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REVIEW

Neutrophil Myeloperoxidase: Soldier and Statesman

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Abstract Myeloperoxidase (MPO) is a major protein constituent of the primary granules of vertebrate neutrophils. It catalyses the hydrogen peroxide-mediated oxidation of halide ions to hypohalous acids, especially HOCl. These reactive oxygen species can participate in a variety of secondary reactions, leading to modifications of amino acids and many types of biological macromolecules. The classic paradigm views MPO as a component of the phagocyte oxygen-dependent intracellular microbicidal system, and thus an important arm of the effector phase of innate immune responses. However, the limited immunodeficiency associated with lack of MPO in mouse and human models has challenged this paradigm. In this review we examine more recent information on the interaction between MPO, its bioreactive reaction products, and targets within the inflammatory microenvironment. We propose that two assumptions of the current model may require revisiting. First, many important targets of MPO modification are extracellular, rather than present only within the phagolysosome, such as various components of neutrophil extracellular traps. Second, we suggest that the pro-inflammatory pathological role of MPO may be a particular feature of chronic inflammation. In the physiological setting of acute neutrophil-mediated inflammation MPO may also form part of a negative feedback loop which down-regulates inflammation, limits tissue damage, and facilitates the switch from innate to adaptive immunity. This different perspective on this well-studied enzyme may usefully inform further research into its function in health and disease.

Keywords Myeloperoxidase · Hypochlorous acid · Neutrophils · Acute and chronic inflammation

Abbreviations

ANCA	Anti-neutrophil cytoplasmic autoantibodies
DC	Dendritic cell
ER	Endoplasmic reticulum
HDL	High density lipoprotein
HOCl	Hypochlorous acid
HOBr	Hypobromous acid
H_2O_2	Hydrogen peroxide
LDL	Low density lipoprotein
MMP	Matrix metalloproteinase
MPO	Myeloperoxidase
NADPH	Nicotinamide adenine dinucleotide phosphate
NET	Neutrophil extracellular trap
PAMP	Pathogen-associated molecular pattern
PRR	Pattern recognition receptor
TLRs	Toll-like receptors
SVV	Small-vessel vasculitides

Introduction

Myeloperoxidase (MPO) belongs to the mammalian heme peroxidase enzyme family. This enzyme, discovered over 40 years ago, was long believed to be a key component of the phagocyte oxygen-dependent intracellular microbicidal system, and thus to play an important part in the effector phase of innate immune responses (Klebanoff 1967).

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However, the limited immunodeficiency associated with lack of MPO in mouse and human models (Aratani et al. 2000, 2002; Eyerich et al. 2007; Lanza 1998; Parry et al. 1981) has challenged this paradigm. Two recent reviews summarize many of the different possible additional functions suggested for MPO (Arnhold and Flemmig 2010; Van der Veen et al. 2009). In this review we examine more recent information on the interaction between MPO, its bio-reactive reaction products, and targets within the inflammatory microenvironment from a different perspective. We propose that two assumptions of the current model may require revisiting. First, many important targets of MPO products may be extracellular, rather than present within the phagolysosome. Second, MPO products may also contribute to a negative feedback loop, down-regulating inflammation, limiting tissue damage, and facilitating the switch from innate to adaptive immunity. This different perspective on this well-studied enzyme may usefully inform further research into its function in health and disease. Several other functions for MPO which are independent of its enzymatic activity have been described (e.g. Klinke et al. 2011), but these lie outside the ambit of this review.

Synthesis and Tissue Distribution

It has been known for many years that neutrophils contain several types of cytoplasmic granules, and it is in the so-called "primary" granules that MPO is found (Borregaard and Cowland 1997). Primary granules are synthesized in the promyelocyte stage of development, and are no longer produced after the start of the myelocyte stage (Klebanoff 2005). In the circulation MPO is found predominantly in neutrophils, and is also present in monocytes. However, MPO is lost when monocytes differentiate into tissue macrophages (Deimann 1984). In the absence of inflammation, MPO is not found in extravascular tissue, while tissue MPO activity is one of the earliest consequences of neutrophil infiltration at sites of inflammation (Nauseef 2007). This activity of MPO is connected with three major antimicrobial strategies of neutrophils: phagocytosis, which involves the uptake of pathogens, degranulation, which results in release of antimicrobial molecules and the recently discovered formation of neutrophil extracellular traps (NETs) (Papayannopoulos and Zychlinsky 2009). NETs are composed of de-condensed chromatin and antimicrobial proteins such as citrullinated histones, MPO and neutrophil elastase (Papayannopoulos et al. 2010). Moreover, it has been reported that MPO is required for NET formation (Metzler et al. 2011).

Like many glycoprotein enzymes, MPO biosynthesis is complex, and includes a series of proteolytic cleavage and processing events. The first of these is conversion of the 80 kDa primary translation product to 90 kDa apoproMPO. There are three distinct steps that are recognized: first, cleavage of a signal peptide; second, N-linked glycosylation; and third, limited de-glucosylation of high mannose oligosaccharide side chains (Hansson et al. 2006). ApoproMPO is the enzymatically inactive heme-free form, and has a relatively long half time in the endoplasmic reticulum (ER) (Hansson et al. 2006). The inactive form interacts with ER molecular chaperones calreticulin (Nauseef et al. 1995), calnexin (Nauseef et al. 1998) and ERp57 (Goedken et al. 2007) via the oligosaccharides attached to the protein. These transitory associations with apoproMPO promote proper folding, but it is unclear whether or not they are necessary for the final MPO maturation process.

In the ER the next important event is the insertion of the iron containing (heme) prosthetic group. This results in the formation of an enzymatically active form, proMPO (Nauseef et al. 1992). Although heme incorporation is essential for exit from the ER, and for proMPO reaching its destination in the primary granules, the final maturation requires a further series of proteolytic events. First, a short (125 amino acid) peptide is cleaved off from proMPO to form a 74 kDa short-lived temporary product that can undergo secondary cleavage into a 59 kDa heavy subunit and a 13.5 kDa light subunit (Olsson et al. 1984). A combination of two heavy–light subunit pair homodimerises (linked by a disulfide bond between the heavy chains) forms the mature MPO with a molecular weight of about 150 kDa (Andrews and Krinsky 1981). It is this protein that is stored and then released into the phagosome when the neutrophil is activated.

Enzymology and the Production of Oxidative Species

The key substrate of MPO is hydrogen peroxide (H_2O_2), itself the product of molecular oxygen via the NADPH oxidase "respiratory burst" complex (Babior 2004). The function of H_2O_2 itself was long thought to be microbicidal. For example, the activation of the neutrophil respiratory burst activates an oxidant sensing transcription factor in *Escherichia coli* bacteria, which initiates a protective transcriptional response specifically directed for preventing oxidative damage (Staudinger et al. 2002). However, this view has been challenged, with the suggestion that the function of H_2O_2 is to act not as a microbicidal effector, but rather to act as a second messenger, regulating changes in pH and ion flux, and triggering proteolytic enzyme release within the phagolysosome (Segal 2005, 2006). These arguments will not be rehearsed here, since they are not directly relevant to the function of MPO. Instead, we will focus on the several

different oxidants, which have been shown to be possible downstream products, either directly or indirectly, of the interaction between MPO and H_2O_2. These include hypochlorous (or hypobromous) acid, chloramines and aldehydes; hydroxyl radicals, singlet oxygen and ozone.

Hypochlorous Acid

A key feature of MPO is its ability to catalyze the two electron oxidation of Cl^- to hypochlorous acid [HOCl; Cl(−1) to Cl(+1)] by hydrogen peroxide at neutral pH and physiological plasma concentrations of halide (Harrison and Schultz 1976; Klebanoff 1999). Approximately 45% of the H_2O_2 consumed by MPO is converted to HOCl (van Dalen et al. 1997). The key redox intermediate in this reaction is formed by the reaction of H_2O_2 with the heme component of MPO. This iron, present normally in ferric form, is converted to oxy-ferryl heme. The resultant primary catalytic complex of MPO can then react with halides to form hypohalous acid, with associated iron regeneration. However, alternative competing reactions have been described. The MPO–H_2O_2 complex can react with excess H_2O_2 to form a further complex, which retains the oxyl-ferryl center. Reducing agents, including superoxide, can convert this complex back to native enzyme, consuming H_2O_2 but not generating HOCl. A third proposed alternative is direct interaction of superoxide with MPO to form a short-lived compound, which has oxygen attached to the heme center (Klebanoff 2005).

Because chloride is normally present at much higher concentrations than other halides, there is general agreement that HOCl is the major product of neutrophil MPO. The enzyme, however, can also catalyze oxidation of significant amounts of bromide anions to hypothiocyanate acid (HOBr) (Chapman et al. 2009) either directly (Senthilmohan and Kettle 2006), or indirectly by reaction of HOCl with bromide (Henderson et al. 2001). MPO can also catalyze the formation of the pseudohalous hypothiocyanous acid (Hawkins et al. 2008).

The extremely high reactivity of HOCl has led to some debate about whether the MPO–H_2O_2–Cl^- system produces free HOCl, which can subsequently diffuse away and react with targets at a distance, or whether the complex reacts directly with targets which access the active site of the enzyme. This important debate is based upon the scientific evidence showing free HOCl in the system (Spalteholz et al. 2006). Early studies of chlorination of small molecules such as taurine proposed the action of free HOCl, but later kinetic studies suggested that taurine interacts at low pH with the enzyme bound intermediate, and that it is this intermediate that is responsible for taurine oxidation (Marquez and Dunford 1994). Other more recent studies support the original model. For example, Davies et al. (2008) observed that extracellular matrix polysaccharides, such as heparin sulfate, are chlorinated efficiently by MPO–H_2O_2–Cl^-. In view of their bulk, steric considerations make it unlikely that these targets can have efficient access to the MPO active site, thus suggesting that free HOCl must be the oxidizing agent in these cases. Indeed, a recent study (Ramos et al. 2008) suggests that even targets as small as a tri-peptide are unable to access the active site. A synthesis of the available data might suggest that small molecules, particularly the amino acid taurine which is present at mM concentrations within the neutrophil cytoplasm, access the enzyme active site and in effect act to limit reactivity with other targets (including MPO itself, see below) within the cell (Marcinkiewicz et al. 1995). However, in the phagolysosome and the extracellular environment, where taurine concentrations are lower, MPO may catalyze the formation of free HOCl, which will then react rapidly with multiple targets within the immediate microenvironment.

Macromolecular Targets of HOCl Modification

The downstream macromolecular targets of HOCl oxidation have also been investigated in great detail (Pattison and Davies 2006). HOCl reacts rapidly with proteins, DNA (Prutz 1996) and lipids (Winterbourn et al. 1992). However, proteins are likely to be a major target and therefore the consequences of this process have been studied in most detail. Novel mass spectrometry techniques have contributed very successfully to this area of research (Pitt and Spickett 2008), and the detection of specific end products (e.g. 3-chlorotyrosine) by either immunohistochemistry or biochemical techniques is widely used as surrogate biological marker to follow MPO activity in vivo.

HOCl is a selective oxidant that modifies particular amino acid residues preferentially (Hawkins et al. 2003). Recently, second order rate constants have been determined for the reactions of HOCl at physiological pH with all of the potential reactive sites within a protein (Pattison and Davies 2001). The order of reactivity for the side chain was Met > Cys ≫ Cystine ∼ His ∼ α-amino > Trp > Lys ≫ Tyr > Arg > Gln ∼ Asn. Reaction with some of these groups is complex and leads to unstable intermediates. For example, interaction with Cys forms an unstable compound, sulfenyl chloride, that reacts either with water to form cysteic acid or with other Cys groups to form cystine. Met is oxidized directly to stable methionine sulfoxide, and Tyr side chains either react at the ∼α-amino group to form *p*-hydroxyphenylacetaldehyde or via the aromatic ring to give 3-chlorotyrosine (Hawkins et al. 2003). Amines, especially the lysine ε-amino group, and

N-terminal α-amino groups are first converted to mono- and dichloramines. These products are themselves important oxidant species and their reactions are discussed below.

Other Products of MPO

Chloramines and Aldehydes

At neutral pH HOCl is in equilibrium with hypochlorite (OCl^-) at approximately equimolar concentrations. At lower more acidic pH, as in phagosomes, HOCl is the dominant form and may form chlorine (Cl_2) which is also a powerful antimicrobial agent, and which can damage host tissues. Although a relatively strong oxidant, HOCl itself is short lived. However, as stated above, HOCl will react rapidly with protein amines, either at N-terminal, or on the side chains of His, Lys and Arg to form mono- and dichloro-amines (Hawkins and Davies 1998). These chloramines retain oxidant activity, but are much more long-lived than either HOCl itself or free amino acid chloramines (Hawkins et al. 2003). The chloramines can also slowly undergo a variety of rearrangement reactions, which can give rise to aldehydes, which are also oxidizing species. Thus, although HOCl (together with varying amounts of HOBr and HOthiocyanate) is almost certainly very short-lived oxidative species, they can give rise to a large variety of secondary products, which retain oxidative potential over longer periods.

Hydroxyl Radical

Production of the hydroxyl radical can result from the Haber–Weiss reaction, the iron catalyzed interaction between H_2O_2 and superoxide (Haber and Weiss 1934). This reaction, however, is probably limited by the low concentration of free iron in biological fluids. A second possible mechanism (Ramos et al. 1992) may be that HOCl, produced via the MPO–H_2O_2–Cl^- system, reacts directly with superoxide to form hydroxyl radical. However, hydroxyl radical is a major product only if there are no proteins or other targets for HOCl. The importance of this alternative under physiological conditions has therefore been questioned (Winterbourn et al. 2006).

Singlet Oxygen

Singlet oxygen is a highly reactive form of oxygen. Several groups have shown that activated neutrophils generate this product at least in vitro. During the first minutes of an antimicrobial response the pH within the phagosome increases from ~5.7 to 7 (Segal et al. 1981) and the rate of formation of singlet oxygen rises, while chloramine formation decreases and eventually is abolished (Arisawa et al. 2003). These findings have been correlated with observations that within 2 min of engulfment by *Staphylococcus aureus* into a phagosome almost all bacteria are killed (Segal et al. 1981). Formation of singlet oxygen has also been linked to the MPO–H_2O_2–Cl^- system, via direct interaction of H_2O_2 with HOCl (Allen et al. 1972). However, as for OH the relative importance of this pathway in vivo, among the many possible competing targets for HOCl, remains open to question (Winterbourn et al. 2006).

Ozone

There has been considerable debate about generation of ozone by activated neutrophils (Babior et al. 2003; Wentworth et al. 2002), largely based on the assumption that singlet oxygen may be present at high concentrations within the phagosome (Babior et al. 2003). As outlined above, this is now thought to be less likely, and therefore, despite the identification of ozone using sensitive traps, this species does not seem to be a major oxidant product of MPO catalyzed reactions.

How Important Is the MPO Pathway for Microbial Killing within Neutrophils?

The contribution of MPO, and of the MPO–H_2O_2–Cl^- system, to bacterial killing has been explored more than any other aspect of this pathway. This has included both in vitro and in vivo experiments, in both human and animal models. It is important to note that in this particular system the mouse model may not mirror the human accurately. Most studies isolate mouse neutrophils from tissue or peritoneal exudate, while those on human neutrophils isolate them from blood. Mice have a much lower proportion of circulating neutrophils than humans (von Vietinghoff and Ley 2008). Furthermore, in the mouse the MPO gene lacks the AluRRE element in the promoter that is seen in primates (Hansson et al. 2006). In humans an allelic polymorphism in this region (−463G/A) affects the binding of the SP1 transcription factor (Piedrafita et al. 1996). Individuals with two copies of the G-allele have a 25-fold greater rate of MPO transcription than those with −463A/A (Piedrafita et al. 1996). The significance of this finding remains unclear and also two other studies have failed to observe this association (Hoy et al. 2001; Rutgers et al. 2003a).

The original studies in vitro demonstrated that purified MPO, in the presence of H_2O_2 and halide ions, can kill bacteria effectively (Klebanoff 1967). Two different approaches were used to follow-up on these studies. In one approach the phagosome content was subjected to careful scrutiny and HOCl was shown to be present, to be active in chlorination, and to be effective in *S. aureus* killing

(Chapman et al. 2002). Interestingly, however, using isotopically labeled tyrosine this study also showed that the vast majority of chlorination occurs on neutrophil rather than on bacterial proteins. The other approach used MPO-deficient mice. These MPO-deficient animals were more susceptible to infection and death following *Klebsiella pneumoniae* infection (Hirche et al. 2005). However, microorganisms differ in their susceptibility to MPO-dependent antimicrobial killing. In a more detailed study, MPO-knockout mice did have increased susceptibility to infections caused by *Candida albicans* (Aratani et al. 1999), *Candida tropicalis, Trichosporon asahii* and *Pseudomonas aeruginosa* (Aratani et al. 2000), whereas responses to *S. aureus, Streptococcus pneumoniae, Candida glabrata* and *Cryptococcus neoformans* were comparable to those seen in wild-type mice (Aratani et al. 2000). In addition, the relative killing defects do not translate necessarily into spread of infection and disease, e.g. the consequences of failure to kill *C. albicans* did not seem to be as serious as expected (Lanza 1998).

In humans, primary (inherited) MPO deficiency is relatively common, with an incidence of between 1:2,000 and 1:4,000 in Europe and the United States (Parry et al. 1981). If MPO is an important element in neutrophil microbicidal activity, MPO deficiency might result in severe impairment of the host response, but this is not the case. A number of older case studies reported isolated examples of infections associated with the absence of MPO (Lehrer and Cline 1969). A more recent study of 100 MPO-deficient individuals also found an increased incidence of infection and chronic inflammation (Kutter et al. 2000). However, it has become clear that human MPO deficiency is rarely associated with severe immunodeficiency (van der Veen et al. 2009). Several possible explanations may be advanced to explain this apparent paradox. MPO deficiency impairment of antimicrobial activity may be restricted to a few specific classes of pathogen. Moreover, several MPO-independent mechanisms can potentially compensate for absence of the peroxidase in the phagosomes, such as prolongation of the respiratory burst (Cramer et al. 1982; Kitahara et al. 1981). Thus, the lack of a major phenotype observed may be another example of the well-known redundancy which characterizes many facets of the human immune system. Despite all these possible explanations, the mild infectious disease phenotype of MPO deficiency in both the human and mouse setting begs the question of whether MPO may have an additional function over and above that of microbicidal killing.

Extracellular Targets of MPO

As discussed above, there is clear evidence showing that proteins within the neutrophil are chemically modified as a result of the generation of HOCl and perhaps other reactive oxygen species which are the consequence of MPO activity. However, there is extensive evidence that MPO can also be released from neutrophils at a site of inflammation leading to modification of macromolecules within the local extracellular microenvironment. The discovery of MPO participation in the NETs formation strongly supports this thesis (Metzler et al. 2011). Nevertheless, much of the evidence for extracellular MPO targets comes from studies of tissue damage associated with chronic inflammatory disease, especially atherosclerosis. Neutrophils have been observed infiltrating atherosclerotic plaques in both humans and mice (de Boer et al. 2010; van Leeuwen et al. 2008). It has been recognized for many years that low density lipoprotein (LDL) oxidation is one of the earliest events, and most important steps, in atherogenesis (Matsuura et al. 2008). There is strong evidence implicating MPO in the oxidation of LDL which has been reviewed recently (Malle et al. 2006).The effects of HOCl release on other proteins have not received the same attention as LDL. However, mass spectrometry of high density lipoprotein (HDL) demonstrated far more HDL-associated 3-chlorotyrosine in samples derived from areas of the aorta where there was atheroma than there was in the plasma of the same patients (Bergt et al. 2004). Plasma levels were also increased in patients with coronary artery disease compared to controls. HOCl has also been shown to oxidize extracellular matrix proteins (Woods and Davies 2003) within blood vessel wall. Thus, a variety of methods can detect both MPO and the presence of MPO-dependent modifications in lipids, proteins and extracellular matrix components within the atherosclerotic plaque. These data provide compelling evidence that MPO products modify macromolecular targets outside the neutrophil phagolysosome. Similar evidence for extracellular modification of target macromolecules by MPO has been documented in many other chronic inflammatory diseases including cystic fibrosis (Kettle et al. 2004) and arthritis (Schiller et al. 2003).

The Role of MPO in Inflammatory Pathophysiology

The increased levels of MPO found in a wide variety of inflammatory diseases have led to the hypothesis that MPO-mediated oxidation is causally implicated in driving the disease process. Some of the major evidence for this is reviewed below.

Atherosclerosis

Elevated levels of MPO are biomarkers which predict adverse clinical outcome in the setting of several acute

coronary syndromes, including acute myocardial infarction, reperfusion injury and stroke (Loria et al. 2008). All these acute syndromes are linked to underlying chronic damage to blood vessels, caused by plaque formation and atherosclerosis, and there is much evidence that infiltrating macrophages and neutrophils participate in the transformation of stable coronary artery plaques to unstable lesions (Naruko et al. 2002; Sugiyama et al. 2001). In the context of plaque formation, the implications of MPO-mediated LDL oxidation discussed above have prompted a wide range of studies, exploring the biochemistry (Pattison and Davies 2006), the cell biology (Podrez et al. 2000) and the immune components (Hansson 1999) of the process, and have also influenced therapeutic approaches (Aratani et al. 1999). Uptake of HOCl-altered LDL is mediated via the CD36 and scavenger receptors (Adachi and Tsujimoto 2006) but recent work has suggested that there are alternative receptors, such as the soluble form of the receptor for advanced glycation products, which bind HOCl-modified LDL (Marsche et al. 2007). The effects of oxidized LDL on cells within the microenvironment are also diverse. For example, at low concentrations, oxidized LDL was found to activate dendritic cells (DCs), and hence T cells, potentially contributing to the documented autoantibody response, while at higher concentrations, oxidized LDL induced apoptosis (Alderman et al. 2002a). HOCl can also cause damage and death of endothelial cells, an effect which is reversed by the antioxidant compound probucol (Russell et al. 1998). HOCl modification may also counteract the protective effects of HDL.

The overall etiological role of MPO-mediated oxidation in atherosclerosis remains unclear. Interestingly, MPO-deficient mice actually develop more serious cardiovascular disease (larger legions) than wild-type controls, although as predicted no chlorination could be detected (Brennan et al. 2001a). These studies provide some suggestive evidence that MPO may function in an anti-inflammatory, as well as pro-inflammatory role.

Cystic Fibrosis

In contrast to atherosclerosis, cystic fibrosis is a chronic inflammatory disease with a strong link to underlying bacterial infection, and consequent neutrophil infiltration. Sputum from cystic fibrosis was found to contain elevated levels of both MPO and 3-chlorotyrosine modified proteins. Furthermore, the high expression G/G MPO promoter polymorphism was associated with more severe chronic lung infections in these patients (Reynolds et al. 2006). Thus in this case, overproduction of MPO is implicated in causing lung damage, which is the most common lethal manifestation of this disease (Kettle et al. 2004). This striking and unopposed pro-inflammatory effect of MPO may be related to the underlying pathological defect in ion exchange. No studies have been reported which directly address the effects of absence of MPO in this disease.

Renal Disease

In renal disease MPO has been identified in the inflamed glomeruli seen in membranous glomerulonephritis (Grone et al. 2002). Indirect effects, mediated via the excretion of HOCl-modified proteins, may be implicated more generally in renal pathology. In vitro HOCl-modified proteins induced upregulation of transcription of genes involved in reactive oxygen species metabolism and cell stress (e.g. heme oxygenase-1, thioredoxin reductase, cytochrome b5 reductase, Gadd 153, amino acid transporter E16, and heat shock protein 70) in a kidney cell line. Genes involved in tissue remodeling, which encode for both pro-inflammatory and anti-inflammatory proteins, e.g. connective tissue growth factor, vascular cell adhesion molecule 1, interleukin 1β, matrix metalloproteinase 7 (MMP-7), and vascular endothelial growth factor, were also up-regulated (Porubsky et al. 2004).

Follow-up of this work, using quantitative RT-PCR, verified the differential expression of a subset of these genes in microdissected tubule-interstitial tissue from patients with a range of renal conditions—acute tubular damage, progressive proteinuric renal disease, and membranous glomerulonephritis (with declining renal function) (Porubsky et al. 2004). These findings were not seen in stable patients with proteinuria caused by minimal change disease. This demonstration of selective up-regulation of a subgroup of genes specifically when proteinuria is accompanied by the presence of HOCl-modified (lipo)proteins supports a potential pathophysiological role for the MPO–H_2O_2–Cl^- system as a component in renal disease, even when it is not suggested to be part of the primary insult.

Arthritis and Vasculitis

Cartilage components in patients with rheumatoid arthritis have been shown to be damaged by HOCl from activated neutrophils present in the synovial fluid (Schiller et al. 2003). However, the effects of HOCl in joints are not confined to rheumatoid arthritis. For example, activation of the MPO–H_2O_2–Cl^- pathway, releasing HOCl, contributes to the inflammatory process in gout, where HOCl uric acid complexes are formed (Thomas 1992). The systemic small-vessel vasculitides (SVV) are associated with the presence of anti-neutrophil cytoplasmic autoantibodies (ANCA) (Davies et al. 1982) directed against MPO (MPO-ANCA) (Falk and Jennette 1988), proteinase 3 (Goldschmeding et al. 1989) or lysosomal membrane protein-2 (Kain et al. 2008).

In vivo studies on mouse and rat models have provided evidence that MPO-ANCA is a primary pathogenic factor in SVV (Little et al. 2005; Xiao et al. 2002). Direct binding of MPO-ANCA auto-antibodies can activate neutrophils resulting in degranulation and HOCl production, which, in turn, can contribute to the destructive inflammatory response damaging the vessel wall (Rutgers et al. 2003b). However, other studies have found that in some cases antibodies to MPO can inhibit, rather than activate enzyme activity. The authors propose the hypothesis that MPO may have both pro-inflammatory and anti-inflammatory activity, and that antiMPO antibodies may play a role in disturbing this balance (Guilpain et al. 2008).

Neurological Disease

The MPO–H_2O_2–Cl^- system has been invoked as a final common pathological component for several neurodegenerative diseases including Alzheimer's disease (Reynolds et al. 1999), multiple sclerosis (Nagra et al. 1997) and Parkinson's disease (Choi et al. 2005). Several pieces of evidence have contributed to these hypotheses. Firstly, with respect to disease susceptibility, in both Alzheimer's disease and multiple sclerosis the G/G MPO polymorphism in a promoter region correlates with increased risk of disease development in female patients (Nagra et al. 1997; Reynolds et al. 1999). Furthermore, MPO has been found to be present in brain aggregates of senile plaques (which is a hallmark of Alzheimer's disease), co localizing with the main plaque component, amyloid β peptide (Reynolds et al. 1999) and has also been found in the plaque lesions of multiple sclerosis (Nagra et al. 1997). Paradoxically, as in the atheromatous plaque, there is MPO present in these plaques in the absence of neutrophils. MPO-derived oxidants, such as chloramines, are also involved in metabolic pathways in both diseases: there is inhibition of the α-ketoglutarate dehydrogenase complex, which affects cerebral energy metabolism, in Alzheimer's patients (Jeitner et al. 2005), and in multiple sclerosis MPO-derived oxidants may play a role in damaging the myelin sheath (Nagra et al. 1997).

In Parkinson's disease the role of MPO and its products has been assumed to be indirect: the presence of an MPO-associated inflammatory reaction has been suggested to increase the risk of subsequent Parkinson's development (Chen et al. 2003). In a mouse model an increased level of MPO expression in the substantia nigra pars compacta region was observed, and it is in this area where the loss of dopaminergic neurons characteristic for Parkinson's development occurs (Choi et al. 2005).

Neoplastic Disease

MPO has been linked to neoplasia either directly, via its ability to damage/mutate cellular DNA (Prutz 1996; Rainis et al. 2007), or indirectly via its pro-inflammatory role, which is now a well-established risk factor for many forms of cancer (Ostrand-Rosenberg 2008). High levels of MPO expression have been correlated with acute promyelocytic leukemia (APL-M3) (Reynolds et al. 1997), while low expression MPO genotypes have been associated with reduced risk of lung cancer (Schabath et al. 2002) and hepatoblastoma (Pakakasama et al. 2003). However, these genetic linkages have not always been replicated (He et al. 2009), perhaps because of differing levels of plasma antioxidants such as carotenoids or diet. Oxidation of tumor cells by HOCl can, however, enhance their immunogenicity, an observation discussed further below (Chiang et al. 2006).

The conclusion from the large number of studies implicating MPO and its products in a wide variety of disease processes can be summarized as follows. There is unequivocal evidence that enhanced MPO-mediated oxidation does indeed occur in a wide variety of diseases, especially those involving chronic inflammation and/or prolonged neutrophil invasion. Furthermore, the targets of these modifications frequently include extracellular proteins and other macromolecules, and can lead to tissue damage. However, the causal links between MPO oxidation, and the disease processes are complex, and both over- and under-expression of MPO has been linked to worse disease outcome. While prolonged overproduction of MPO is likely to lead to tissue damage, MPO may play an anti-inflammatory role in some cases. Possible mechanisms for this effect are discussed further below.

Additional Roles of MPO-Mediated Oxidation

Enzyme Inactivation

HOCl is a selective oxidant, and several groups have shown that it inactivates enzymes by specific oxidation of methionine and/or cysteine side chains. Interestingly, many of these targets are themselves important components of the inflammatory process, and include matrilysin MMP-7 (Fu et al. 2003), the tissue inhibitor of metalloproteinase 1 (Wang et al. 2007), the neutrophil proteinase cathepsin G (Shao et al. 2005) and lysozyme (Hawkins and Davies 2005). MPO itself can be inactivated by neutrophil-mediated oxidation (King et al. 1997). Thus, HOCl can form part of a negative feedback loop, which can potentially contribute to a regulatory process for switching off the inflammatory process. The concentrations required for Cys/Met modification are very low thus raising the probability that these reactions will indeed take place within inflammatory microenvironment.

Pathogen-Associated Molecular Pattern Inactivation

The activation of innate immunity and inflammation by microbial pathogens occurs via the specific interaction between pathogen-associated molecular patterns (PAMPs) on the microbes, and pattern recognition receptors (PRRs) on host cells. The paradigm for this interaction is between lipopolysaccharide on the surface of gram negative bacteria and Toll-like receptor 4 (TLR4) on macrophages, which stimulates the production of pro-inflammatory cytokines and the oxygen burst. However, pretreatment of whole bacteria (*E. coli* and *S. aureus*) with HOCl (at low micromolar concentrations) strongly inhibited the ability of the organisms to stimulate macrophage activation, nitric oxide production and tumor necrosis factor α release (Marcinkiewicz et al. 1994). Remarkably, this observation has not been followed up, and the nature of the chemical reactions involved, and the extent with which this phenomenon is observed in other PAMP/PRR interactions remains unknown. The study does provide support for a role for HOCl in down-regulation of inflammation.

MPO, HOCl and the Enhancement of Adaptive Immunity

As outlined above, proteins are major targets of HOCl. Neutrophil recruitment and activation are among the earliest events in many immune responses, so protein antigens are likely to be exposed to oxidation via MPO either within the neutrophil phagosome or in the immediate vicinity of neutrophil infiltration. Many neutrophils die during this initial inflammatory process, and the cell remnants can be taken up by surrounding antigen-presenting DC. Thus, it is likely that many of the antigens being processed and presented will be in a modified form after pre-exposure to oxidative processes (Fig. 1).

As an in vitro model of these oxidative processes, others and we have studied the adaptive response of protein antigens exposed to HOCl. Early work showed that HOCl oxidation lowered the threshold of ovalbumin required to stimulate both cellular and humoral immune responses. Enhanced responses were due to increased T cell responses, which, in turn, derived from improved processing and presentation (Marcinkiewicz et al. 1991, 1992). Oxidation of hen egg lysozyme and bovine α-lactalbumin, either via chlorination or via performic acid, induced higher T cell activation responses when presented by macrophages (Carrasco-Marin et al. 1998). The release of oxidants can lead to the formation of reactive advanced oxidation products, cross-linked proteins that may act as superantigens (Alderman et al. 2002b) and will activate DCs (Alderman et al. 2002b). Similarly, aldehyde modifications

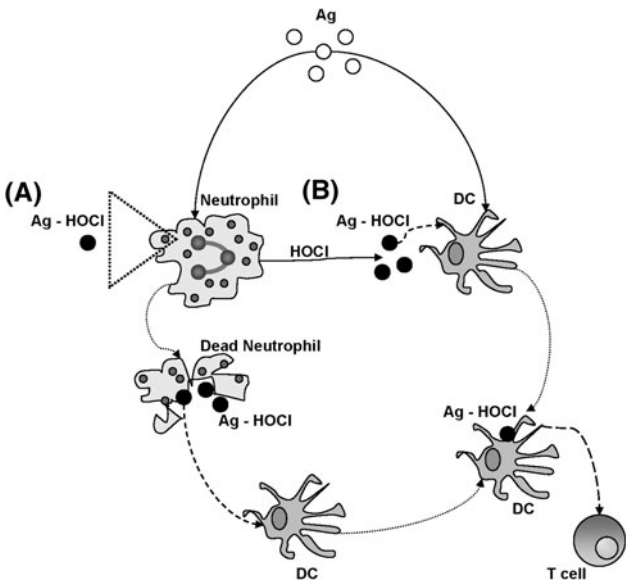

Fig. 1 Model for the enhancement of adaptive immunity by MPO-derived HOCl. Protein antigens (Ag) are likely to be exposed to HOCl oxidation via two ways: (*A*) oxidation occurs within the neutrophil phagosome, dead neutrophil are then phagocytosed by antigen-presenting cells (*DC* dendritic cells) that are processing and presenting HOCl-oxidized antigens (Ag-HOCl); (*B*) oxidation occurs in the immediate vicinity of neutrophil infiltration, HOCl-oxidized antigens are directly taken up, processed and presented by DCs

of protein antigens by glycolaldehyde or by oxidation with NaIO4 were found to render antigens highly immunogenic (Allison and Fearon 2000). We have recently applied these findings to a preclinical tumor immunotherapy model, and have shown that HOCl oxidation in vitro strongly enhances processing/presentation of tumor cells by human DCs (Chiang et al. 2006, 2008). The mechanisms underlying the phenomenon include increased DC activation (Alderman et al. 2002a) and enhanced uptake of antigen. These studies are now being moved toward a phase I clinical trial in the ovarian cancer setting. Moreover, our recent studies have shown that HOCl-modified OVA is taken up more efficiently by antigen-presenting cells and degraded more efficiently by proteases. The enhancement of immunogenic properties of chlorinated OVA was mediated via modification of the N-linked carbohydrate side chain rather than formation of protein aldehydes or chloramines (Prokopowicz et al. 2010). Therefore, all these data suggest that HOCl may act as a natural adjuvant of immune responses to proteins that facilitates antigen processing, presentation, cross-priming and effector responses of adaptive immunity.

Hypothesis and Conclusion

Previous work on the MPO-mediated neutrophil oxidation system has focused on two functional outcomes. The first,

and historically the original function assigned to MPO is as part of the armory of microbicidal oxidative species generated by myeloid lineage phagocytes. A role in microbial killing remains a likely function of MPO, but the extent to which it contributes to the overall human innate immune response remains open—at any rate it is not essential for survival. The second role for MPO oxidation has been in mediating tissue damage associated with chronic inflammatory diseases. It is undoubtedly the case that MPO oxidation can contribute to tissue damage, although its role in the etiology of specific pathologies is complex and difficult to determine. However, the potential for causing host tissue damage and pathology itself highlights the question of MPO's physiological function. From an evolutionary perspective, the benefits from a largely redundant role of MPO in bactericidal killing need to be balanced against the potential handicap arising from its role in immunopathology. In this current pro-inflammatory paradigm, the evolutionary forces, which have retained MPO as a major protein component of granulocytes in all major vertebrate groups, seem obscure.

The hypothesis put forward in this review is that MPO has an additional potential anti-inflammatory role, which can contribute both to down-regulating innate immunity and facilitating the crucial switch to adaptive immunity (Marcinkiewicz 1997). A negative regulatory role for MPO may explain why the enzyme is expressed in neutrophils, whose role in innate immunity is rapid but short lived, and not in macrophages, which play an important role in mediating long-term protection.

We propose that HOCl, the major oxidative product of MPO, may act to regulate several distinct steps. Reaction of HOCl with taurine, an abundant amino acid in neutrophils, leads to the formation of taurine chloramine, which has anti-inflammatory as well as bactericidal properties (Kontny et al. 2003; Muz et al. 2008). HOCl selective modification of amino acid side chains (with methionine as the primary target) may contribute to the inactivation of key enzymes, which mediate both bacterial killing and tissue remodeling (Fu et al. 2003; Hawkins and Davies 2005; Shao et al. 2005; Wang et al. 2007). One specific example is the inactivation of MMP-7 discussed above. Reaction of HOCl with microbial components may modify the interactions between microbial PAMPs and innate immune PPRs, such as TLRs, and thus dampen the subsequent innate immune response. And finally, HOCl modification of protein antigens may act as a neutrophil-dependent molecular tagging system (Marcinkiewicz 1997), by which sentinel DCs can recognize pathogen-derived antigens, and subsequently drive effective adaptive immunity. The evidence that HOCl modification enhances antigen processing and presentation has been reviewed above. However, HOCl may also inhibit adaptive T cell immune responses within inflammatory sites, either by direct cytotoxicity or via conversion to taurine chloramine (Brennan et al. 2001b; Milla et al. 2004).

The evidence supporting this hypothesis remains fragmentary and contradictory pieces of evidence certainly exist. But we believe that by directing attention to a little studied aspect of MPO physiology, it may stimulate further research, which will help to answer some of the unresolved questions, which still surround this intensely studied, but persistently paradoxical neutrophil enzyme.

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Conflict of interest The authors declare that they have no conflict of interest.

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