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Role of reactive oxygen species and iron in host defense against infection

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1. ABSTRACT

Reactive oxygen species (ROS) and iron play important roles in the innate immune response. ROS are released by immune cells and are highly reactive and indiscriminately destructive in response to pathogens. In addition, ROS act as signaling molecules and play a role in apoptosis, therefore excessive ROS production can damage host molecules, leading to more harm than benefit for the host. Iron acts as a catalyst for the formation of ROS, therefore, manipulation of iron levels is a way in controlling ROS production. Iron metabolism and ROS production may affect many disease processes and must be tightly regulated for the host to generate an appropriate response. Current researches examine the roles of iron and ROS in various conditions, including neurodegeneration, inflammation, infection and cancer. Therapies directed at regulating ROS production through regulating iron levels are a major focus in these fields today.

2. INTRODUCTION

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen, which are typically by-products of aerobic metabolism. ROS is a categorical term, encompassing oxygen radicals and other reactive species including superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH•) (1). ROS have a range of activities including pathogen defense, stress adaptation, and mediation of signaling processes (2,3). ROS is released by innate immune cells to destroy pathogens and can be produced in an "on-demand" fashion in response to the detection of a pathogenic threat. This process is critical for immune function as patients with impaired ROS mediated oxidative bursts, such as in Chronic Granulomatous Disease, suffer recurrent infections (4). However, since ROS can also damage health tissue, over production of ROS can

be detrimental for the host.

Iron is an essential trace metal required for almost all forms of life. It plays a critical role in various physiologic and basic metabolism in living organisms, and frequently acts as a catalyst for metabolic processes and various enzymatic reactions (1). Iron is a critical catalyst in ROS production, and therefore iron levels must remain balanced in order to maintain the immune system to function properly. It has been shown that low iron levels are associated with impaired immune function, reducing both phagocytic activity and oxidative bursts of immune cells (5). Unbalanced levels of iron can result the overproduction of ROS, leading oxidative stress and tissue damage. Because of the critical roles of ROS and iron in host defense and survival, modulation of ROS and iron to benefit the host may have therapeutic potential in host defense against infection. This review aims to provide an overview of sources of ROS with its mechanism in the innate immune response and a link to iron levels.

3. THE CHEMISTRY OF ROS AND IRON

ROS, including both free radicals and non-radicals, are characterized by their highly reactive nature. Free radicals such as superoxide anions and hydroxyl radicals, have an unpaired valence electron, or incomplete electron shells, which are unstable and actively seek an electron for shell completion. They will accept or capture any electron non-selectively from surrounding molecules, and this electron capture may generate an additional free radical. A cascade of radical formation results eventually causing damage to biological molecules including proteins, lipids and DNA. Non-radical ROS species include hydrogen peroxide and hypochlorous acid. They are also considered to be highly reactive since they can cross biological membranes and form the highly reactive hydroxyl radicals by interaction with transition metal ions such as Fe^{2+} and Cu^{+}. Therefore, iron levels play a role in radical ROS production.

In healthy states, most iron is present in a protein-bound form, such as transferrin-bound iron, to maintain steady-state, while free iron or labile iron serves as a catalyst for the generation of hydroxyl radicals from superoxide anions and hydrogen peroxide. Free iron has three main states: ferrous bivalent (Fe^{2+}), ferric trivalent (Fe^{3+}) and ferryl tetravalent (Fe^{4+}). Switching between these states occurs by their electron transfers properties (ability to accept or donate electrons) and provides the primary catalytic function. As illustrated in Figure 1, the Haber-Weiss reaction reduces ferric iron to ferrous iron. A second reaction, termed the Fenton reaction, oxidizes this iron again resulting in the production of hydroxyl radicals (Figure 1). Together these reactions are referred to as Fenton Chemistry and have been well described historically (1, 2). Cu^{2+} may also act as the transition metal required for catalysis (3). Hypochlorous acid may be substituted for hydrogen peroxide, with hydroxyl radicals resulting from this modified reaction (4). Importantly, this system can be catalyzed by low levels of free iron due to redox cycling.

The Fenton and Haber-Weiss reactions result in the generation of a highly reactive hydroxyl radical from hydrogen peroxide and a superoxide anion. The process is catalyzed by iron.

4. PHYSIOLOGICAL SOURCES OF ROS

Most cell types share common pathways for ROS production. Aerobic respiration within the mitochondria of cells is the primary source of physiological ROS as a normal by-product. The mitochondrial electron transport chain (mETC), which consists of four membrane-bound complexes (I, II, III and IV), uses oxygen for energy production. A series of redox reactions occur along the mETC at these complexes to generate a proton gradient for ATP generation. Redox reactions involve a transfer of electrons between molecules, which changes the oxidation state of the molecules involved. If reduction is incomplete during oxidative phosphorylation, the superoxide anion is produced via univalent reduction. Mitochondrial ROS are primarily produced at complexes I and III, but there is some evidence in murine models of production at complex II (5). The localization of these complexes determines where ROS are released and the resulting damage. Complexes I and III release ROS into the mitochondrial matrix, where mitochondrial DNA is easily damaged, while complex III also releases them

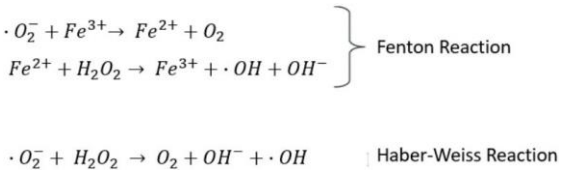

Figure 1. Fenton and Haber-Weiss reactions.

into the intermembrane space, which provides a route to the cytosol (5).

The complexes of the mETC can efficiently carry out redox reactions due to their metal centres, which are primarily in the form of iron-sulfur clusters. Iron homeostasis is critical for the appropriate function of these enzymes, with reduced concentrations impairing the function of enzymes that require iron as a cofactor or structural element while increased concentrations lead to toxic ROS generation (6). Recent evidence suggests that these iron-sulfur complexes may actually act as a sensor for appropriate iron levels within mitochondria (7). While these complexes are the best described sources of ROS, any protein capable of redox reactions could contribute to ROS production. Monoamine oxidases, flavoenzymes of the outer mitochondrial membrane, may produce the highest levels of H_2O_2 of endogenous sources within brain mitochondria (8). The presence of iron results in hydroxyl radical production which exacerbates neurologic damage due to membrane lipid peroxidation and DNA damage (8).

Several other intracellular sources, such as peroxisomes and the endoplasmic reticulum (ER), can also contribute to ROS production. Peroxisomes are small cytosolic organelles associated with various catabolic pathways. Similar to mitochondria, oxidative metabolism in peroxisomes occurs along their electron transport chain on the membrane complexes, which possess cytochrome c reductase and ferricyanide reductase activities that contribute to superoxide anion production (9). Catalase is present in peroxisomes to breakdown the H_2O_2 and 20-60% of produced H_2O_2 will cross the peroxisomal membrane (5). One interesting property of peroxisomes is that the number and size of peroxisomes can be modulated, via activation of peroxisome proliferator activated receptors (PPARs), which in turn alters ROS production. An excess of peroxisomes results in peroxisome-induced oxidative stress, which can be exacerbated by the liberation of complexed transition metals within the peroxisome and lead to peroxisome damage (10). Minor amounts of ROS are produced by various oxidase enzymes in both peroxisomes and the ER (10, 11).

Specific cell types can have unique mechanisms for producing ROS. One such mechanism is a specialized system for drug metabolism bound to their ER, termed the membrane-bound microsomal monooxygenase (MMO) system (12). This system is found in many tissues, but has the highest concentration in liver cells due to their role in drug detoxification (12). The MMO system contains many heme-thiolate enzymes from the cytochrome P-450 superfamily which consist of a conserved core heme and associated iron atom which confers their oxidative properties (13). As the level of oxidation reactions controls ROS production, changes in the activity of these enzymes as a result of various conditions can result in ROS-mediated damage. As an example, enzyme CYP1B1 in pulmonary cells is associated with oxygen-mediated pulmonary toxicity in cases of hyperoxia where its activity increases and ROS production rises (14). Enzyme CYP2E1, found in hepatocytes, shows an increase in activity after heavy alcohol consumption, leading to enhanced ROS production (15).

5. ROLE AND MECHANISM OF ROS AND IRON IN THE IMMUNE SYSTEM

5.1. Role of ROS in the immune system

ROS production is central to our current understanding of how the innate immune system defends against invading pathogens. Phagocytic immune cells, such as neutrophils and macrophages, harness ROS production for host defense. Phagocytic cells recognize pathogens through their pattern recognition receptors (PRRs), and internalize the microbes into internal compartments, termed phagosomes, for degradation via oxidative burst (16). These immune cells possess a unique oxidase enzyme responsible for ROS generation during an oxidative burst. Components of nicotinamide adenine

dinucleotide phosphate (NADPH) oxidase scattered in the cytosol of resting cells are activated by recognition of infection and translocate from the cytosol to the phagosome membrane to form the active enzyme. Electrons are pumped across the membrane where they participate in the univalent reduction of oxygen to superoxide anion (5). NADPH oxidase 2 (NOX2) is the catalytic subunit of the enzyme found in phagocytes, while other cell types possess other isoforms of the enzyme (5) Electrons are transferred from NADPH, facilitated by NOX2, via flavin adenine nucleotide (FAD) and heme to molecular oxygen. Because this is an oxygen-dependent process, triggered ROS production results in a large linear increase in oxygen consumption by the responding cell (17). ROS production in mitochondria also plays an essential role in the innate immune response (18, 19). Evidence showed that in macrophages, stimulated Toll-like receptors (TLRs) recruit mitochondria to the phagosomal membrane and induce mitochondrial ROS production by signaling for the assembly of the mitochondrial respiratory chain (16). Because of the importance of mitochondrial ROS, mitochondrial density can affect the function of innate immune cells (18).

ROS production also plays a role in regulation of the life cycle of macrophages. In the absence of NADPH oxidase, monocytes are unable to differentiate into macrophages (20). In, addition, macrophage polarization is also impacted by ROS. Macrophages can be polarized into different inflammatory profiles, such as pro-inflammatory M1 or anti-inflammatory M2 phenotypes, in response to the microenvironment. The pro- or anti-inflammatory phenotypes are mediated by neutrophils which typically shift the macrophage profile to M1 phenotype during infection (21). Polarization to an anti-inflammatory M2 phenotype is triggered by apoptotic cells to reduce levels of inflammation and to facilitate tissue repairing. ROS production by NADPH oxidase can promote an M1 phenotype, where NADPH oxidase inhibition resulted in an M2-like phenotype in polarizable microglia (22). However, a study by Xu *et al*. found that the deletion of NADPH oxidase impaired M2 phenotype differentiation, but not the M1 phenotype, by affecting the MAPK pathway (20). As evidenced by these variable results, the complex role of ROS in macrophage polarization remains to be fully elucidated.

Additional roles for ROS have been described during apoptosis in lymphocytes. The release of Granzyme B from lymphocytic granules induces the mitochondrial ROS production, infectedwhich is essential for apoptotic signalling in the target cells through cytochrome *c* release and caspase activation (23, 24). High doses of ROS, regardless of the site of generation, can provide an activation signal for apoptosis. Cleavage of the NDUFS1, an iron-containing protein associated with the mETC, enhances mitochondrial ROS production, resulting in amplifying apoptotic signaling (23). NOX2-derived ROS are also involved in induction of apoptosis in infected cells under oxidative stress, although the importance of this is pathogen-specific as ROS production can be induced or repressed dependent on the species of bacteria (24).

Because of the unselective nature of ROS, granulocytes require compensatory mechanisms to protect themselves during respiratory bursts. Rapid acidification occurs during the respiratory burst, which is regulated by a voltage-gated H^+ channel (Hv1) which functions to balance the intracellular charge and is essential for innate immune function (25). No conclusion has been reached on the exact role of this channel in innate immunity. One study suggests NADPH activity becomes suboptimal when the Hv1 channel is absent, but some function is still possible (25). A recent study by Levine *et al.* noted Hv1 functions to optimize the pH of the intracellular vacuole to facilitate killing by proteases, cathepsin G and elastase (26). Notably, Hv1 knock out mice were able to extrude protons, but vacuoles became over-acidified and ROS production was impaired (26). Eosinophils also possess NADPH oxidase systems and can produce ROS. Unlike neutrophils, eosinophils generate ROS predominantly extracellularly (27).

Due to the small size of ROS, they can target local and distal sites acting as both first messengers and secondary messengers in the immune cell signaling (28). ROS can activate local TRPM2 channels to induce Ca^{2+} influx in immune

cells, including macrophages, monocytes, neutrophils, lymphocytes and microglia (29). This Ca^{2+} influx is involved in the nuclear translocation of the transcription factor NF-κB, which is responsible for the production of the human chemokine CXCL2 by monocytes, and ultimately the recruitment of neutrophils to the site of infection (29). TRPM2-mediated Ca^{2+} influx can also associated with NLRP3 inflammasome activation but the mechanism is not well understood (29). It was suggested that the TRPM2 channel is involved in membrane depolarization, and may inhibit NADPH oxidase as a form of negative feedback to control ROS-related tissue damage (29).

5.2. Immune action of specific species of ROS

As mentioned above, ROS encompass oxygen radicals and other reactive species including superoxide anions, hydrogen peroxide and hydroxyl radicals. The immune action of these species of ROS will be described below.

Superoxide anions are highly reactive molecules produced by aerobic cells. Despite this reactivity, most of the antibacterial effects of superoxide anions comes from their role in the pathway to hydrogen peroxide and hypochlorous acid generation (30). Superoxide dismutase (SOD) is an important antioxidant enzyme with a dual role. It controls the level of superoxide anion within the cell by catalyzing the dismutation of superoxide anion to either molecular oxygen or hydrogen peroxide. *Escherichia. coli* contains both iron and manganese forms of SOD, with single mutants growing normally while double mutants are conditionally sensitive to aerobic environments. SOD may be dispensable with respective to the overall lifespan of certain organisms, but it is crucial for response to acute stressors and managing ROS toxicity (31). *E. coli* undergoing oxidative stress from superoxide anion due to high oxygen levels experience impaired amino acid biosynthesis and require supplemented media for growth (32). The superoxide anion destroys the catalytic center of dihydroxy-acid dehydratase and other dehydratase family members, leading to the disruption in amino acid biosynthesis (33).

Hydrogen peroxide is produced by the dismutation reaction of oxygen, the superoxide anion and water, catalyzed by SOD. Hydrogen peroxide is only weakly reactive *in vivo* towards most molecules, with the exception of various H_2O_2-sensitive proteins such as cysteines (34). Hydrogen peroxide can be harmful as it is able to be converted to hydroxyl radicals in the presence of transition metals, including copper or iron, or UV light (35, 36). Recent evidence shows H_2O_2 can target many mononuclear enzymes in *E. coli*, which have iron as a co-factor *in vitro* suggesting H_2O_2 may disrupt more metabolic pathways than previously elucidated (37).

The production of hydrogen peroxide is important for the formation of hypochlorous acid (HOCl), a weak acid, via the peroxidation of chloride ions by myeloperoxidase (MPO), a cationic heme-containing enzyme (38). MPO is abundantly expressed in primary azurophilic granules of neutrophils and the primary lysosomes of monocytes, although this is lost upon differentiation into macrophages (39). HOCl is a major bactericidal product released during an oxidative burst that degrades essential components of mETCs including cytochromes, iron-sulfur proteins and nucleotides (40, 41). On ingestion into the phagosome, any nearby proteins from both the phagocyte and pathogen are rapidly chlorinated due to the non-selective nature of the HOCl (42). Detection and monitoring of highly reactive species is challenging, but the recent development of intracellular probes have allowed in depth examination of the production and function of HOCl (43). Usually a lack of effective bacterial defence against chlorine oxidants and inactivation of other microbial antioxidant systems by HOCl gives an advantage in host microbial defence (44). However, bacteria such as *Pseudomonas aeruginosa* may possess alginate coats which scavenge hypochlorite and inhibit phagocytosis, preventing the rapid clearance of infection (45). *P. aeruginosa* is an opportunistic pathogen and typically only causes infections in the immunocompromised. HOCl production is critical to immune function since deficiencies in MPO increasing susceptibility to infection. MPO knock-out mice show impaired defence against *Candida albicans*, a phenotype which is seen in up to 1 in 4000 humans (39, 46). *C. albicans* is most commonly associated with yeast

infections, but can cause other infections in immunocompromised people. Patients with hereditary MPO deficiencies are usually clinically healthy as they still produce some ROS, but show enhanced susceptibility to *C. albicans* (47).

In addition to their direct actions, various species of ROS can degrade the heme moiety of hemoglobin, a porphyrin which coordinates a molecule of iron. This process results in the generation of free iron which can be used to catalyze the generation of additional ROS. The conjugate base of hydrogen peroxide (HO_2^-) mediates heme degradation via the major chemical pathway, and the production of additional superoxide anions via a minor pathway (48). HOCl mediates the oxidative degradation of heme, which results in both the release of complexed iron and the production of toxic protein aggregates (49). HOCl also exerts negative feedback on MPO by mediating both enzyme inactivation and destruction on the heme moiety, but this process is regulated by both hydrogen peroxide and various scavenging enzymes (50).

5.3. Role of iron in immune defense

Because iron is central to many aspects of the innate immune response, including ROS generation, both the pathogen and the host seek to modulate it in their favor. Hosts attempt to reduce free iron available for pathogens by sequestering it intracellularly, via a process known as nutritional immunity. This prevents pathogen access while ensuring iron is still available to the host in a quantity sufficient for normal immune function. Mechanisms of nutritional immunity include the secretion of iron-binding lactoferrin and the degradation of the iron exporter ferroportin (51). Iron status is known to play an important role in susceptibility to infection which is well described in various studies. Historically, iron overload disorders have been shown to lead to an increased susceptibility to infection, as the oversaturation of host transferrin leads to defective nutritional immunity (52). As iron is also essential for bacterial pathogenicity, iron overload is clearly undesirable. The situation is more complex for iron deficiencies. One recent clinical study observed an increased incidence of bloodstream infections in those with very low iron levels (53). This work suggests that infection susceptibility may be dependent on the level of iron deficiency. This has been described as a U-shaped relationship, with infection risk being increased from either extreme of iron levels (54). The plateau of the U likely represents the desirable level of iron which maintains immune function without promoting infection.

Bacteria have various methods of circumventing iron-withholding to ensure proliferation including iron-uptake systems and siderophore production. Siderophores are low molecular weight iron-binding molecules secreted by bacteria to chelate iron from the system, often increasing the virulence of the pathogen. *Yersinia pestis* possesses a High Pathogenicity Island (HPI) which encodes the siderophore yersiniabactin, essential for the high pathogenicity observed with this species and other Enterobacteriaceae (55). *Y. pestis* is highly virulent and is most famously known for causing the “Black Death” plague. Because yersiniabactin is a better chelator than lactoferrin, it outcompetes host iron acquisition methods and provides iron to the bacterium while reducing its availability for ROS production by neutrophils, monocytes and macrophages (55). The secretion of lipocalin 2 by neutrophils attempts to combat iron acquisition by binding and sequestering siderophore-iron complexes, common to many Gram-negative pathogens (52). Mucosal surfaces often contain high concentrations of lipocalin 2, lactoferrin and other antimicrobial molecules, such as defensins, to restrict iron and contribute to overall bacterial killing (56). Other bacteria may have reduced iron requirements or utilize other metal co-factors. Lactobacilli are widely accepted to have extremely low iron requirements but may utilize many iron sources to promote survival during the stationary growth phase (57). *Borrelia burgdorferi*, a tick-borne pathogen responsible for Lyme disease, is unable to transport iron and instead uses manganese as a co-factor for SOD activity (58).

6. CLINICAL RELEVANCE

In order to avoid the threat of ROS, pathogens possess unique features to modify host ROS production to favor their survival. An understanding of the interactions between the innate

immune system and pathogens is critical for developing appropriate therapies.

Tuberculosis (TB) is a highly infectious respiratory illness, with an estimated 10 million new cases and 1.3 million deaths in 2017 (59). The causative agent of TB, *Mycobacterium tuberculosis*, is an intracellular pathogen that infects alveolar macrophages and activates an adaptive immune response. The main characteristics of TB is formation of granuloma, where the pathogen is surrounded by macrophages, lymphocytes and fibroblasts (60). The formation of granulomas presents a unique challenge for immune eradication, as the pathogen is sequestered but may persist within the granuloma. ROS is suggested to play an important role in the immune response to TB since NADPH oxidase deficiencies, as in CGD, lead to more severe mycobacterial infections and impaired granuloma formation (61). This may be explained by a role for ROS in the promotion of autophagy during intracellular infections (62). *M. tuberculosis* also lacks conventional systems to prevent oxidative stress, making it critical that this pathogen effectively reduces ROS levels (63). In addition, *M. tuberculosis* are able to produce secretory proteins to downregulate ROS production and inhibit NF-κB gene expression within the infected macrophage (63). This inhibition may provide a survival advantage for the pathogen.

Other pathogens also have well described mechanisms for downregulating ROS production as a mechanism for immune evasion. Several common pathogens including *Neisseria gonorrhoeae,* causative agent of gonorrhea, and *C. albicans* are able to suppress the oxidative burst, contributing to pathogenesis (64, 65). *Francisella tularensis* is a highly virulent pathogen, which is considered a security-sensitive biological agent due to its potential for use in bioterrorism (67-69). It is the causative agent of tularemia, which is often fatal when it manifests in its pneumonic form. To avoid the oxidative burst, *F. tularensis*, can interfere with the assembly of NADPH oxidase at two separate points; p47 subunit phosphorylation and $gp91^{phox}$/$gp22^{phox}$ heterodimer formation (66, 67). In addition, *F. tularensis* can produce an acid phosphatase, a soluble enzyme to inhibit the respiratory burst of neutrophils (68). Novel antimicrobials that combine cationic liposome DNA complexes and bacterial membrane protein fractions to stimulate ROS production have been shown to attenuate infections from *F. tularensis*, as well as *Burkholderia pseudomallei*, *Y. pestis*, and *Brucella abortus* (69).

The historical view of ROS-pathogen interactions focuses on the destruction of pathogens by ROS. However, some pathogens seek to increase ROS production for their own benefit. For example, *Entamoeba histolytica* is a parasite responsible for amoebic dysentery in humans. *E. histolytica* induces neutrophil apoptosis by the activation of ERK1/2 by ROS derived from NADPH oxidase (70). This signaling pathway is advantageous to the pathogen since apoptotic neutrophil can no longer respond to an infection. Pathogens such as *Trypanosoma cruzi*, a parasite responsible for Chagas disease, and Japanese Encephalitis Virus (JEV) are also able to enhance ROS production to induce host cell death (71). Other pathogens are well adapted to survive oxidative stress. The superoxide anion provides a germination trigger for spores of *Bacillus anthracis*, another security-sensitive biological agent responsible for anthrax (72). Pathogens that benefit from ROS all feature special adaptations that allow them to survive or at least tolerate these normally toxic molecules, but these mechanisms are yet to be fully described for most mentioned pathogens (62).

7. OXIDATIVE STRESS: BALANCING ROS LEVELS

While ROS are essential for normal physiological functioning, levels must be tightly controlled. Low levels of ROS can serve as mediators, adapting to stress within the system and activating signalling pathways. Higher levels of ROS production can be triggered to respond to pathogenic threats, but this system is meant as a temporary defence. Within the host, physiological scavenging systems exist to mediate levels of ROS and prevent damage to host molecules. These include enzymes, such as superoxide dismutase (SOD), which scavenges superoxide anions, and catalase, which breaks down hydrogen peroxide. Other more specific enzymatic defenses was also reported in specific systems, including the pulmonary system (73). In

addition to enzymatic defenses, non-enzymatic antioxidants exist, including various vitamins, glutathione and carotenoids, such as β-carotene (73). If homeostasis is disrupted, then ROS may become detrimental. This results in oxidative stress, where there is an imbalance between ROS production and detoxifying systems. Because ROS cause indiscriminate damage to many biomolecules, oxidative stress has been linked to diseases including coronary diseases, neurodegenerative disorders and the development of cancer (74).

Bacteria are also susceptible to oxidative stress and possess ROS scavenging enzymes to protect against damage, but the efficacy of these defense systems is limited. *E. coli* only possess enough H_2O_2-scavenging enzymes such as alkyl hydroperoxide and catalase to maintain tolerable intracellular concentrations while bacteria with mutated scavenging systems are rapidly overcome by endogenous ROS (75). This results in most bacteria being very susceptible to immune attack. Bacteria may possess inducible systems, such as the SoxRS and OxyR redox-sensitive transcription factors in *E. coli*, to cope with exogenous stress by promoting gene expression. The Cys residues of OxyR react preferentially with H_2O_2, which regulates genes for peroxide scavenging, while the Fe-S clusters of SoxRS react with the superoxide anion to activate genes for superoxide scavenging (76). These systems are activated to maintain homeostasis when constitutive systems become overwhelmed, with analogous systems being found in many bacteria. PerR serves as a similar function to OxyR in many Gram-positive bacteria (77). *Salmonella typhimurium*, often implicated in food poisoning, possesses the Salmonella Pathogenicity Island 2 (SPI2) that activates a Type III secretion system, which secretes proteins from the bacterial cell directly into the eukaryotic host cell to prevent the trafficking of NADPH oxidase within phagocytes (78). This system allows the pathogen to avoid the NADPH-dependent respiratory burst and may explain how SoxRS mutant *S. typhimurium* maintain virulence (79). Bacteria also scavenge iron internally to reduce ROS levels. Dps is an iron-binding protein identified in over 100 species of bacteria, which scavenges iron and is involved in H_2O_2 detoxification (80). These systems present a challenge to immune cells attempting to combat infections via ROS production.

8. CURRENT DIRECTIONS

ROS continues to be a topic of interest in the field of immunology due to its potential for immune response modulation. The role of ROS in various diseases and illnesses is well-described, so current research focuses primarily interactions with other systems and molecules and on the applications of anti-oxidative therapies. ROS have been observed interacting with many other biomolecules. For example, hydrogen sulfide has recently been identified as a signaling molecule that works in combination with both ROS and nitric oxide, modulating vascular tone (81). Interactions between ROS and zinc have also been observed, amplifying damage during cerebral ischemia (82).

One major line of research relates to the role of ROS in neurodegenerative diseases, such as Alzheimer's disease, which has long been speculated on but is challenging to confirm in patients. Yang *et al*. recently designed a chemical probe, allowing for the detection of high levels of ROS in diseased brains (83). Other detection methods are currently under development, including radiometric fluorescent probes and nanoparticle-based techniques (84, 85). This probe will allow confirmation of increased ROS levels in patients, which is a key piece of knowledge needed for developing targeted therapies. Recent evidence suggests that ROS generation in the brain may result from an increase in ROS-active metal ions, such as iron or copper (86). This suggests that modulation of iron levels may have therapeutic benefit in this setting. Iron chelation, which temporarily binds excess iron in the system, makes iron unavailable to both the host and the pathogen. Potential benefits of iron chelation include a reduction in ROS generation by preventing iron catalysis of the Fenton reaction and reducing iron availability for bacterial metabolism and growth. Part *et al*. recently demonstrated that intracellular iron chelation can reduce the production of ROS by amyloid peptides (87).

Iron chelation has been classically used to treat iron overload disorders, and have been recently

studied in anti-inflammatory, anti-microbial and anti-cancer settings. Novel chelator DIBI has been shown to have anti-inflammatory effects in an experimental sepsis model, likely mediated through a reduction in ROS production (88). Further studies using DIBI have demonstrated potent anti-microbial activity against Gram-positive and Gram-negative bacteria and fungi (89). Iron chelation may also have a role to play in cancer treatments. Iron chelator Deferasirox has been shown to have anti-cancer activities against acute myeloid leukemia, possibly mediated by a transient increase in ROS levels (90). Recent advancements include the development of a ROS-responsive degradable nano-gel, which improves excretion of chelated iron to reduce toxicity associated with iron chelation (91).

As seen in the case of the anti-cancer activity of Deferasirox, an iron chelators, research is not always directed at reducing ROS levels. Cancer cells are often undergoing oxidative stress, resulting in increased ROS production. Leinamycin, generated by *Streptomyces atroolivaceous*, is an anti-tumor antibiotic that is activated by ROS molecules (92). ROS are also harnessed by some types of antibiotics to combat invading pathogens. ROS are known to be important for the bactericidal activity of quinolone antibiotics (93). Not all antibiotic killing is dependent on ROS production, but they can play a role in secondary damage and pathogen demise when the bacterium being targeted begins to experience oxidative stress (94). These are just some examples of the broad range of applications emerging in the fields of inflammation, infection and cancer research.

9. CONCLUSIONS

ROS is critical for the host response to invading pathogens, playing a role in the oxidative burst of innate immune cells and in various signaling pathways. One key component to this ROS response is iron, which plays a central role in ROS generation and bacterial pathogenesis. This relationship is well described in the literature. Changes in iron levels can modulate ROS production, so both the host and pathogen have evolved mechanisms to shift levels of both iron and ROS in their favor. As these mechanisms are pathogen specific, they may be important for the development of effective therapeutics. Despite its importance in pathogen destruction, ROS can be detrimental if oxidative stress occurs. Both pathogens and hosts have developed mechanisms to manage ROS production and reduce damage, including altering iron levels. Recent evidence indicates that ROS may not always be harmful to the pathogen, so more research is necessary to fully understand the complex interactions between the host and pathogen during infection. Current lines of research are examining how iron and ROS may play a role in other biological systems and how these interactions can be modulated clinically. These processes can be targeted to exert anti-inflammatory, anti-bacterial and anti-cancer actions.

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Abbreviations: CYP: Cytochrome P450, H_2O_2: Hydrogen peroxide, HOCl: Hypochlorous acid, mETC: Mitochondrial electron transport chain, MPO: Myeloperoxidase, NADPH: Nicotinamide adenine dinucleotide phosphate, NFkappaB: Nuclear factor-kappa B, OHo: Hydroxyl radical, O^{2-}: Superoxide anion, ROS: Reactive oxygen species, TRPM2: Transient receptor potential cation channel 2

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