIP is originally located at the nucleus. Notwithstanding, when the cell is exposed to massive oxidative and nitrosative stress this protein is translocated to the mitochondria activating the cytochrome c release, caspase activation, and apoptosis (Chen et al. 2010; Saxena et al. 2010). Although peroxynitrite is very useful for efficient intramacrophage amastigote killing (Hölscher et al. 1998; Talvani et al. 2002), *T. cruzi* expresses a peroxyredoxin (tryparedoxin peroxidase) which deactivates nitrogen free radicals enhancing parasite survival and infection (Alvarez et al. 2011). The most important oxidative/nitrosative stress evasion mechanisms are listed in Table 1.

Conclusions

Oxidation and nitroxidation phagocyte reactions are essential in microbial killing. Pathogens display many different antioxidant systems to deactivate phagocyte free radicals. Considering that the majority of randomized clinical trials with antioxidant therapy in healthy subjects or infectious diseases patients had no effects on morbidity

and mortality or had negative outcomes (Ackerman et al. 2009; Biesalski et al. 2010; Charunwatthana et al. 2009; Lykkesfeldt and Poulsen 2009), and that in chronic hepatitis C antioxidant therapy had just mild positive effects for patients (Gabbay et al. 2007), the concept of antioxidant therapy is questionable and should regard the role of phagocyte oxidative/nitrosative burst in infectious and parasitic diseases.

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