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Review article

Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function

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ABSTRACT

Myeloperoxidase (MPO) is a heme-containing peroxidase expressed mainly in neutrophils and to a lesser degree in monocytes. In the presence of hydrogen peroxide and halides, MPO catalyzes the formation of reactive oxygen intermediates, including hypochlorous acid (HOCl). The MPO/HOCl system plays an important role in microbial killing by neutrophils. In addition, MPO has been demonstrated to be a local mediator of tissue damage and the resulting inflammation in various inflammatory diseases. These findings have implicated MPO as an important therapeutic target in the treatment of inflammatory conditions. In contrast to its injurious effects at sites of inflammation, recent studies using animal models of various inflammatory diseases have demonstrated that MPO deficiency results in the exaggeration of inflammatory response, and that it affects neutrophil functions including cytokine production. Given these diverse effects, a growing interest has emerged in the role of this well-studied enzyme in health and disease.

1. Introduction

Neutrophils play a vital role in host defense against pathogens such as bacteria and fungi [1,2]. They exhibit potent microbicidal activity through generation of reactive oxygen species (ROS). Neutrophils generate superoxide anion (O_2^-) through the activity of phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase present at the plasma membrane, resulting in a respiratory burst [3,4]. Subsequently, O_2^- dismutates to hydrogen peroxide (H_2O_2). Myeloperoxidase (MPO, E.C.1.11.1.7) is a cationic heme-containing enzyme found in primary azurophilic granules of neutrophils, and to a lesser degree in primary lysosomes of monocytes. In the presence of H_2O_2 and a halide—chloride, bromide, or thiocyanate, MPO catalyzes the formation of reactive oxygen intermediates, including hypochlorous (HOCl), hypobromous, and hypothiocyanous acids, respectively [5,6]. Upon activation of neutrophils in peripheral blood and tissues, MPO is released into both the phagolysosomal compartment and the extracellular environment. The classic paradigm views MPO as a component of the intracellular microbicidal system of phagocytes and thus part of an important innate immune system for host defense against invading microorganisms [7,8]. To define the *in vivo* role of MPO in host defense, MPO-knockout (MPO-KO) mice were created by two independent research groups [9,10] and have been extensively studied for their susceptibility to infections. In addition, recent studies of the MPO-KO mice have implicated MPO in the pathogenesis of multiple inflammatory diseases, including atherosclerosis and cardiovascular disease, kidney disease, pulmonary inflammation, rheumatoid arthritis, skin inflammation, neuronal disease, and metabolic syndrome. This review highlights the key aspects of pathophysiological properties of MPO and summarizes the findings obtained from extensive studies with various disease models using MPO-KO mice. Of note, the history [7], enzymology [2,5,6], clinical studies [8,11,12] of MPO, and new drug development [13] are topics omitted here but explored in depth in other papers in this issue.

1.1. Role of MPO in host defense

Patients with chronic granulomatous disease (CGD), in which granulocytes are unable to produce O_2^- due to deficiency in phagocyte NADPH oxidase activity, are particularly susceptible to fungal infection, typically *Aspergillus* species, but also catalase positive bacteria including *Staphylococcus aureus* and *Burkholderia cepacia*. The majority of affected individuals are diagnosed early in life, although patients may remain undiagnosed until adult life despite the early onset of symptoms [14].

Abbreviations: ANCA, anti-neutrophil cytoplasmic antibodies; CGD, chronic granulomatous disease; ERK, extracellular-signal regulated kinase; IFN, interferon; IL, interleukin; IκBα, inhibitor of κBα; iNOS, inducible nitric oxide synthase; KC, keratinocyte-derived chemokine; LDLR, low-density lipoprotein receptor; LPS, lipopolysaccharide; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; MPO, myeloperoxidase; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor kappa-light chain enhancer of activated B cells; RANTES, regulated on activation, normal T cell expressed and secreted; ROS, reactive oxygen species; Th1, T helper 1 cell; TLR, toll-like receptor; TNF, tumor necrosis factor

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In a mouse model of CGD, intratracheal challenge with *A. fumigatus* results in high rates of mortality [15,16]. In contrast to the clinical phenotype of patients with CGD, human MPO deficiency is rarely associated with severe immunodeficiency, and impairment of antimicrobial activity is restricted only to specific types of pathogens, such as *Candida albicans* [8]. MPO-KO mice provide insights on the involvement of MPO in host defense against invading pathogens. Mutant mice exhibit increased susceptibility to infection with *C. albicans* [9] and *Klebsiella pneumoniae* compared to infected wild-type (WT) mice [17]. These mice are also considerably more susceptible to the intranasal instillation of *C. tropicalis*, *Trichosporon asahii*, and *Pseudomonas aeruginosa*, whereas susceptibility to infection with *A. fumigatus* and *Cryptococcus neoformans* is increased to a lesser degree and susceptibility to *S. aureus* and *Streptococcus pneumoniae* is comparable to that of the WT strain [18–20]. In contrast, MPO-KO mice have improved survival, lower bacterial colonization, and less lung injury after intraperitoneal *Escherichia coli* challenge due to augmented expression of inducible nitric oxide synthase (iNOS) and nitric oxide (NO) production in the mutant mice [21]. Similarly, lung viral load is lower in the MPO-KO mice intranasally infected with influenza virus [22]. Parasite clearance after *Plasmodium yoelii* infection occurs more rapidly in MPO-KO mice than in WT mice, leading to the hypothesis that decreases in parasite levels might depend more on the adaptive immune system than on MPO-mediated responses [23]. Thus, the MPO-dependent oxidative system is important for host defense against fungi and bacteria, although the effect varies by pathogen species. The level of susceptibility to intraperitoneal *C. albicans* infection was compared in WT, MPO-KO, and CGD mice, and it was observed that when the fungal load was low, ROS formed by the neutrophil NADPH oxidase were adequate to control infection even in the absence of MPO, whereas at high fungal load, respiratory burst products and MPO were essential [24]. These findings with MPO-KO mice demonstrate that MPO plays an important role in overcoming large challenges with infectious fungi.

When interpreting data obtained from mouse studies, including those involving MPO-KO mice, it is important to note that mouse neutrophils lack MPO-independent antimicrobial agents such as defensins [25], and their MPO level is estimated to be around 10–20% of that in human neutrophils [26]. In addition, MPO-KO mice express higher levels of iNOS [21]. These observations should be taken into account when translating results obtained in mice to the human situation. Nevertheless, MPO-KO mice have been instrumental in dissecting the role of MPO and MPO-derived oxidants in various disease models.

1.2. Role of MPO in organ inflammation

MPO can damage host tissue through the generation of reactive halogenating and nitrating agents [27,28]. Indeed, decreased levels of 3-chlorotyrosine, 3-bromotyrosine, 3-nitrotyrosine, and protein carbamylation are seen at the inflammation sites of MPO-KO mice compared to WT mice [29–31]. Thus, based on the assumption that MPO has a detrimental effect during chronic inflammation, it can be expected that inflammation would be reduced under MPO-deficient conditions. Indeed, this has been observed in many acute and chronic inflammatory diseases. However, in the cases of inflammatory response to non-infectious stimuli or chronic inflammation in the absence of viable pathogens, several recent studies have shown increased levels of inflammatory process in MPO-KO mice as described below as well as summarized in Table 1.

1.2.1. Atherosclerosis and cardiovascular disease

MPO has been widely linked to many aspects of human cardiovascular disease and it is believed that this enzyme acts on both the initiation and propagation of cardiac pathologies [32]. Early studies demonstrated an unexpected modest increase in atherosclerotic lesion size in atherosclerosis-susceptible low-density lipoprotein receptor (LDLR)-KO mice after transplantation with bone marrow from MPO-KO mice. Similar changes were observed when MPO-KO mice were crossed with LDLR-KO mice [10]. Subsequent studies show that transgenic mice expressing human MPO experience accelerated atherosclerotic plaque development [31,33], perhaps reflecting species-specific differences in critical determinants for atherogenesis. The latter findings agree with results of many other studies that support the concept that MPO plays an important role in the pathogenesis of atherosclerosis and cardiovascular disease. A challenge with mouse models still lies ahead in better understanding of what extent human-mouse differences are contributing to unexpected outcomes of animal experiments [34–37]. However, substantial evidence supports the concept that MPO plays a very important role in the pathogenesis of atherosclerosis. In contrast to WT mice, MPO-KO mice are resistant to the compromise of acetylcholine-dependent vascular relaxation induced by LPS treatment, suggesting that MPO contributes to vascular dysfunction during acute inflammation by modulating endothelial NO bioavailability [38]. MPO-KO mice exhibit less left ventricle dilation and impairment in systolic left ventricular function [39]. Transplantation of MPO-KO cells into irradiated apolipoprotein E-KO mice alleviates inflammation, decreases oxidative damage, and increases endothelial function with a significant impact on plaque formation [40]. MPO-dependent formation of NO-derived oxidants serves as a pathway for the initiation of lipid peroxidation in a peritonitis model in MPO-KO mice [41]. Vasculitis formation induced by injection of *C. albicans*-derived substances is significantly reduced in MPO-KO mice [42]. Comparison of diabetic WT and MPO-KO mice shows that an increased MPO release in the former induces over-expression of vascular adenosine A_3 receptor that may be responsible for vascular dysfunction by promoting vasoconstriction [43]. MPO-KO mice pretreated with angiotensin II to provoke leukocyte activation show blunted atrial fibrosis, accompanied by lower atrial tissue abundance of 3-chlorotyrosine, a specific footprint of MPO-dependent HOCl formation, and by reduced activity of matrix metalloproteinase (MMP) in atrial tissue as compared to angiotensin II-treated WT mice [44]. These results are consistent with the idea that MPO-dependent regulation of MMP activity is a key contributor to increase atrial fibrosis [45]. A more recent study in an animal model of ischemia-related myocardial damage revealed that MPO augments arrhythmogenic left ventricular remodeling, as manifested in breakdown of connexin 43 by activation of MMP-7, and enhanced ventricular post ischemic fibrosis [46]. Thus, MPO contributes to vascular dysfunction by virtue of its capacity to generate potent ROS and to promote activity of MMPs.

1.2.2. Kidney disease

Although MPO has been implicated in the pathogenesis of kidney disease, as both MPO and HOCl-modified proteins have been detected in diseased renal tissue [47], functional studies examining the role of endogenous MPO in renal inflammation are limited. In a mouse model of chronic kidney disease, MPO-KO mice develop significantly less glomerular injury than do WT mice, which is accompanied by a lower infiltration of monocyte/macrophages and T cells and lower expression of the fibrosis marker genes MMP-2 and -9 [48]. A significant reduction in renal function loss was observed after reperfusion of is chemically damaged kidneys in MPO-KO mice compared with WT mice, demonstrating an important contribution of MPO in the induction of organ damage after renal ischemia-reperfusion by influencing critical factors such as neutrophil extravasation [49]. Proof of the concept that immunity to MPO can induce crescentic glomerulonephritis was provided by Xiao et al. [50], who demonstrated that splenocyte transfer from MPO-immunized MPO-KO mice to immune-deficient mice results in pauci-immune crescentic glomerulonephritis and circulating MPO-*anti*-neutrophil cytoplasmic antibodies (MPO-ANCA). These data implicate MPO-ANCA as a major determinant of disease pathology. Further studies demonstrated that sera from MPO-immunized MPO-KO mice induce crescent formation in a lipopolysaccharide (LPS)- and neutrophil-dependent manner [51,52]. MPO-KO mice show significantly reduced

Table 1
Pathophysiological role of MPO in various inflammatory disease models.

Disease model	Findings with MPO deficiency	Ref.	Risk/benefit of MPO deficiency
Atherosclerosis/Cardiovascular diseases			
Endotoxemia	Resistant to vascular endothelial dysfunction induced by LPS	[28]	Benefit
Myocardial infarction/ischemia	Decreased left ventricle dilation associated with improved function	[39]	
	Less ventricular postischemic fibrosis	[46]	
Vascular disfunction	Reduced vasoconstriction in response to adenosine A_3 receptor agonists	[43]	
Atrial fibrillation	Protected from angiotensin II-induced atrial fibrillation	[44]	
Atherosclerosis	Increased atherosclerosis	[10,33]	Risk
Kidney diseases			
Chronic kidney disease	Less albuminuria, glomerular injury, and renal inflammation	[48]	Benefit
Ischemia/reperfusion injury	Decreased neutrophil influx and improved renal function	[49]	
Glomerulonephritis	Decreased neutrophil-mediated injury	[55]	
	Enhanced glomerular accumulation of T cells	[55]	Risk
Lupus nephritis	More severe nephritis	[56]	
Pulmonary inflammation			
Sepsis-induced injury	Reduced *E.coli* sepsis-induced lung inflammation and injury	[21]	Benefit
Influenza virus-induced injury	Reduced lung injury	[22]	
Asbestos-induced injury	Delayed lung inflammation	[59]	
Acute lung inflammation	Less severe LPS-induced lung neutrophilia with peaked earlier than that in WT mice	[60]	
	More severe LPS-induced lung neutrophilia	[61]	Risk
	More severe zymosan-induced lung neutrophilia	[64,65]	
	More severe nonviable *C. albicans*-induced lung neutrophilia	[66]	
Carbon nanotubes-induced fibrosis	Stronger fibrogenic response	[63]	
Bone marrow-transplantation– induced injury	Increased lung inflammation and impaired lung function	[67]	
Rheumatoid arthritis	Attenuated development of K/BxN serum-transfer arthritis	[72]	Benefit
	Attenuated development of collagen-induced arthritis despite enhanced adaptive immunity	[72]	
	Enhanced development of antigen-induced arthritis due to enhanced adaptive immunity	[57]	Risk
Skin inflammation	Attenuated development of nitrogen mustard-induced injury	[74]	Benefit
	Early onset of ultraviolet -induced skin inflammation	[75]	Risk
	Increased ovalbumin-induced delayed-type hypersensitivity	[57]	
Neurological diseases			
Spinal cord injury	Reduced damage, and better functional recovery	[77]	Benefit
Blood-brain barrier dysfunction	Attenuated LPS-induced blood-brain barrier permeability	[79]	
Parkinson's disease	Less neurotoxicity	[82]	
Ischemia-reperfusion injury	Increased infarct volume and nitrotyrosine formation	[78]	Risk
Multiple sclerosis	Increased susceptibility to hindlimb paralysis	[84]	
Metabolic syndrome	Protection from high-fat diet-induced obesity and insulin resistance	[86]	Benefit
	Reduced development of non-alcoholic steatohepatitis and diminishes adipose tissue inflammation	[87]	

glomerular injury, since the target autoantigen is not present to bind ANCA [53]. Thus, MPO can potentially modulate or mediate glomerular injury in glomerulonephritis.

On the other hand, recent reports have demonstrated anti-inflammatory roles of MPO in pathologies characterized by complex inflammatory response in the absence of infectious agents [54]. Endogenous MPO locally contributes to glomerular damage during neutrophil-mediated glomerulonephritis, whereas it attenuates initiation of the adaptive immune response inducing crescentic, autologous-phase glomerulonephritis by suppressing T cell proliferation, cytokine production, and T helper 1 cell (Th1)/T helper 2 cell ratio [55]. In a murine lupus nephritis model, an enhanced glomerular accumulation of leukocytes was observed in MPO-KO mice. The enhancement of the renal disease in MPO-KO mice correlates with increased accumulation of $CD4^+$ T cells, macrophages and neutrophils in glomeruli, which is associated with augmented generation of $CD4^+$ T cell responses [56]. This in turn correlates with enhanced activation of dendritic cells and increased T cell autoimmunity in lymph nodes and spleen [57]. Therefore, in experimental lupus nephritis, MPO mediated suppression of pathogenic T cell autoimmunity overrides the local damaging effects of MPO in the kidney. These results are concordant with observations in humans showing an increased incidence of lupus nephritis in patients with a polymorphism causing reduced MPO expression [58].

1.2.3. *Pulmonary inflammation*

Increased neutrophils in lung tissues are a feature of animal models of oxidant lung injury and in patients with inflammatory lung diseases. While it is clear that the accumulated neutrophils in the lungs play a key role in pulmonary failure caused by chronic inflammation, its mechanism of action remains incompletely characterized. Asbestos-associated lung inflammation is reduced in MPO-KO mice in comparison with asbestos-exposed WT mice at a relatively early stage of inflammation. Increases in cell cycle reentry in the distal bronchiolar epithelium accompany inflammation in MPO-KO mice, thereby supporting the hypothesis that MPO induces early asbestos-induced oxidative stress, initial epithelial cell injury, and inflammation [59]. Compared to WT mice, MPO-KO mice exhibit less lung inflammation after intranasal challenge with LPS, which correlates with decreased levels of proinflammatory cytokines and chemokines, such as interleukin (IL)-6, interferon (IFN)-γ, and keratinocyte-derived chemokine (KC) [60]. Despite employing a similar model of acute lung inflammation induced by LPS, a different research group showed enhanced lung inflammation in MPO-KO mice, accompanied by higher levels of regulated on activation, normal T cell expressed and secreted (RANTES) in bronchoalveolar lavage fluid [61]. These two studies used different doses of LPS and the seemingly contradictory results might hint that the effect of MPO-deficiency on LPS-induced lung inflammation is dependent on the dosage of the inflammatory stimulus.

Recent evidence from studies in murine models of inflammation shows that MPO has an anti-inflammatory role. In a sepsis model, MPO-KO mice develop significantly increased hypothermia and mortality in response to intraperitoneally injected LPS [62]. MPO-KO mice are defective in the oxidation and clearance of single-walled carbon nanotubes from the lungs, whereas the inflammatory response is more

robust than that of WT mice [63]. More severe neutrophil-mediated lung inflammation has also been observed in MPO-KO mice exposed to zymosan compared to the zymosan-exposed WT mice, which correlates with higher levels of macrophage inflammatory protein (MIP)-2 and KC at an early phase of inflammation [64,65]. A similar study by the same group but employing a model of lung inflammation induced by non-viable *C. albicans* demonstrated that the MPO-KO mice exhibit a more severe lung inflammation than do WT mice, which is associated with a higher production of MIP-2 and KC at an early stage of inflammation as well as significantly higher lung concentrations of tumor necrosis factor (TNF)-α and IL-1β than in the WT mice at a later stage of inflammation [66]. Similarly, MPO-KO recipient mice exhibit increased lung inflammation after allogeneic bone marrow transplantation, which is associated with the higher lung concentrations of TNF-α and monocyte chemoattractant protein (MCP)-1 compared with responses in WT mice. Suppressed apoptosis of inflammatory cells recovered from bronchoalveolar lavage fluid parallels the enhanced lung inflammation in MPO-KO mice [67]. *In vitro*, MPO-KO neutrophils reveal a decreased rate of cell death characterized by phosphatidylserine surface expression [61,68].

1.2.4. Rheumatoid arthritis

Rheumatoid arthritis is a common chronic autoimmune disease characterized by inflammation and destruction of joints. Although the role of neutrophils in rheumatoid arthritis has been less extensively investigated than has the contribution of other leukocytes, critical roles of neutrophils as effector cells mediating joint inflammation and damage have been demonstrated in K/BxN serum–transfer arthritis [69] and collagen-induced arthritis [70]. 3-Chlorotyrosine formation is upregulated at sites of inflammation in rheumatoid arthritis joints, suggesting that MPO contributes to the progression of the disease [71]. MPO deficiency attenuates the severity of the disease in both K/BxN arthritis and collagen-induced arthritis models [72]. In contrast, MPO-KO mice have enhanced $CD4^+$ T cell responses in the lymph nodes, causing methylated bovine serum albumin-induced arthritis [57].

1.2.5. Skin inflammation and injury

Nitrogen mustard is a highly toxic alkylating agent that causes severe skin injury and neutrophilic inflammation [73]. Nitrogen mustard exposure elicits significantly more skin epidermal thickening, DNA damage, apoptosis, and expression of inflammatory and proteolytic mediators including MMP-9 in WT mice compared to MPO-KO mice, indicating that MPO plays an important role in nitrogen mustard-induced skin injuries [74]. In a model of ultraviolet-induced skin inflammation, the lack of MPO results in an early onset of inflammation in dorsal skin of mice irradiated with ultraviolet, accompanied by neutrophil infiltration and upregulation of MIP-2 production [75]. Lack of MPO causes enhanced T cell-mediated delayed-type hypersensitivity in skin [57]. T cell activation and proliferation are enhanced in both the lymph node and spleen in the ovalbumin-immunized MPO-KO mice compared with immunized WT mice [57]. When the mechanism of MPO-mediated suppression of adaptive immunity was investigated, it was found that neutrophils and MPO directly interact with tissue dendritic cells, and that enzymatically active MPO inhibits dendritic cell activation, as measured by decreased IL-12 production and CD86 expression [57]. These data demonstrate that MPO can limit the extent of an adaptive immune response by attenuation of dendritic cell activation, thereby decreasing both their migration to the draining lymph node and their antigen presentation capacity.

1.2.6. Neurological diseases

The recognition of associations between MPO and the neuroinflammation seen in neurodegenerative diseases has recently gained impetus [76]. Although corroborating experimental and clinical evidence suggests a possible deleterious role of MPO in various neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Multiple sclerosis, the basic understanding of the injurious effects of MPO on neurological disease outcome remain distant. In MPO-KO mice, attenuated neutrophil infiltration and tissue damage, as well as better functional recovery were observed after spinal cord injury compared with the WT mice, indicating that MPO exacerbates secondary injury not only by generating the strongly neurotoxic oxidant HOCl, but also by enhancing the neutrophil infiltration after spinal cord injury [77]. Infarct volume and nitrotyrosine formation are enhanced in MPO-KO mice in contrast to WT mice after ischemic brain injury [78]. LPS-induced blood-brain barrier dysfunction is significantly lower in MPO-KO mice as compared to WT littermates [79]. Secosterols, cholesterol ozonolysis products, are detected in human brain specimens from patients with Alzheimer's disease [80]. The secosterol formation is decreased in the culture of phorbol myristate acetate-activated neutrophils isolated from MPO-KO mice, strongly suggesting that secoterols are formed through the MPO-dependent mechanism *in vivo* [81].

On the other hand, deletion of MPO results in decreased loss of neurons in the substantia nigra of MPO-KO mice in response to a Parkinsonian agent [82]. Studies with MPO inhibitors and MPO-KO mice reveal that MPO deficiency potentiates, rather than inhibits, the rotenone-induced activated state of glia and promotes glial cell death. Furthermore, rotenone-triggered neuronal injury is more apparent in co-cultures with glial cells from MPO-KO mice than in those from WT mice [83]. MPO-KO mice are more susceptible to experimental autoimmune encephalomyelitis [84]. Experimental autoimmune encephalomyelitis is an inflammatory disease of the central nervous system mediated by $CD4^+$ Th1 cells, suggesting the activation of acquired immunity due to MPO deficiency.

1.2.7. Metabolic syndrome

Adipose tissue of obese mice and humans is infiltrated with many immune cells, including macrophages, which secrete a variety of cytokines, such as TNF-α and IL-1β that directly impair insulin signaling. In the early stage of obesity, neutrophils are recruited to adipose tissue where they produce chemokines and cytokines, thereby promoting macrophage infiltration [85]. MPO-KO mice are protected from high-fat diet-enhanced body weight gain and insulin resistance. MPO deficiency causes high body temperature via upregulation of uncoupling protein-1 and mitochondrial oxygen consumption in brown adipose tissue. Lack of MPO also attenuates high-fat diet-induced macrophage infiltration and expression of proinflammatory cytokines and chemokines, such as TNF-α, IL-1β, IL-6, and MCP-1, demonstrating that activation of MPO in adipose tissue contributes to the development of obesity and obesity-associated insulin resistance [86]. LDLR-KO mice that were reconstituted with bone marrow from MPO-KO mice show attenuated development of non-alcoholic steatohepatitis and diminished adipose tissue inflammation in response to high-fat diet, which is associated strong down-regulation of proinflammatory genes such as TNF-α and IL-6 in comparison with non-reconstituted LDLR-KO mice, thereby suggesting an important role for MPO in the pathogenesis of metabolic disease [87]. In studies employing MPO-KO mice and an acute inflammation model, free and protein-bound 2-aminoadipic acid, which is elevated in subjects at risk for diabetes, are formed by MPO during inflammation, further supporting the involvement of MPO-catalyzed oxidative processes as risk factors for the development of diabetes [88].

1.3. Role of MPO for neutrophil function

Increasing evidence has indicated that ROS derived from neutrophils act as mediators in cell signaling [89]. When stimulated with toll-like receptor (TLR)-4 agonist LPS *in vitro*, MPO-KO neutrophils express decreased levels of KC and MIP-1α, while IL-6, IL-10 and TNF-α are increased [60]. In contrast, the mutant neutrophils stimulated *in vitro* with zymosan, an agonist for TLR-2, dectin-1, and complement receptor 3, produce more of MIP-1α, MIP-1β, IL-1α, IL-1β, and TNF-α [64] as well as MIP-2 [64,65,90] as compared to WT neutrophils. MPO-

KO neutrophils stimulated *in vitro* with nonviable *C. albicans* produce a higher level of MIP-2 compared to the stimulated WT neutrophils [66]. Thus, cytokine production by MPO-KO neutrophils varies substantially depending on the type of pathogen associated molecular pattern engaged. In the case of zymosan stimulation, MPO-KO neutrophils enhance extracellular-signal regulated kinase (ERK) 1/2 activation as well as nuclear factor kappa-light chain enhancer of activated B cells (NF-κB) and inhibitor of κBα (IκBα) compared to response of WT neutrophils [90]. Since the ERK/NF-κB pathway plays an important role in regulating gene expression of proinflammatory cytokines and chemokines [91,92], a stronger activation of this pathway could result in greater production of MIP-2 than by WT cells [64,90]. ERK1/2 are activated by treatment with exogenous H_2O_2 [93]. Since MPO is the enzyme catalyzing the reaction to produce HOCl from H_2O_2 and Cl^-, it is possible that activated MPO-KO neutrophils accumulate higher intracellular levels of H_2O_2 relative to those in WT neutrophils [94]. On the other hand, HOCl and its derivatives, such as taurine chloramine [95] and monochloramine [96], strongly inhibit the NF-κB pathway by blocking the oxidation of IκBα [97]. Tateno et al. speculate that both the lack of HOCl and the accumulation of H_2O_2 due to MPO deficiency contribute to the upregulation of MIP-2 production in mouse MPO-KO neutrophils stimulated with zymosan [90]. On the other hand, MPO-KO neutrophils engulf more zymosan than do WT neutrophils. Importantly, cell surface expression of CD11b and phosphorylation of ERK1/2 are significantly higher in zymosan-stimulated MPO-KO neutrophils than in zymosan-stimulated WT neutrophils, suggesting that upregulation of CD11b/ERK signaling pathway due to absence of MPO enhances the zymosan phagocytic activity of mouse neutrophils [98]. Together, these results demonstrate that neutrophil-derived HOCl should no longer be considered as exclusively a cytotoxic oxidant but rather as a mediator of subtle reactivity that is capable of modulating cellular signaling mechanisms.

2. Concluding remarks

Modern hygienic environments, free of the high microbial burdens, may render MPO-dependent host defense less important than it was earlier in human evolution. However, unequivocal evidence implicates MPO or MPO-derived ROS as contributing factors in a wide variety of inflammatory diseases. 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. Whereas prolonged overproduction of MPO likely leads to tissue damage, MPO may play an anti-inflammatory role in selected situations, depending on the type of the inflammation. Keeping in mind that mice are not human and that murine disease models do not always faithfully mirror human diseases, I strongly believe that we can answer some of the unresolved questions regarding this persistently paradoxical neutrophil enzyme by directing attention to the pathophysiology in MPO deficiency through research with animal models.

Conflicts of interest

The author declares that I have no conflict of interest.

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