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Microreview

Myeloperoxidase in human neutrophil host defence

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Summary

Human neutrophils represent the predominant leucocyte in circulation and the first responder to infection. Concurrent with ingestion of microorganisms, neutrophils activate and assemble the NADPH oxidase at the phagosome, thereby generating superoxide anion and hydrogen peroxide. Concomitantly, granules release their contents into the phagosome, where the antimicrobial proteins and enzymes synergize with oxidants to create an environment toxic to the captured microbe. The most rapid and complete antimicrobial action by human neutrophils against many organisms relies on the combined efforts of the azurophilic granule protein myeloperoxidase and hydrogen peroxide from the NADPH oxidase to oxidize chloride, thereby generating hypochlorous acid and a host of downstream reaction products. Although individual components of the neutrophil antimicrobial response exhibit specific activities in isolation, the situation in the environment of the phagosome is far more complicated, a consequence of multiple and complex interactions among oxidants, proteins and their by-products. In most cases, the cooperative interactions among the phagosomal contents, both from the host and the microbe, culminate in loss of viability of the ingested organism.

Background

While in search of a tissue source of catalase that would lack the biliverdin that contaminated liver preparations, Kjell Agner discovered in tuberculous empyema a green enzyme that was structurally different from other proteins known at that time (Agner, 1941). Agner described many of the structural and enzymatic properties of the green enzyme, verdoperoxidase, in his doctoral thesis for medical school (Agner, 1941). Two years later, in 1943, Theorell and Åkeson purified a brown-green peroxidase from cow's milk and demonstrated that it had spectral properties distinct from those of Agner's leucocyte-derived verdoperoxidase (Theorell and Åkeson, 1943). Recognizing that despite both enzymes being green, they originated from different sources, Theorell and Åkeson proposed the names myelo-peroxidase for Agner's enzyme and lacto-peroxidase for the milk enzyme.

Whereas Agner's subsequent studies detailed how myeloperoxidase (MPO) inactivated bacterial toxins (Agner, 1947; 1950; 1955), Schultz initiated seminal studies to define the biological relevance of MPO in a broader context. Noting that MPO represented $>5\%$ of the dry weight of human neutrophils (Schultz and Kaminker, 1962), Schultz purified the protein from neutrophils isolated from human blood and defined many of the structural and enzymatic features of MPO. Pioneering studies by Zgliczynski, Schultz and others elucidated much of the chemistry supported by MPO and speculated how it might participate in human host defence (Schultz and Kaminker, 1962; Zgliczynski *et al.*, 1968; 1971; Harrison and Schultz, 1976; Zgliczynski and Stelmaxzynska, 1979; Zgliczynski, 1980). Seminal studies by Klebanoff and colleagues established the critical links between the presence of MPO in neutrophils, halogenation of ingested bacteria in phagosomes, and the microbicidal action of the MPO-H_2O_2-Cl system (Klebanoff, 1967; 1968; 1970a,b; 2005; Klebanoff and Hamon, 1972).

Based on a comprehensive analysis of the evolutionary relationships among sequences of peroxidase proteins in the plant and animal kingdoms, the Obinger laboratory has recently categorized MPO and other proteins previously called animal peroxidases as members of Chordata peroxidase subfamily (Zamocky *et al.*, 2008). All members expressed in mammals, including MPO, EPO, LPO and TPO, contain conserved motifs on both proximal and distal sides of the essential haem prosthetic group, a calcium binding site, and at least two covalent bonds linking the haem group to the protein backbone. In

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addition to the conserved ester linkages in the haem pocket, MPO contains a third, a sulfonium linkage between the 2-vinyl group and methionine 409. The presence of the sulfonium linkage empowers MPO with unique spectral qualities as well as greater oxidizing potential, rendering MPO the only member of the protein superfamily able to oxidize Cl^- to Cl^+ and thereby generate HOCl at physiologic pH (Furtmuller *et al.*, 2003; Arnhold *et al.*, 2006; Zederbauer *et al.*, 2007).

Collectively, all peroxidases support the same general chemistry; they catalyse one- and two-electron oxidations of susceptible substrates, both organic (AH_2) and inorganic, such as halides (X^-), to promote structural changes with physiological consequences:

$$H_2O_2 + 2\,AH_2 \text{ ---peroxidase----} \rightarrow 2\,H_2O + 2\,{}^{\bullet}AH \text{ (reaction 1)}$$

$$H_2O_2 + X^- + H^+ \text{ ---peroxidase----} \rightarrow H_2O + HOX \text{ (reaction 2)}$$

Peroxidase-mediated post-translational modifications underlie a diverse set of biological phenomena, from thyroid hormone synthesis (Ruf and Carayon, 2006) to pathophysiologic lipoprotein modification (Undurti *et al.*, 2009), to cross-linking of fertilized sea urchin eggs to block entry of sperm arriving too late (Foerder and Shapiro, 1977). In all situations, the principles remain the same, only the substrate and context differ. This review focuses attention on MPO-mediated events in neutrophil phagosomes, the highly specialized space where ingested microbes are confined, and in the context of their contribution to host defence against infection. Extensive and elegant studies have examined MPO-dependent biochemistry in other inflammatory diseases, most notably cardiovascular disease, and the interested reader will be richly rewarded by reading any of several recent and excellent reviews (Malle *et al.*, 2003; Nicholls and Hazen, 2009; Shao *et al.*, 2012; Nussbaum *et al.*, 2013).

Players

The essential elements of haem peroxidase-based biochemistry include the peroxidase, a source for H_2O_2, and substrates that can undergo one or two electron oxidation (Dunford, 1999). In the context of events within the phagosome of human neutrophils, the identities of the peroxidase and the H_2O_2 source, MPO and the phagocyte NADPH oxidase, respectively, are well established (Fig. 1). In addition, the voltage-gated proton channel, Hv1, provides the charge compensation required to sustain NADPH oxidase activity (DeCoursey, 2013). Less

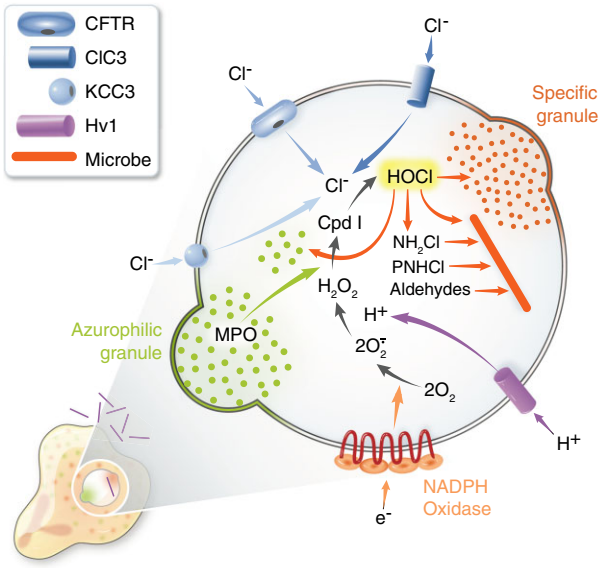

Fig. 1. MPO-dependent events in human neutrophil phagosomes. Concomitant with phagocytosis, human neutrophils assemble and activate the NADPH oxidase and recruit granules to fuse with nascent phagosomes. The NADPH oxidase transfers electrons into the phagosome, generating superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) from molecular oxygen. The voltage-gated proton channel Hv1 provides the bulk of charge compensation necessitated by electron transfer from cytoplasm, thereby allowing sustained generation of oxidants in close proximity to the ingested microbe. Chloride (Cl^-) from the neutrophil cytoplasm enters phagosomes predominantly via cystic fibrosis conductance regulator (CFTR), although ClC3 and KCC3 support some Cl^- influx. MPO, provided by azurophilic granules, and H_2O_2 react to yield Compound I (Cpd I), which catalyses the two electron oxidation of Cl^- to Cl^+ to produce hypochlorous acid (HOCl) or bleach. HOCl reacts with peptides and proteins from host and microbe to generate an array of products, including monochloramines (NH_2Cl), protein chloramines (PNHCl), which can then decompose to form aldehydes. Granule proteins, both native and oxidant-modified, HOCl and its derivatives attack microbial targets, with the outcome dependent both on the number and accessibility of vulnerable targets as well on the defensive responses specific to the ingested organism.

well characterized is the source of chloride to support HOCl production. The MPO-H_2O_2-Cl system requires a continuous supply of Cl, as the small amounts internalized incidentally during phagocytosis are rapidly consumed as degranulation and NADPH oxidase activation occur (Winterbourn *et al.*, 2006). The unusually high chloride concentration in neutrophil cytoplasm provides a reservoir to meet intraphagosomal needs, and several different transporters serve to deliver the needed chloride. Cystic fibrosis transmembrane conductance regulator (CFTR) transports the bulk of the chloride from cytoplasm into the phagosomal lumen (Aiken *et al.*, 2012; Zhou *et al.*, 2013). CFTR, a constituent of secretory vesicle, but not plasma, membrane in resting neutrophils, reaches the nascent phagosomes as granules fuse during phagocytosis, thereby providing the means to transfer chloride from the

cytoplasmic reserves (Painter *et al*., 2006; Zhou *et al*., 2013). The steady state concentrations of chloride in phagosomes of patients with cystic fibrosis (25.5 ± 3.3 mM, $n = 5$) or normal neutrophils treated with the CFTR inhibitor GlyH-101 (27.7 ± 4.4 mM, $n = 5$) are significantly lower than that in normal neutrophil phagosomes (67.7 ± 7.3 mM, $n = 5$) (Painter *et al*., 2010). Although the measured values may be higher than the *bona fide* steady state concentration due to technical reasons,[1] optimal MPO activity in phagosomes requires chloride transport via CFTR; phagosomes containing genetically or pharmacologically dysfunctional CFTR exhibit reduced MPO-dependent modification of targets (*vide infra*) and killing of ingested *Pseudomonas aeruginosa* (Painter *et al*., 2008). In addition to CFTR, the anion transporter ClC3 (Moreland *et al*., 2006) and the K^+/Cl^- co-transporter KCC3 (Sun *et al*., 2012) also contribute to chloride transport into neutrophil phagosomes.

Products of the MPO-H_2O_2-Cl system

Compound I, the Fe(IV) cation radical produced by the reaction between H_2O_2 and native MPO (Marquez *et al*., 1994; Furtmüller *et al*., 1998), can support one-and two-electron oxidations of an array of substrates, including halides, the pseudohalide thiocyanate, amino acids, proteins, lipids and nucleic acids (reviewed comprehensively in Pattison and Davies, 2006; Summers *et al*., 2008). Even after restricting consideration to the chemistry plausible in the phagosome, the number of potential reactions exceeds facile comprehension. Rather than catalogue the multiple and diverse possibilities, I will summarize key reactions likely to occur in human neutrophils and contribute, directly or indirectly, to killing ingested organisms. Many of the details of reactions and their kinetics will be omitted here, instead directing the interested reader to recently published excellent reviews (Hurst, 2012; Klebanoff *et al*., 2013; Winterbourn and Kettle, 2013).

For the sake of simplifying the presentation, consider HOCl as the most proximal effector product of the MPO-H_2O_2-Cl system. The product of the two-electron oxidation of Cl^-, HOCl exists in equilibrium with OCl^- at the near neutral pH in neutrophil phagosomes, as the pK_a is 7.53 at 30°C. Under conditions that include substrates that would compete with Cl^- for oxidation by Compound I, the number of potential reaction substrates multiplies. The reactions of HOCl with amino acids, peptides, and proteins have been extensively studied (for review see Hawkins *et al*., 2003) and generate products, including chloramines and eventually aldehydes (Zgliczynski *et al*., 1971) that can exert antimicrobial activity as well (Thomas *et al*., 1986), and can generate tyrosyl radicals that prompt protein dimerization and aggregation (Vissers and Winterbourn, 1991). HOCl generated by MPO produces mono- and dichloramines of primary amines, including those on neutrophil granule proteins present within phagosomes (Thomas *et al*., 1982). Although these represent quantitatively minor reactions, they can be long-lived and, depending on the concentration and structural features, can contribute to antimicrobial action. Some products, such as the monochloramine NH_2Cl generated by the reaction of HOCl and NH_4^+, can penetrate the hydrophobic surfaces of particular microbes and thereby gain access to vulnerable intracellular targets (Thomas, 1979b). In the case of *E. coli*, MPO-dependent generation of long-lived chloramines and chloramide derivatives correlates with oxidation of critical bacterial sulfhydryl groups and loss of microbial viability (Thomas, 1979a,b). The magnitude of the contribution of chloramines and other HOCl-derived agents to antimicrobial action varies as a function of the size, charge, and polarity of the toxin produced, the chemical composition of the surface of the ingested microbe, and the specific vulnerable targets in the organism.

The abundant supply of MPO ideally equips neutrophils for service in innate immune defence against microbes, converting the H_2O_2 produced by the NADPH oxidase into the potent microbicide HOCl. H_2O_2 possesses greater redox potential than does HOCl (1.776V versus 1.482V) but exerts much less antimicrobial action (Klebanoff and Clark, 1978; Hurst and Barrette, 1989; Elzanowska *et al*., 1995). For example, *E. coli* washed with EDTA to remove adventitious metal cations that might support Fenton chemistry can tolerate 50 mM H_2O_2 for more than 40 min without a loss in viability (Elzanowska *et al*., 1995). In part, this tolerance reflects the detoxifying action of peroxide-consuming enzymes present in aerobic organisms, as without such enzymes, even endogenously produced H_2O_2 can exert substantial DNA damage (Park *et al*., 2005). H_2O_2 oxidizes cysteine and methionine residues most readily but at relatively slow rates compared with the activity of HOCl. For example, the rate constant for thiol oxidation by H_2O_2 is 2.9 M^{-1} s^{-1} (Imlay, 2003), whereas that for HOCl is ~a million-fold faster, 3.0×10^7 M^{-1} s^{-1} (Pattison and Davies, 2001). The rapid reaction kinetics of HOCl enhances its capacity to attack microbial targets that may be accessible only briefly. Supporting that prediction, HOCl, but not H_2O_2, prompts protein aggregation *in vitro* (Winter *et al*., 2008), presumably by irreversibly modifying vulnerable targets that are exposed only transiently during the continuous folding and unfolding of protein conformations.

[1]Note that these measurements likely overestimate the true chloride concentration, because the intrinsic susceptibility of the fluorescent probe used to detect chloride requires inclusion of sodium azide in experimental conditions. Consequently, chloride consumption by MPO is inhibited under the experimental conditions.

Targets of the MPO-H_2O_2-Cl system

The specific target and the HOCl-mediated changes that culminate in microbial death have not been defined and vary among organisms (Hurst, 2012). All things being equal with respect to inherent susceptibility of microbial components to modification, spatial arrangements dictate that surface structures would be the first to encounter MPO-derived toxins. However, in many cases, HOCl attacks surface structures but with little consequence on bacterial viability. Instead, loss of function supported by proteins in bacterial inner membranes correlates with microbial death (reviewed in Hurst, 2012), as does HOCl-dependent oxidation of methionine residues in cytosolic and inner membrane proteins of *E. coli* (Rosen *et al*., 2009). Whatever the underlying chemistry, HOCl kills within milliseconds (Albrich and Hurst, 1982) and more efficiently than does H_2O_2 in a variety of experimental settings. Based on the number of molecules of oxidant/organism needed to kill *E. coli*, HOCl is ~ 1000-fold more potent than is H_2O_2 ($0.4–5.0 \times 10^8$ versus $> 3 \times 10^{11}$) (Lymar and Hurst, 1995). Similarly, the relative dose-dependent killing of *K. pneumoniae* demonstrates 50% reduction in viability at 60 min for 1 μM HOCl versus 1 mM H_2O_2 (Hirche *et al*., 2005). Like many experimental systems, both of these examples utilized buffer systems without protein and were thus free of competing substrates for the oxidants. As discussed later, teasing out 'lethal hits' from the array of competing reactions with inadvertant targets that constitute complex biological systems, as in neutrophil phagosomes, represents a formidable challenge to constructing *in vitro* conditions that accurately mirror event in neutrophils and be used to assess how neutrophils kill.

Diversity among the microbes with respect to surface composition (*e.g.* presence or absence of capsule, composition of capsule if present, lipopolysaccharides in enteric Gram negative rods versus lipoteichoic acid in Gram positive bacteria) undermine the notion of the existence of a single microbial target. Furthermore, microbes sense attack by antimicrobial agents and respond to threats to their viability by evading toxins, enduring the modifications to structure or function, repairing host-induced damage, or all of the aforementioned actions. In some cases, the bacterial responses match predictions based on current understanding of events in phagosomes. The different transcriptional responses of *E. coli* ingested by neutrophils from normal individuals versus those with chronic granulomatous disease (CGD), an inherited deficiency in NADPH oxidase activity, illustrate the remarkable specificity and speed with which an organism can respond to its environment. For example, *E. coli* fed to normal neutrophils modulate expression of > 70 genes, including upregulation of genes such as *OxyRS* that respond to H_2O_2 (Staudinger *et al*., 2002). In contrast, the same *E. coli* fed to CGD neutrophils failed to upregulate H_2O_2-responsive genes, as anticipated given the absence of oxidants in CGD phagosomes. In other settings, bacterial responses affirm the complexity of biochemistry in normal neutrophils. MPO-mediated HOCl oxidizes methionine residues and methionine sulfoxide (MeO) production correlates with loss of viability of *E. coli* (Rosen *et al*., 2009). Deletion of methionine sulfoxide reductase (*msr*) from *E. coli* eliminates the capacity to regenerate methionine from MeO and increases susceptibility to HOCl. Likewise, *msr*-deletion renders *S. aureus* more susceptible to oxidant and neutrophil killing (Pang *et al*., 2014). These data jibe with expectations, given the recognized importance of oxidants in killing and the role of msr in the repair of oxidant damage. Whereas *S. aureus* ingested by normal neutrophils increase expression of *msr,* upregulation is far greater in neutrophils without NADPH oxidase activity. Furthermore, purified neutrophil granules alone, without added oxidants, promote *msr* upregulation in *S. aureus*. Collectively, these data demonstrate that ingested bacteria react promptly and selectively to neutrophil-derived toxins but often in ways not readily explained by current models of antimicrobial activity. However, the unexpected observation that *S. aureus* upregulates *msr* under conditions free of exogenous oxidants illustrates the complex interactions among phagosomal contents.

In situ

A significant fraction of the oxygen consumed by activated neutrophils can be recovered as HOCl, and a sensitive, specific, and readily performed assay can be used to quantify extracellular HOCl production by stimulated neutrophils (Dypbukt *et al*., 2005). HOCl represents from 28% to 72% of the oxygen consumed by phorbol myristate acetate (PMA)-stimulated neutrophils (Weiss *et al*., 1982; Foote *et al*., 1983; Thomas *et al*., 1983; Chapman *et al*., 2002). The susceptibility of the fluorophores green fluorescent protein (GFP) to bleaching by chlorination of Y66 (Espey *et al*., 2002) provides a specific and selective tool to monitor HOCl generation with neutrophil phagosomes. Both *E. coli* (Palazzolo *et al*., 2005) and *S. aureus* (Schwartz *et al*., 2009) stably expressing GFP have served as probes for neutrophil HOCl production. In addition to HOCl being generated within phagosomes, the loss of viability directly correlates with MPO-dependent modification of ingested bacteria.

Compartmentalization of ingested microorganisms within phagosomes not only limits their access to vital nutrients but also creates conditions that enhance toxicity

of antimicrobial agents present. From the perspective of chemistry, the close approximation of phagosomal membrane to the microbial surface creates a relatively small reaction zone for oxidants to attack soluble substrates in phagosomal fluid. Consequently, the tight quarters favour reactions between oxidants and microbe even in an environment rich in competing substrates (Lymar and Hurst, 1995). Additionally, the very small volume of the phagosome, estimated to be 1.2 fl (Winterbourn *et al*., 2006), allows high concentrations of oxidants to be achieved, and the ability of MPO to adhere to some bacteria enhances the effective concentration of toxic agents at the site of attack.

Adopting a reductionist approach to examine *in vitro* the antimicrobial activity of MPO, most investigators utilize the MPO-H_2O_2-Cl system (Klebanoff, 1968) free of other neutrophil granule proteins. However, during phagocytosis, fusion of neutrophil granules delivers agents, including proteolytic enzymes and proteins with direct antimicrobial activity (reviewed in Rorvig *et al*., 2013), which contribute to the antimicrobial activity and can serve as substrate for HOCl. Accessibility and inherent susceptibility determine sites for attack by the MPO-H_2O_2-Cl system, rendering both exogenous substrates originating from ingested microbes and endogenous neutrophil-derived molecules released into phagosomes during degranulation equally eligible targets.

The complex mix of synergistic as well as antagonistic interactions dictates the fate and overall survival of bacteria in neutrophils (Nauseef, 2007; Hurst, 2012; Winterbourn and Kettle, 2013). Neutrophil elastase (NE) enhances the killing of *S. aureus* as well as *E. coli* by MPO-H_2O_2-Cl by a mechanism that is independent of protease activity, as heating NE does not compromise its synergistic influence (Odeberg and Olsson, 1976). NE augments cathepsin G-mediated killing as well, but cathepsin G does not synergize with the MPO-H_2O_2-Cl system. Adding to the complexity of so many interactions among effector molecules, MPO-mediated modifications can inactivate many of the granule proteins in neutrophils (Voetman *et al*., 1981). Although only some have been examined, many granule proteins undergo irreversible inactivation in the presence of HOCl or MPO-H_2O_2-Cl, including acid phosphatase, N-acetyl-β-glucosaminidase, β-glucuronidase, α-fucosidase, α-mannosidase, lysozyme, vitamin B_{12}-binding protein, MMP-7, 8, and 9, cathepsin G and NE (Kobayashi *et al*., 1982; Clark and Borregaard, 1985; Fu *et al*., 2001; 2003; Hawkins and Davies, 2005; Hirche *et al*., 2005). Whereas HOCl or the MPO-H_2O_2-Cl system inactivates NE, cathepsin G and MMP-7, H_2O_2 alone does not (Fu *et al*., 2003; Hirche *et al*., 2005; Shao *et al*., 2005). Furthermore, HOCl at low concentrations inactivates NE without prompting changes in apparent size after SDS-PAGE, whereas at high concentrations of HOCl NE undergoes complete degradation (Hirche *et al*., 2005), suggesting that extensive oxidation of NE by HOCl may induce its autodegradation.

MPO can mediate the same inactivation of lysosomal enzymes in more complex biological settings, as in supernatants from stimulated neutrophils. Supernatants from stimulated neutrophils from patients with CGD or MPO deficiency, cells lacking essential elements for MPO-mediated chemistry, contain more lysosomal enzyme activity than do secretions from normal neutrophils, and the activity of lysosomal enzymes released by normal neutrophils are increased under anaerobic conditions, in the presence of inhibitors of MPO or the NADPH oxidase, or by adding agents that scavenge oxidants (Kobayashi *et al*., 1982; Clark and Borregaard, 1985; Weiss *et al*., 1985; Fu *et al*., 2003; Hirche *et al*., 2005). Not directly relevant to host–pathogen interactions but pertinent to inflammation in general, MPO can oxidatively inactivate plasma protease inhibitors and thereby modulate extracellular tissue damage at inflammatory sites (Clark and Klebanoff, 1979; Clark *et al*., 1980; 1981; Clark, 1983; Hawkins and Davies, 2005). As a result of egalitarian attack of the MPO-H_2O_2-Cl system on substrates whether from man or microbe, the granule proteins released into phagosomes can be chemically modified and functionally modulated, enhanced as well as reduced, depending on the particular agent and the specific modification. Recognition of such interactions and their consequences figures into the final analysis of overall events in the neutrophil phagosome.

Role for MPO in the antimicrobial activity of intact neutrophils

Neutrophils possess MPO and can generate HOCl, which can exert rapid microbicidal action on a wide variety of microorganisms, but do they rely on MPO to kill ingested microbes trapped in phagosomes?

Studied *in vitro* and compared with normal neutrophils, those deficient in MPO have defective killing of bacteria as well as fungi, with variation among organisms in the degree of the defect. For example, killing of *S. aureus* after 30 min by MPO-deficient neutrophils is ~ 30% of that by normal neutrophils but ~ 80% of normal when *E. coli* are fed to neutrophils (Klebanoff and Hamon, 1972; Klebanoff *et al*., 2013). Furthermore, MPO-deficient neutrophils kill susceptible bacteria more slowly than do normal neutrophils, but in some cases achieve the same success in the end (Lehrer *et al*., 1969; Hampton *et al*., 1996; Decleva *et al*., 2006). In contrast to the slow but eventually effective killing of selected bacteria, MPO-deficient neutrophils lack the capacity to kill *Candida albicans* (Decleva *et al*., 2006), a defect that parallels

the clinical picture of some individuals with MPO deficiency (*vide infra*). The variation in antimicrobial activity as a function of the specific organism is not unique to MPO deficiency but seen as well in CGD (Holland, 2013). In fact, normal, CGD and MPO-deficient neutrophils kill *E. coli* equally well (Rosen and Michel, 1997).

Experimental infection models using MPO knock-out mice have demonstrated increased susceptibility of deficient mice to challenges with a variety of organisms, including *Klebsiella* and *Candida* (Aratani *et al*., 1999; 2000; 2006; Hirche *et al*., 2005), and to the caecal ligation and puncture model (Gaut *et al*., 2001). Data from some of the animal studies highlight a relationship between organism load and the impact of MPO on outcome of infection. When the inoculum of infection with fungi is high, eradication of infection and survival require MPO. In contrast, oxidants generated by the NADPH oxidase suffice in responding effectively to challenges with low inocula (Aratani *et al*., 2002a,b). Although the validity of extrapolations from MPO knock-out mice to humans can be undermined by inherent differences between phagocytes from the two species with respect to NADPH oxidase activity, MPO content and the repertoire of MPO-independent antimicrobial agents such as defensins (Rausch and Moore, 1975; Eisenhauer and Lehrer, 1992; Mestas and Hughes, 2004; Rittirsch *et al*., 2007), the contributions of MPO to the inflammatory process *per se*, independent of direct antimicrobial action, also obfuscate interpretation of the results of experimental models. MPO knock-out animals express higher levels of induced nitric oxide synthase, generate more nitric oxide, and exhibit less lung injury in response to sepsis or endotoxin (Brovkovych *et al*., 2008; Haegens *et al*., 2009; Takeuchi *et al*., 2012), demonstrating that overall survival from infection reflects important factors in addition to the eradication of viable organisms.

As reviewed recently (Klebanoff *et al*., 2013), widespread application of flow cytometry in clinical laboratories upended appreciation of the prevalence and clinical phenotype of inherited MPO deficiency in humans. Only two patients with MPO deficiency had been reported (Grignaschi *et al*., 1963; Undritz, 1966) prior 1969 when Lehrer and Cline described a young diabetic male with MPO deficiency and disseminated candidiasis (Lehrer and Cline, 1969) and reports of MPO deficiency were infrequent until haematology laboratories adopted peroxidase staining and flow cytometry to identify and enumerate cells in peripheral blood. Subsequently, studies demonstrate that MPO deficiency occurs relatively commonly, affecting 1 in 2000 to 4000 healthy individuals in North America and Europe (Kitahara *et al*., 1981; Kutter *et al*., 1994; Kutter, 1998) and 1 in 57 000 in Japan (Nunoi *et al*., 2003). With the exception of systemic candidiasis in the presence of diabetes mellitus, few MPO-deficient patients with increased or unusual infections have been reported. Although the paucity of infectious complications in MPO-deficient subjects could be offered as evidence against the importance of MPO in host response to infection, that would be a superficial interpretation of the data, as MPO-deficient neutrophils are not equivalent to neutrophils that lack only MPO (Klebanoff, 1970b). Klebanoff demonstrated that MPO-deficient neutrophils kill test microbes more efficiently than do normal neutrophils treated with azide (to inhibit MPO) and that killing by MPO-deficient neutrophils is not inhibited by azide treatment, together suggesting that myeloid cells in MPO-deficient subjects may adapt to the deficiency by enlisting antimicrobial systems that compensate. However, the absence of MPO has consequences for antimicrobial action that reflect the loss of HOCl-dependent modification of neutrophil granule proteins.

The many synergies among antimicrobial toxins acting in phagosomes sabotage reaching a straightforward answer to questions about the role of MPO in human host defence. The absence of MPO eliminates not only the capacity to deliver lethal damage to target microbes but also to modify the structure and activity of other antimicrobial elements present in phagosomes. Without MPO, granule proteins such as NE, cathepsin G, and MMP-7, agents otherwise inactivated by the MPO-H_2O_2-chloride system, will be functional and exert antimicrobial action not present in the phagosomes of normal neutrophils. Furthermore, the ambient H_2O_2 concentration in phagosomes lacking MPO will be higher than that in normal neutrophils for two reasons. MPO terminates activity of the NADPH oxidase (Jandl *et al*., 1978) and MPO-deficient neutrophils have prolonged oxidase activity and generate more H_2O_2 than do normal neutrophils (Klebanoff and Pincus, 1971; Klebanoff and Hamon, 1972; Rosen and Klebanoff, 1976; Nauseef *et al*., 1983). In addition, the absence of MPO, which normally rapidly consumes H_2O_2 to generate HOCl, will further increase the concentration of H_2O_2 (Winterbourn *et al*., 2006). The altered oxidant composition and tone in MPO-deficient phagosomes modifies not only the available effector molecules but also substrates on and in microbes that constitute vulnerable targets. Microbial sites may be more or less susceptible to attack after modification by H_2O_2 versus HOCl. Taken together, these factors suggest that contrasting the prevalence of infection *in vivo* or antimicrobial action *in vitro* of normal and MPO-deficient neutrophils overlooks the downstream effects of MPO-H_2O_2-Cl on the collective antimicrobial activity of other oxidants and granule proteins in phagosomes and offers only limited insight into the contribution of MPO to normal neutrophil function.

Take home

As noted nearly four decades ago, initial neutrophil-dependent killing of microbes depends on a system that can be inhibited by azide (Koch, 1974). The most rapid and complete antimicrobial action by human neutrophils against many organisms relies on H_2O_2 from the phagocyte oxidase and MPO from azurophilic granules. MPO supports production of lethal amounts of HOCl in human neutrophils, bacteria recovered from phagosomes have MPO-specific post-translational modifications, MPO-mediated changes in bacteria correlate with loss of their viability, and MPO-deficient neutrophils kill many species of microorganisms more slowly than do normal cells. Collectively, these data suggest that efficient antimicrobial activity by neutrophils depends on the MPO-H_2O_2-Cl system.

The strikingly different clinical phenotypes of humans lacking the NADPH oxidase or MPO highlights the complexity of interactions within phagosomes that culminate in the death of ingested microbes. The dominant oxidant present in normal (HOCl), MPO-deficient (H_2O_2), and CGD (none) neutrophils reacts differently with both microbial targets as well as vulnerable sites on granule proteins, thereby altering the overall antimicrobial 'tone' in phagosomes in the distinctly different settings.

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Disclosures

The author has nothing to disclose.

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