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Myeloperoxidase: A new player in autoimmunity

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Abstract

Myeloperoxidase (MPO) is the most toxic enzyme found in the azurophilic granules of neutrophils. MPO utilizes H_2O_2 to generate hypochlorous acid (HClO) and other reactive moieties, which kill pathogens during infections. In contrast, in the setting of sterile inflammation, MPO and MPO-derived oxidants are thought to be pathogenic, promoting inflammation and causing tissue damage. In contrast, evidence also exists that MPO can limit the extent of immune responses. Elevated MPO levels and activity are observed in a number of autoimmune diseases including in the central nervous system (CNS) of multiple sclerosis (MS) and the joints of rheumatoid arthritis (RA) patients. A pathogenic role for MPO in driving autoimmune inflammation was demonstrated using mouse models. Mechanisms whereby MPO is thought to contribute to disease pathogenesis include tuning of adaptive immune responses and/or the induction of vascular permeability.

Keywords

Autoimmunity; Myeloperoxidase; Oxidative stress; Multiple sclerosis; Rheumatoid arthritis; Neutrophil

1. Introduction

MPO is a myeloid-lineage restricted enzyme with strong antibacterial properties. During myeloid cell differentiation, MPO is largely expressed by neutrophils [1] where is located within azurophilic granules [2]. In addition, studies have also shown that MPO is produced by monocytes/macrophages including peritoneal macrophages and central nervous system (CNS) microglial cells, at least during pathological conditions [3–5]. In bacterial infection, neutrophil-derived MPO generates the potent bactericidal compound hypochlorous acid (HClO) [2,6]. Since neutrophils are important contributors to autoimmune disease pathogenesis it is not surprising that MPO is generally regarded as pathogenic during

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autoimmune disease progression, although some studies have reported contrary results [7]. The importance of neutrophils in the pathogenesis of a number of autoimmune diseases and the lack of safe and effective strategies to specifically target them, makes MPO a potential therapeutic target.

2. MPO localization to azurophilic granules

The MPO gene is located on the long arm of human chromosome 17 and spans 14 kbp and is induced in a tissue- and differentiation-specific manner [8–10]. Neutrophils are the main source of MPO where it accounts for 5% of the dry weight of the cell [11], making MPO the most abundant protein in neutrophils. MPO is transcribed only in promyelocytes during neutrophil differentiation in the bone marrow [12]. MPO expression is induced by G-CSF, which promotes differentiation of multipotential progenitor cells to granulocytes by regulating the transcription factors C-EBP and PU.1, which drive commitment of the granulocyte-macrophage progenitor to neutrophils and macrophages [13,14]. Neutrophil differentiation leads to decreased MPO transcription, which is undetectable in mature cells [12].

In promyelocytes MPO is packaged into azurophilic granules along with several other antimicrobial proteins, serine proteases and lysosome hydrolases [15]. The packaging of proteins in the azurophilic granules is asynchronous creating heterogeneous populations of azurophilic granules. In promyelocytes there are at least three categories of azurophilic granules. Small electron dense and nucleated azurophilic granules have uniform MPO distribution, while MPO in large spherical granules is detected at the granule rim. In addition, MPO is synthesized by promyelomonocytes, where it accounts for 1% of total cell protein [16]. Monocytes released into the circulation turn off MPO synthesis, which is further downregulated during their differentiation into macrophages [10,15].

3. MPO as a bactericidal compound

Neutrophils are a first line of defense against pathogens and upon entering tissues phagocytize bacteria localizing them to phagosomes where most bacteria are killed and digested [16]. In neutrophils, MPO-containing azurophilic granules fuse with the phagosome and when the common membrane is ruptured the granule contents, including MPO, are released into the phagosome [17]. Myeloid cells, after encountering pathogens, generate a superoxide burst that following dismutation leads to the generation of antibacterial hydrogen peroxide (H_2O_2) [2,6,16]. MPO utilizes H_2O_2 in the peroxidation (two subsequent one-electron reactions) and *ortho*-halogenation (two-electron reaction) cycles to generate the extremely potent bactericidal hypochlorous acid (HClO) along with other toxic oxidants [18,19]. In the peroxidation cycle, MPO oxidizes organic substrates, such as tyrosine (Tyr) and tryptophan, and ionic species (nitrite (NO_2^-), ascorbate, and urate), creating reactive radicals [18]. The halogenation cycle is unique to MPO due to its high oxidative potential that is needed to oxidize (pseudo)halides, such as chloride (Cl^-), bromide (Br^-), and thiocyanate (SCN^-), to HClO, hypobromous acid, and hypothiocynate, respectively [18–21]. Since MPO preferentially oxidizes Cl^-, and because Cl^- has the highest blood concentrations of all pseudohalides, HOCI is a primary product of MPO activity [16].

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Bactericidal activities of HClO and its toxicity for surrounding tissues occur because of its potential to modify lipids, DNA, amines and Tyr forming halohydrins, 5-chlorouracil, chloroamines and 3-chlorotyrosine, respectively [22]. The usual consequence of MPO-mediated macromolecule modification is their fragmentation. Hypochlorite was shown to modify side chains and peptide bonds of proteins leading to formation of chloramides and chloramines, which after conversion to radicals form enhanced peptide bond fragmentation [23–26]. HClO was shown to modify and then induce fragmentation of albumin, ribonuclease A and myoglobin in a concentration and time-dependent manner [26]. Similarly, HClO mediates modification of nucleotides in DNA through the creation of chloramines that undergo decomposition with radical formation leading to crosslinking and DNA denaturation [27,28]. Additionally, hypochlorite targets polysaccharides by interacting with nitrogen in amino sugars. Sugar derivatives undergo decomposition through radical intermediates, ultimately leading to fragmentation of the polysaccharide chain [29,30]. The main effect is the fragmentation of glycosaminoglycans and extracellular matrix [31]. Fragmentation of macromolecules through radical intermediates is relatively fast due to their instability. The presence of chlorinated tyrosines is a stable and specific marker of MPO activity [32].

4. Regulation of MPO expression

MPO expression levels depend upon allelic polymorphisms in the promoter region. A G to A substitution at position −463 (G-463A) leads to a 25-fold decrease in MPO transcription [33]. The substitution occurs within the Alu receptor response element (AluRRE), which is a cluster of nuclear receptor biding sites [33]. The substitution changes the consensus sequence of the Sp1 transcription factor, which is essential for enhanced MPO transcription. A G to A substitution, leading to decreased MPO transcription is also found at position −129 (G-129A) [34]. Alternative splicing of the MPO mRNA gives two transcripts of 3.6 and 2.9 kB [35]. The primary translation product is an 80 kDa precursor protein that undergoes a series of modifications including cleavage of a signal peptide, N-linked glycosylation, and limited deglycosylation, to form the catalytically inactive MPO precursor (apoproMPO) [35,36]. In the next step, MPO gains catalytic activity by incorporation of an iron-heme molecule into the catalytic centrum. Heme is covalently attached by two ester bonds and, unique for heme containing enzymes, a third sulfonium linkage, that uniquely orients one heme molecule into the enzyme pocket [36]. The unique configuration of the heme moiety confers MPO with very high oxidative potential, enabling chlorination at physiological pH [36]. Cleavage of proMPO leads to a 59 kDa α-subunit and a 13.5 kDa β-subunit that are covalently attached through the heme moiety [37]. A disulfide bridge joins the two heavy-light protomers in mature 150 kDa MPO [36,37].

5. Health consequences of MPO deficiency

MPO production is influenced by several genetic mutations and deletions [36]. Prior to automatization of hematological assays, inherited MPO deficiency was regarded as rare and having serious health consequences, such as increased susceptibility to infection, with *Candida* being the most common, especially in individuals who developed diabetes [38–41]. Now it is known that the incidence of MPO deficiency is quite high affecting 1 in 2000–

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4000 people in the United States and Europe and 1 in 55,000 people in the Japanese population [38,42]. MPO-deficiency is still associated with a higher occurrence of severe infections and chronic inflammatory process; however, ~50% of individuals remain asymptomatic [43,44]. Of interest, MPO deficiency is highly beneficial in cardiovascular integrity as demonstrated by decreased occurrence of myocardial infarction [44]. No association of MPO-deficiency with cancer susceptibility has been reported [44].

The lack of severe infections in some MPO-deficient individuals is not completely surprising when the role of H_2O_2 as a bactericidal compound is taken into account. H_2O_2 is produced during the oxidative burst by NADPH oxidase. Impaired assembly of NADPH oxidase due to a mutation in the NADPH-oxidase subunit gp94 gene thereby blocking production of H_2O_2 is observed in chronic granulomatous disease (CGD) patients [45]. These patients suffer from fatal infections, which are prevented when patients are infected with H_2O_2-producing bacteria, such as strains of *streptococci*, *pneumococci*, and *lactobacilli* [46]. These data indicate that even low levels of H_2O_2 are sufficient to activate H_2O_2-dependent killing mechanisms. Strains of *Streptococcus faecalis* and *Streptococcus pneumoniae* that produce diminished levels of H_2O_2, are killed less efficiently than WT strains by CGD leukocytes [22]. An intact respiratory burst in MPO-deficiency leads to abnormally slow killing of *Serratia marcescens* and *Staphylococcus aureus* 502A [41]. These data indicate that H_2O_2 is a critical anti-bacterial compound as it mediates killing of pathogens directly and indirectly by activating an MPO-dependent mechanism. While the presence of MPO is not crucial for pathogen elimination, it greatly increases the efficacy of the process.

6. The impact of MPO on neutrophil function

Besides antimicrobial molecules found in neutrophil granules, neutrophils also generate neutrophil extracellular traps (NETs) that are DNA structures released due to decondensation of chromatin [47,48] (Fig. 1). A number of proteins are bound to the NETs including histones and granule proteins including elastase, cathepsin G and MPO [47,48]. There are three described models of NETosis [48]. The first model is suicidal NETosis and is induced by various stimuli that activate neutrophils leading to the activation of the NADPH oxidase complex and increased cytosolic Ca^{2+} that ultimately results in deamination of histones allowing the decondensation of chromatin and is dependent upon ROS and PKC/Raf/MERK/ERK [48,49]. In vital NETosis occurs in response to recognition of stimuli through toll-like receptors and the C3 complement receptor and is characterized by release of nuclear DNA without loss of the nuclear or plasma membrane [48,50,51]. In a second vital NETosis model, mitochondrial DNA is released through the recognition of C5a or LPS [52].

NETs not only trap bacteria, but also modulate many aspects of both the innate and adaptive immune response [53]. In the innate immune response, NETs were shown to activate the NLRP3 inflammasome that utilized P2X7 receptor-mediated potassium efflux, resulting in the release of IL-1β and IL-18 [54]. NETs were reported to modulate adaptive immune responses by inhibiting LPS-induced monocyte DC maturation, thereby suppressing T cell activation [55]. Activated neutrophils release NETs 2–4 h after activation by a variety of stimuli and is associated with a novel form of cell death that is dependent upon the

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generation of reactive oxygen species (ROS) by NADPH oxidase as indicated by the lack of NET formation in patients with CGD that carry mutations in NADPH oxidase [56]. The downstream events leading to cell death and NET formation was shown to be mediated by neutrophil elastase, a component of azurophilic granules, that upon translocation to the nucleus partially degrades specific histones leading to chromatin decondensation [57]. The role of MPO in the process is to synergize with elastase to drive chromatin decondensation in a manner independent of its enzymatic activity [57]. More recently it was shown that intragranular MPO processing of ROS was required to trigger NET formation [58]. Mechanistically, MPO-derived HClO was shown to be the required ROS for NET formation in human neutrophils [59]. The above findings are consistent with neutrophils from *MPO*$^{-/-}$ humans lacking NET formation following activation with PMA [60]. In a separate study, the requirement for MPO following PMA activation of neutrophils was also shown, but bacteria were able to induce NETs independent of MPO. Thus the precise inflammatory conditions that promote a particular NETosis mechanism in an MPO-dependent manner is not clear [61].

7. The influence of MPO on the immune responses

An important step in the initiation of the adaptive immune response is the activation and migration of tissue dendritic cells (DC) to the draining lymph node (Fig. 1). To investigate whether MPO impacts adaptive immune responses, WT and *Mpo*$^{-/-}$ mice were immunized with OVA emulsified in CFA and T cell responses were measured both in vivo and in vitro (Fig. 1). While T cell activation and proliferation were not different in unimmunized mice, they were both increased in *Mpo*$^{-/-}$ mice in the lymph node and spleen 6 days after immunization [62] (Fig. 1). When splenocytes were stimulated with OVA in vitro, *Mpo*$^{-/-}$ T cells produced higher levels of IFN-γ and IL-17A [62]. These cumulative data indicate that MPO suppresses adaptive immune responses, which was further shown by enhanced T cell-mediated skin delayed type hypersensitivity and antigen-induced arthritis in *Mpo*$^{-/-}$ mice [62] (Fig. 1). When the mechanism of MPO suppression of adaptive immunity was investigated, it was found that neutrophils and MPO directly interacted with DC in the lymph node [62]. In addition, it was found that co-culture of neutrophils with DC increased their production of MPO and that enzymatically active MPO inhibited LPS-induced DC activation in a dose-dependent manner as measured by decreased IL-12 production and CD86 expression [62] (Fig. 1). Using in vitro assays, the mechanism whereby MPO suppresses DC activation was determined to be dependent upon MPO-generated oxidants and DC CD11b expression [62]. These data demonstrate that MPO can limit the extent of an adaptive immune response by attenuation of DC activation thereby reducing both their migration to the draining lymph node and antigen presentation capacity (Fig. 1). However, it is worth noting that DC activation, characterized by increased IL-12 production, is also inhibited by nitric oxide (NO) [63]. NO is consumed by MPO, which is known as an NO-oxidase, implying that MPO could lead to the activation of DC by limiting NO availability [64] (Fig. 2).

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8. MPO and the vasculature

Leukocyte entry into the site of inflammation is regulated by the endothelial barrier [65]. A series of well described events, rolling, binding and diapedesis lead to extravasation into the tissues [65] (Fig. 2). Transmigration is supported by weakened interactions between adjacent endothelial cells [65]. MPO is regarded as an important modulator of vasculature functioning, being associated with chronic vascular diseases such as atherosclerosis and chronic coronary disease [66]. After being released, MPO interaction with the vasculature is supported by electrostatic forces between positively charged MPO and negatively charged heparin-sulfate proteoglycans (HSPG) associated with the endothelium and negatively charged albumin [67,68] (Fig. 2). Both MPO transcytosis and deposition in the vascular extracellular matrix (ECM) was shown to occur independently of neutrophil transmigration [69] (Fig. 2). In the vascular ECM, MPO works as an NO-oxidase, consuming NO that leads to impaired endothelial relaxation [64] (Fig. 2). An additional mechanism whereby MPO likely alters endothelium function is through its nitrotyrosination of the ECM component fibronectin [70] (Fig. 2). How this impacts endothelial barrier function is unknown. Interestingly, neutrophils were shown to deliver MPO directly to endothelial cells that required cell-cell interactions dependent upon the integrin Mac-1 (CD11b/CD18) [71].

9. Influence of MPO on cytokine production during lung inflammation

Cytokine production by *Mpo*$^{-/-}$ neutrophils varies substantially depending on the type of pathogen associated molecular pattern signal received. *Mpo*$^{-/-}$ peritoneal cavity neutrophils stimulated in vitro with the toll-like receptor (TLR) 2 agonist zymosan, had increased production of proinflammatory MIP-1α, MIP-1β, IL-1α, IL-1β, and TNF-α as compared to WT [72]. This finding correlated to in vivo findings as indicated by a similar increase in the same cytokines in *Mpo*$^{-/-}$ mice following zymosan administration [72]. Similarly, it was found that *Mpo*$^{-/-}$ mice with zymosan-induced lung inflammation exhibited elevated levels of MIP-2, which correlated with increased number of neutrophils in the lung [73]. In contrast, when the TLR4 agonist LPS was used to induce acute lung inflammation, neutrophil numbers were not increased in the lung of *Mpo*$^{-/-}$ mice as was observed in WT mice [74]. This finding correlated with decreased levels of the cyokines IL-4, IL-6, IL-9, IL-13 and IFN-γ and the chemokines KC, MIP-1α and RANTES in the lung of *Mpo*$^{-/-}$ mice [74]. When stimulated with LPS in vitro, *Mpo*$^{-/-}$ neutrophils expressed decreased levels of KC and MIP-1α as observed in the lung, while IL-6, IL-10 and TNF-α were increased [74]. Of interest, the reduced numbers of neutrophils in the lung of *Mpo*$^{-/-}$ mice was suggested to be due to decreased migration to KC, as indicated by in vitro migration assays [74]. It is not clear why MPO-deficiency differentially alters responsiveness to TLR ligands in lung injury, but it could be due to either direct or indirect stimulation of different cells types that produce the cytokines. This was addressed using alveolar macrophages from rats stimulated with either active or enzymatically inactive MPO [75]. Both forms of MPO increased macrophage respiratory burst, intracellular killing of *Candida albicans* and TFN-α production [75]. MPO also induced the production of IL-1α and IL-1β message in the alveolar macrophages [75]. These data demonstrate that MPO could directly induce the production of proinflammatory cytokines by alveolar macrophages in the lung.

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10. MPO and rheumatoid arthritis (RA)

RA is a chronic autoimmune disease leading to joint damage and cartilage erosion. The inflammatory response in RA is complex being dependent on both T cells and antibodies [76]. In numerous RA mouse models, neutrophils have been shown to be a prominent cellular component inside inflamed joints [77]. In addition, neutrophils from RA patients were shown to exhibit an increase in the spontaneous generation of NETs in vitro that was associated with MPO expression [78]. Pathogenesis of neutrophils in arthritis was demonstrated by attenuation of disease severity due to their depletion in both the K/BxN antibody-mediated arthritis and collagen-induced arthritis (CIA) models [79–81]. A specific role for MPO in both the K/BxN and CIA models was indicated by reduced disease severity in *Mpo*$^{-/-}$ mice [82] (Fig. 2). Of particular interest is the finding that adaptive immune responses were enhanced in *Mpo*$^{-/-}$ mice with CIA (Fig. 2). These data suggest that MPO may have a direct impact on inflammation in the joint that is independent of the underlying immune response. This concept is supported by the finding that both intracellular and extracellular MPO was shown to be present in the synovium of RA patients [82]. In addition, RA patients were shown to have increased plasma levels of MPO compared to healthy controls [83,84]. However, there was inconsistency as to the correlation between MPO levels in the plasma and disease activity score, as one study showed correlation, while another did not [83,84]. Additionally, RA patients exhibited higher levels of MPO and its product Cl-Tyr in synovial fluids compared to osteoarthritis (OA) patients [85]. RA patients treated with disease-modifying antirheumatic drugs and/or biological agents exhibited decreased MPO-specific activity in the joints [85]. These data correlate well with murine studies, where injection of active or inactive MPO into the joints exacerbated disease, while decreased MPO levels in affected joints, observed after treatment with atrovastatin or blockade of CXCR1/CXCR2 receptors, correlated with decreased severity disease [86–88].

11. MPO in multiple sclerosis (MS)

MS is an autoimmune disease of the central nervous system (CNS) mediated by T cells recognizing myelin antigens and characterized by immune cell infiltration, demyelization and neuronal damage [89,90]. The animal model that shares features of MS is experimental autoimmune encephalomyelitis (EAE) [91]. Interestingly, EAE was almost completely attenuated by depletion of neutrophils [92–94]. Although, the role of neutrophils in the progression of MS and EAE needs further clarification, it is not surprising that their main constituent MPO is gaining acceptance as an important modulator of disease severity.

An association between MPO and MS is suggested by linkage between the 192 bp allele of the D17S957 microsatellite region of MPO with MS in the British population [95]. However, two other studies showed that the 17q22–23 chromosomal region, which contains the MPO gene, is not strongly associated with MS [96,97]. Since MPO levels are influenced by promoter polymorphisms, several studies have examined its correlation with MS incidence. Early studies correlated the G allele at -463, which is associated with higher levels of MPO, with increased incidence of early onset MS in females in the American population [5]. Similarly, this allele was found in 96% of MS patients in Poland [98]. In contrast, MS in individuals from Olmsted county in the USA carrying the A allele was not associated with

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gender, age at onset, susceptibility or altered course or severity of disease [99]. Since these studies contained less than 200 individuals, a Swedish group reevaluated the association using almost 900 patients and 600 controls and concluded there was no influence of the MPO promoter polymorphisms at -463 and -129 positions on MS susceptibility [100].

Although the association between the level of MPO transcripts and MS is unclear, MPO activity is thought to play a role in MS. Higher serum levels of MPO were found in Japanese patients with conventional and opticospinal MS at remission [101]. In healthy individuals, MPO is not detected in macrophages/microglia cells in the CNS; however, during MS, MPO was detected in microglia/macrophages in and around MS lesions [5,102]. Each subtype of MS (acute, relapsing-remitting, primary and secondary progressive) has a unique pattern of demyelinating lesions. In patients with acute and relapsing MS presents with focal changes in white matter, while progressive MS is characterized by diffused changes in normal-appearing white matter and cortex [103]. In patients with primary and secondary MS, MPO activity is increased in areas of cortical demyelization and in active demyelization in brain white matter, while MPO activity was not significantly increased in non-active lesions [102,104]. In patients with acute or early relapsing MS, active lesions contained macrophage/microglial cells expressing MPO [5]. A unique lesion, characterized by low demyelization and axonal injury but with the presence of MPO-containing macrophage/microglial cells was observed in patients with acute or early relapsing MS [4,105]. It was suggested that the areas of activated microglial cells precede formation of these lesions, which develop into active demyelinated plaques [4].

MS plaques are detected by DTPA-gadolinium (Gd)-enhanced magnetic resonance imaging (MRI) [106]. DTPA-Gd passes through the blood-brain barrier (BBB) disrupted by inflammation. Subtle focal changes in normal-appearing white matter, without overt demyelization and T cell infiltration are not visible using conventional MRI [107]. Since early diagnosis and prompt treatment reduces relapse and slows axonal damage, and production of MPO precedes formation of active plaques with demyelination and overt inflammation, a new method based on MPO-targeted MRI was introduced [108]. In this technique, MPO in the presence of H_2O_2 radicalizes the sensor leading to its oligomerization [108]. The oligomers bind to proteins, retaining it at the site of focal change and enabling its visualization using T1-weighted MRI [108]. Another method for visualization of inflammatory lesions using MPO is based on bioluminescence [109]. Although this method was not tested in a mouse model of MS, it was very successful in the visualization of changes in acute dermatitis, allergic contact hypersensitivity, arthritis and some tumors [109]. Inflammatory lesions were visualized based on detection of blue light, emitted by luminol oxidized by MPO [109]. Interestingly, when the MPO-sensitive probe MPO-Gd was used to visualize EAE severity, it was shown that inhibition of MPO with 4-aminobenzoic acid hydrazide decreased lesion volume, confirming that targeting MPO could be a relevant therapeutic strategy in MS [110]. In addition, MPO-Gd was superior to DTP-Gd at detecting subclinical inflammatory lesions even in the absence of overt BBB breakdown, and showed that the number of MPO-Gd-enhancing lesions correlated with the infiltration of neutrophils and macrophages producing MPO [102,111].

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Although neutrophils are not typically found in MS lesions, likely due to their short-half life in tissues, evidence is mounting supporting their role in MS pathogenesis [112]. Two separate studies reported that neutrophils in MS patients expressing a primed phenotype were increased in the peripheral blood as assessed in vitro by higher levels of degranulation and oxidative burst that correlated with higher levels of NETs in the serum [113,114]. In addition, it was found that the neutrophil-to-lymphocyte ratio was highly increased in MS patients undergoing relapse, and was a predictor of disability progression [115]. Additional evidence for neutrophil activity in MS is the increase in serum CXCL8 (IL-8), a chemoattractant for neutrophils in humans [116]. In a separate study, an increase in the granulocyte recruiting chemokine CXCL5 was reported in the plasma of relapsing MS during new lesion formation [117]. This same study showed that plasma levels of CXCL1 (neutrophil chemoattractant), CXCL5 and neutrophil elastase directly correlated with MS disability and lesion volume [117]. Finally, post-mortem tissue from a MS patient affected by relapse following cessation of natalizumab showed a prominent neutrophil infiltration in regions of BBB leakage [118].

Increased BBB permeability is a feature of EAE, being associated with disease onset and neutrophils are thought to be important contributors to this process, likely by a MPO-dependent mechanism [93,118,119]. Using a primary brain microvascular endothelial cell line, it was shown that MPO can directly alter permeability in vitro [120]. In our studies utilizing the novel MPO inhibitor *N*-acetyl lysyltyrosylcysteine amide (KYC) [121], we found that its daily administration starting at disease initiation did not alter the day of onset or the kinetics of the first seven days of disease progression after which disease progression stalled resulting in a significantly reduced peak and cumulative disease score [122]. These data indicate, that at least in EAE, MPO does not play an essential role in the process of peripheral CD4 T cell priming that is required for EAE onset. When KYC was administered therapeutically starting at the peak of disease there was an immediate and significant decrease in disease severity [122]. After only 5 days of KYC treatment there was a significant reduction in the number of macrophages and neutrophils in the CNS [122]. Interestingly, there was no reduction in the number of IL-17 and/or IFN-γ secreting CD4 T cells [122]. These data further support the concept that MPO can contribute to disease pathogenesis in the absence of specific effects on adaptive immunity. In the same experiment, we showed that the BBB was completely sealed after five days of MPO inhibition [122]. Further data supporting a role for MPO in opening of the BBB is a study that transiently opened the barrier by LPS injection, which lead to significantly less CNS vascular permeability in *Mpo*$^{-/-}$ mice as compared to WT [120]. Cumulatively, these data suggest a role for neutrophil-derived MPO in the breakdown of the BBB contributing to MS disease progression in the absence of a direct impact on adaptive immunity.

12. Conclusion

MPO is the most cytotoxic and abundant enzyme expressed by neutrophils, and in addition to having direct anti-bacterial properties, it has emerged as a modulator of the adaptive immune response in certain contexts. Increased MPO activity and protein levels have been observed in many inflammatory conditions. MS and RA are the most prominent examples among autoimmune disorders, which according to their mouse models are exacerbated by

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MPO activity. Moreover, while MPO was shown to negatively impact the extent of adaptive immune responses, the evidence suggests that it tunes, but does not inhibit, proinflammatory processes. At least in EAE, if negative tuning of the adaptive immune response occurs by MPO, then it must be below the threshold of that which is required for disease onset and progression of disease. Given that elevated MPO activity is associated with a number of inflammatory diseases and the clinical data indicate that MPO deficiency does not necessarily preclude an individual to increased life threatening infections this supports the idea that MPO is a valid and promising therapeutic target. Finally, therapeutic targeting of MPO, a component of innate immunity, has an advantage over therapies that directly and profoundly impact adaptive immunity.

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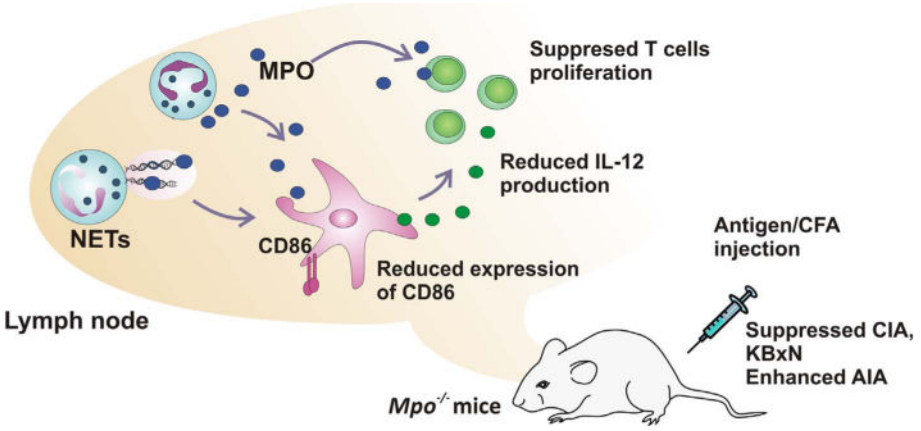

Fig. 1.

The influence of MPO on the immune responses. The immune response induced by subcutaneous injection of OVA/LPS or OVA/CFA, leads to recruitment of neutrophils to the local lymph node where they are activated releasing NETs and MPO. MPO released from neutrophils limits the activation of DC as measured by CD86 expression and reduced IL-12 production. Consequently, T cell proliferation and proinflammatory cytokine production is reduced. Consistent with this, MPO-deficient mice have enhanced antigen induced arthritis (AIA). However, the reduced disease severity in MPO-deficient mice in the K/BxN arthritis and collagen induced arthritis (CIA) mouse models, indicates that MPO can also be pathogenic in disease.

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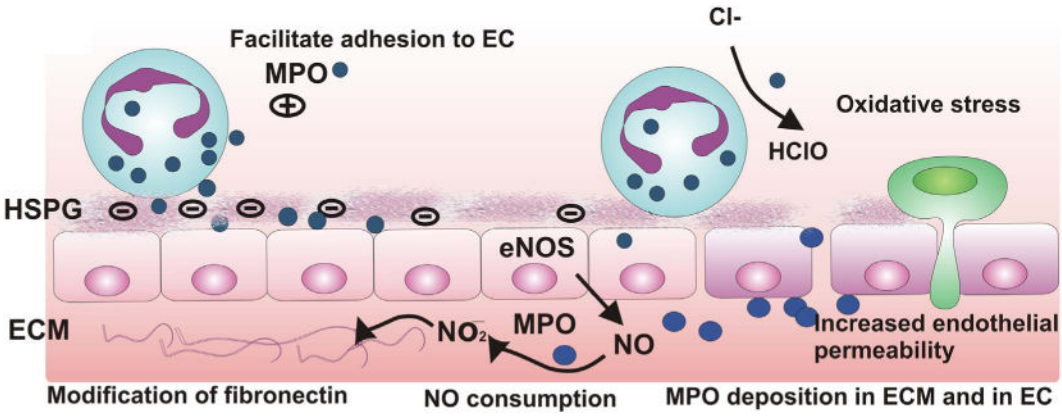

Fig. 2.

MPO and the vasculature. Leukocyte transmigration across the endothelium is supported by electrostatic interactions between positively charged MPO and the cell membrane containing charged heparin-sulfate proteoglycans (HSPG). Additionally, MPO generates HClO that increases endothelial permeability. Released MPO is transcytosed and deposited in the vascular extracellular matrix (ECM). In the ECM, MPO utilizes NO, leading to impaired vascular relaxation and the generation of NO_2^-, which modifies the ECM component fibronectin.