Myeloperoxidase

van der Veen, Betty S.; de Winther, Menno P. J.; Heeringa, Peter

Published in:
Antioxidants & Redox Signaling

DOI:
10.1089/ars.2009.2538

Publication date:
2009

ANTIOXIDANTS & REDOX SIGNALING
Volume 11, Number 11, 2009

DOI: 10.1089/ars.2009.2538

COMPREHENSIVE INVITED REVIEW

Myeloperoxidase: Molecular Mechanisms of Action and Their Relevance to Human Health and Disease

Betty S. van der Veen,[1] Menno P.J. de Winther,[2] and Peter Heeringa[1]

Reviewing Editors: Ohara Augusto, John W. Chen, Michael Davies, Xin-Liang Ma, Ernst Malle, Pasquale Pignatelli, and Tanja Rudolph

[1]Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands.
[2]Department of Molecular Genetics, Cardiovascular Research Institute Maastricht, Maastricht, the Netherlands.

Abstract

Myeloperoxidase (MPO) is a heme-containing peroxidase abundantly expressed in neutrophils and to a lesser extent in monocytes. Enzymatically active MPO, together with hydrogen peroxide and chloride, produces the powerful oxidant hypochlorous acid and is a key contributor to the oxygen-dependent microbicidal activity of phagocytes. In addition, excessive generation of MPO-derived oxidants has been linked to tissue damage in many diseases, especially those characterized by acute or chronic inflammation. It has become increasingly clear that MPO exerts effects that are beyond its oxidative properties. These properties of MPO are, in many cases, independent of its catalytic activity and affect various processes involved in cell signaling and cell–cell interactions and are, as such, capable of modulating inflammatory responses. Given these diverse effects, an increased interest has emerged in the role of MPO and its downstream products in a wide range of inflammatory diseases. In this article, our knowledge pertaining to the biologic role of MPO and its downstream effects and mechanisms of action in health and disease is reviewed and discussed. *Antioxid. Redox Signal.* 11, 2899–2937.

I. Introduction

Almost 70 years ago, Agner (2) described the purification of an intensely green iron-containing protein with peroxidase activity from purulent fluid of patients with tuberculous empyema (2). Because of its green color, the protein was initially named verdoperoxidase, but because subsequent studies showed that the expression of this peroxidase was restricted to cells of the myeloid lineage, this was soon changed to myeloperoxidase (MPO; EC 1.11.1.7) (3, 208, 316, 317). After the initial description of MPO by Agner, it took almost 30 years before the functional role of MPO was elucidated. In seminal studies by Klebanoff (175, 176), MPO, together with its substrate hydrogen peroxide (H_2O_2) and a halide, proved to be a powerful antimicrobial system, and as such, MPO is a key component of the oxygen-dependent microbicidal activity of phagocytes, and in particular, neutrophils.

MPO is a member of the superfamily of mammalian heme peroxidase enzymes, which also includes eosinophil peroxidase (EPO) and lactoperoxidase (LPO) (99, 264). The human genes for these three mammalian heme peroxidases are encoded adjacent to each other on the long arm of chromosome 17, are similar in gene organization, and share considerable sequence homology, suggesting that these genes may have evolved from a common ancestral gene *via* duplication (343). MPO and EPO are highly cationic (P_i of >10), and they readily bind to various negatively charged structures, such as bacterial surfaces (234, 319), components of the extracellular matrix (79, 184), and cellular membranes, including those of endothelial cells (32, 35, 380) and neutrophils themselves (135).

For a long time, MPO has been considered to be a bactericidal enzyme whose main function is to generate reactive oxygen species (ROS) that contribute to the destruction and killing of engulfed pathogens. However, recent evidence has extended this view by demonstrating that MPO is also intimately involved in cellular homeostasis and is an important factor in the initiation and progression of various inflammatory diseases. In this context, MPO and its downstream products and effects have gained prompted considerable interest in various inflammatory diseases, most prominently (cardio)vascular diseases. In this article, recent developments pertaining to our knowledge of the biologic roles of MPO in health and disease, including its role as an autoantigen in small-vessel vasculitides, its mechanism of action, and its potential as a biomarker, are reviewed. The importance of the MPO–H_2O_2 system in bacterial and fungal killing is beyond the scope of this review and is not discussed in depth. The reader is referred to some excellent recent reviews in which these issues are extensively discussed (79, 177).

II. Properties of MPO

A. Cellular origin of MPO

During myelopoiesis in the bone marrow, MPO is actively synthesized in promyelocytes and promonocytes, whereas in fully differentiated myeloid cells, MPO synthesis normally ceases (Fig. 1). The main cellular source of MPO is the neutrophil, and, in humans, MPO levels in these cells range from 2–5% of total cellular protein, which is equivalent to 2–4 μg per 10^6 cells (177). Neutrophils are highly motile phagocytic cells and constitute the primary effector cell type of the innate immune system (75, 160). These cells are traditionally viewed as short-lived, fully differentiated cells whose main function is quickly to find, ingest, and destroy a wide range of invading pathogens, including bacteria, fungi, and protozoa (75, 246). Indeed, in a typical immune response to invading pathogens, neutrophils are the first cells to arrive at sites of infection, but they also leave or die early. This acute neutrophilic response is often accompanied by host-tissue damage (134, 358). However, this view on neutrophils as passive, nonspecific phagocytes has changed considerably in recent years, and neutrophils are now recognized as important cellular players that are essential for the launching and shaping of effective immune responses and tissue repair (246). These immune-modulating effects are in part mediated by neutrophil-derived cytokines and chemokines that affect and attract other immune cells, such as monocyte/macrophages and dendritic cells (39, 67, 347, 348). In addition, the same mediators that are necessary for neutrophil-mediated killing of pathogens can also modulate cell signaling (245). For example, ROS can inactivate tyrosine phosphatases through reversible oxidation of active-site cysteine sulfhydryls (339).

To perform their function, neutrophils engulf and ingest pathogens, after which a phagolysosomal compartment is formed by fusion of the phagosome with intracellular granules that contain various bactericidal components (92). Neutrophils contain at least three different types of granules: the primary (also known as azurophilic granules), secondary (also known as specific granules), and tertiary granules (also

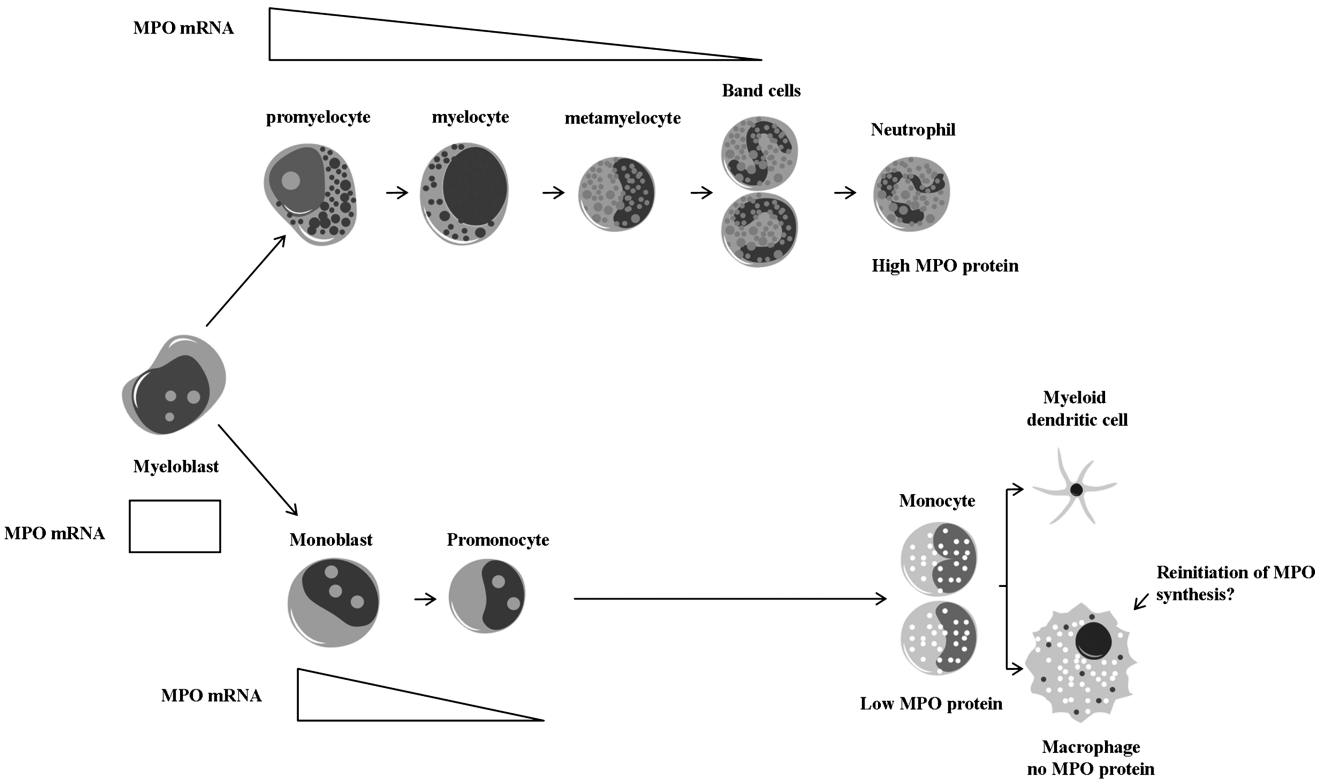

FIG. 1. Expression of myeloperoxidase mRNA during myelopoiesis. Abundant MPO mRNA expression is detected in undifferentiated myeloblasts. During differentiation into neutrophils or monocytes, MPO mRNA levels decrease and are absent in mature neutrophils and monocytes. In mature neutrophils, MPO protein is abundant (2–4 g/10^6 cells), whereas levels in monocytes are much lower. With differentiation into macrophages, MPO protein is lost, although some evidence indicates that, under certain circumstances, MPO synthesis is reinitiated in these cells [based on (73)].

known as gelatinase granules) (42, 43). The secondary and tertiary granules are discharged first, contain overlapping sets of proteins, but are peroxidase negative (92). The primary granules are peroxidase positive because of their high content of MPO, but in addition, they contain various proteolytic enzymes, including defensins, lysosomal hydrolases, and neutral serine proteinases, such as elastase, proteinase 3 (Pr3), and cathepsin G. Within these granules, a matrix exists consisting of negatively charged sulfated proteoglycans, to which these cationic proteins bind. Together with the acidic pH that is maintained within the granules, this creates an intragranular environment in which these proteolytic enzymes are presumably kept in an inactivated state. On neutrophil activation, the primary granules release their contents into the phagosome. At the same time, the various components of the NADPH oxidase complex are recruited to the internal phagolysosomal membrane surface as well as to the plasma membrane (29, 30). The activation of the NADPH complex produces superoxide radicals ($O_2^{\cdot}$), which, through either spontaneous or superoxide dismutase (SOD)-catalyzed dismutation of $O_2^{\cdot}$ also lead to the generation of H_2O_2 (30). As such, the substrate required for the generation of MPO-derived oxidants is formed.

Besides neutrophils, monocytes also contain MPO-positive granules. These MPO-containing granules are formed in promonocytes in the bone marrow, are readily detectable in mature circulating monocytes, but are far fewer in number compared with neutrophils (257) (Fig. 1). Furthermore, when monocytes differentiate into macrophages, expression of MPO is generally lost, although some evidence suggests that MPO expression may be reinitiated in these cells under certain conditions (328). Finally, MPO expression has been detected in Kupffer cells in the liver (51), microglia, granule-containing neurons, and pyramidal neurons of the hippocampus (107, 242).

B. MPO gene

1. Gene structure. The gene encoding for human MPO has been cloned by several research groups (61, 150, 238, 357, 379). It is located on the long arm of chromosome 17 mapped to segment q23.1, is about 11 kb in size, and consists of 11 introns and 12 exons (141, 191, 350). The *MPO* gene has also been cloned from various other species, including pigs (221), mice (353), chickens (188), and fish (60). Furthermore, sequence comparison demonstrated considerable homology among the different species, suggesting that the gene has been highly conserved during evolution.

2. Transcriptional activation. Transcription of the *MPO* gene is highly specific to tissue and maturation stage. Normally, MPO mRNA expression is limited to the myeloid lineage, where it is detected only during the myeloblast and promyelocyte stages of myeloid cell maturation (42, 111). This indicates that *MPO* gene transcription must be highly regulated, and research has shown that this is, to a large extent,

dependent on the interaction of several transcription factors and *cis* elements in or near the promoter region (25, 386). A basal promoter has been identified and characterized in the 5′ flanking region of the *MPO* gene, located between −128 and +11 bp (26, 28). This promoter appears to be responsible for the initiation of the majority of full-length MPO transcripts, although more recently, two additional promoter regions were identified located proximally within 1 Kb from the major MPO promoter (204). In addition, elements that regulate gene transcription have been described for the *MPO* gene. Studies investigating the chromatin structure of the *MPO* gene in the promyelocytic cell line HL60 already suggested the existence of upstream enhancer regions in the *MPO* gene by demonstrating the presence of a DNase 1 hypersensitive site ∼4 kb upstream from the transcription-initiation site (210). Induction of terminal differentiation of these cells by treatment with dimethyl sulfoxide or 1,25-dihydroxyvitamin D_3 reduced the DNase I hypersensitivity, suggesting the presence of regulatory elements that control *MPO* gene transcription within this region (210, 122). Indeed, in later studies, Austin *et al.* (27) identified and characterized a 365-bp MPO enhancer segment ∼4 kb upstream of the *MPO* gene transcription site and found that this enhancer increases the activity of the minimal human MPO promoter in leukemic cells and that it is, in part, responsible for the decrease in promoter activity in HL60 cells on induction of maturation. Further characterization of this enhancer segment showed that it contains binding sites for AML1, c/EBP, and c-Myb, all of which contribute to its functional and tissue-specific activity (381).

3. Genetic polymorphisms. In addition to enhancer regions upstream of the promoter region that regulate transcription activity, a number of single-nucleotide polymorphisms (SNPs) have been identified within or near the promoter region of the *MPO* gene (Fig. 2). The most extensively studied polymorphism lies within a specific Alu-receptor response element in the upstream promoter region, which contains several response elements recognized by ligand-dependent transcription factors, including peroxisome proliferator–activated receptor (PPAR) γ/α, liver X receptor (LXR), estrogen receptor (ER), and SP1 (185, 275, 297). This Alu element has been found to be specific for primates and is lacking in the mouse *MPO* gene (297). Recently, this Alu element with its multiple transcription factor–binding sites has been linked to the aberrant expression of MPO in cells that, under normal circumstances, would not express MPO, including macrophages, astrocytes, and hepatocytes (213, 297). The Alu element consists of four hexamer response elements, of which the first one contains a functional −463G/A SNP. The −463G allele creates an SP1-binding site and increases promoter activity in reporter gene-transfection assays. In primary myeloid leukemic cells and monocytes/macrophages, the −463GG genotype has been linked to higher MPO mRNA and protein levels (295). In contrast, the −463A site enhances ER binding and has been associated with lower MPO expression levels (185). An increasing number of studies have determined −463G/A genotypes in cohorts of normal individuals, demonstrating genotype frequencies in white persons in general of around 60% for −463GG, 35% for −463GA, and 5% for −463AA. Given the importance of MPO in inflammatory diseases and the possible influence of the −463G/A SNP on MPO expression levels, the −463G/A genotype frequencies have been determined in a wide range of diseases. Several of these genetic-association studies report a link between −463G/A polymorphisms and disease risk or severity in a range of diseases, including atherosclerosis (20, 212, 258), Alzheimer's disease (AD) (203, 281), MPO antineutrophil cytoplasmic autoantibody–mediated vasculitis (300), multiple sclerosis (242, 382), hepatitis C virus–induced fibrosis (298), periodontal disease (228), and various forms of cancer (see section on carcinogenesis). However, for some of these associations, contradictory results exist, as is discussed later. Also, caution should be taken when interpreting these results because many of these studies are based on rather small sample sizes, vary in control cases, and are, in some cases, underpowered.

The −463G/A polymorphism does not appear to have an effect on circulating MPO levels or MPO activity, as demonstrated in three independent cohorts of normal individuals

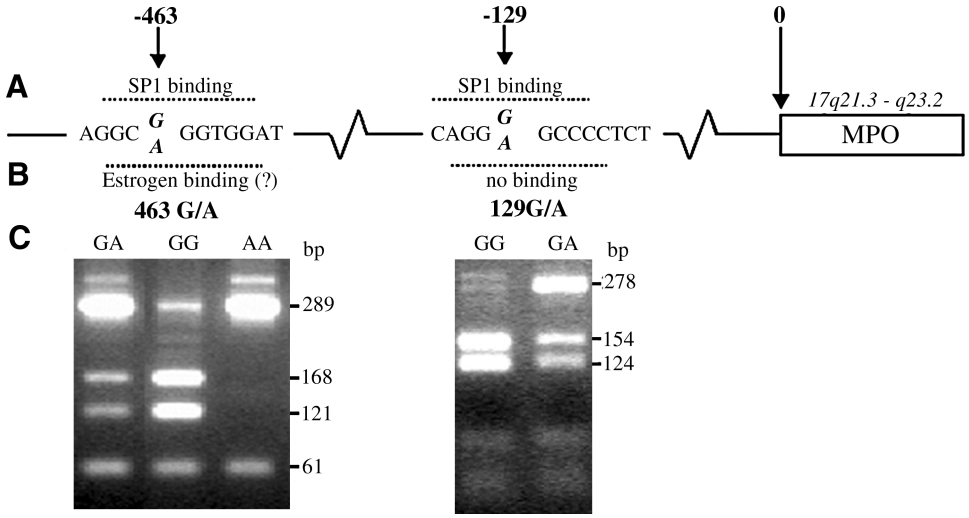

FIG. 2. Schematic representation of two single-nucleotide polymorphisms (SNPs), −463 and −129 G/A, in the promoter region of the *MPO* gene. (**A**) shows (putative) binding sites when both polymorphisms have their wild-type genotype (G allele). (**B**) Effect when both sites are polymorphic (A allele). (**C**) Agarose gels of the PCR digestion products of the two SNPs. The primary PCR products for the two SNPs, −463G/A (350 bp) and −129G/A (289 bp) were subjected to restriction-enzyme analysis. The G-to-A substitution results in the loss of specific restriction sites for the restriction enzymes *Aci*I and *Apa*I for the −463 and −129 G/A polymorphisms, respectively (305).

(69, 136, 305). In one of these studies, a molecular screen of the *MPO* gene identified five additional polymorphisms, one in the 5′ flanking region, three in the intronic flanking regions, and one in the coding region of the gene (136). Further analysis showed that for the −129G/A polymorphism located in the promoter region of the *MPO* gene, the −129A allele abolishes an SP1 binding site and is significantly associated with decreased circulating MPO levels (136) (Fig. 2). This observation was confirmed by Rutgers *et al.* (305), who demonstrated reduced MPO activity and protein levels in neutrophils derived from −129GA individuals. Finally, several additional polymorphisms in the *MPO* gene were identified by Chevrier *et al.* (68), many of which were located in the coding regions (68). In a follow-up study, two of these SNPs, −638C/A located in the 5′ flanking region and V53F located in exon 2, were found to modulate MPO expression. Carriers of the −638A or 53F allele displayed higher MPO activity in their neutrophils, although the molecular basis of these effects is unknown (69).

In summary, several SNPs in the *MPO* gene have been described that could affect MPO protein levels and activity. However, it is generally believed that these molecular variations of the gene have only modest effects on biologic variations in MPO serum concentrations in the healthy population. Other factors that influence serum MPO levels that are likely to be more important include age, gender, smoking in men, and the use of oral contraceptives by women (136, 305). For example, MPO activity in neutrophils is higher in women compared with that in men, whereas in both men and women, serum MPO levels increase with age (136, 154, 305).

C. MPO protein biosynthesis, processing, and structure

The biosynthesis of mature MPO involves a series of complex translational and posttranslational processes [for a detailed review, see (118)]. The initial translation product of the *MPO* gene is a single 80-kDa protein, which in the endoplasmatic reticulum is converted into the 90-kDa apoproMPO through cleavage of the 41 amino acid signal peptide and N-linked glycosylation, resulting in the incorporation of mannose-rich side chains (248, 249, 378). The oligosaccharides added to apoproMPO are important for its transient association with the molecular chaperones calreticulin and calnexin, both of which are calcium-binding proteins and most likely involved in proper protein folding (252, 253). ApoproMPO is enzymatically inactive because it lacks heme. Incorporation of a heme converts apoproMPO into the enzymatically active proMPO, which now enters the Golgi. After proMPO exits the Golgi, various proteolytic modifications occur to generate the full mature protein (5, 180, 267). First, the 125–amino acid propeptide is cleaved off to generate a 74-kDa short-lived intermediate. Next, the 74-kDa intermediate is cleaved into two subunits comprising a 59-kDa heavy (α)-subunit and a 13.5-kDa light (β)-subunit, which are linked by covalent bonds associated with the heme group. Finally, two of these so-called heavy and light protomers combine by a single disulfide bond between the two heavy subunits to produce the mature form of MPO (10, 95, 121). The processed mature MPO protein is thus a glycosylated 146-kDa homodimer, and this is the form found in the primary granules of neutrophils and lysosomes of monocytes.

Within the family of animal peroxidases, MPO is the only member known to form a dimer, although the functional consequences of dimer formation are not known (118). In this respect, it is noteworthy that production of recombinant MPO in Chinese hamster ovary cells generates a monomeric heme-containing single-chain precursor of 84 kDa, which exhibits peroxidase activity and is secreted into the medium (236). Recombinant MPO therefore resembles monomeric and partially unprocessed proMPO, but its enzymatic and functional properties appear to be similar to those of the mature two-chain protein (236). The x-ray crystallographic structure of canine [Protein Data Bank (PDB) code: 1MYP, Fig. 3] and human MPO (PDB: 1CXP) have been reported, as well as structural data on the interaction of human MPO with bromide (Br^-, PDB: 1D2V) and thiocyanate (SCN^-, PDB: 1DNU) (40, 93, 95, 383).

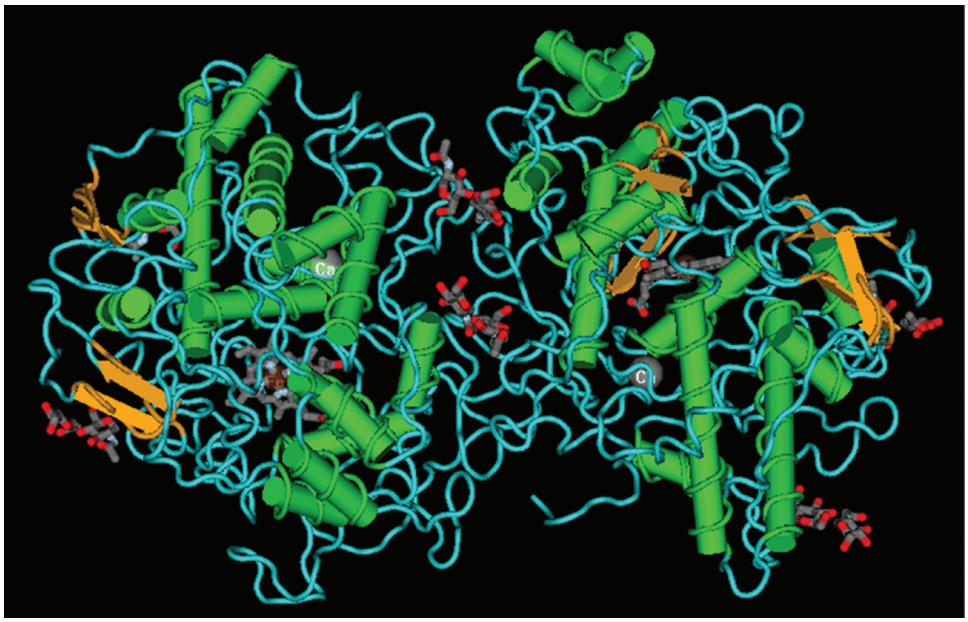

FIG. 3. Three-dimensional structure of the MPO homodimer. Highlighted are α-helices (green) and β-sheets (yellow). Each monomer contains one heme group [modified from (383)]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

D. Enzymatic properties of MPO

1. Catalytic mechanisms and redox intermediates of MPO. The complete MPO system consists of MPO, H_2O_2, and an oxidizable factor. When these components react, at least three redox intermediates of MPO can be formed. The generation of these intermediates and their interaction with products of the respiratory burst of phagocytes are briefly summarized here (Fig. 4; for more detailed information see refs. 99, 79).

In its native form, the heme center in MPO is in its ferric form [MPO-Fe(III)], but in the presence of H_2O_2, a short-lived redox intermediate termed compound I is formed, which contains an oxy-ferryl (MPO-FeIV = O) heme center and a porphyrin π-cation radical. As a result of this oxidation reaction, two electrons are generated that reduce H_2O_2 to H_2O. Compound I is the primary catalytic complex of MPO and is a strong oxidant that readily oxidizes halides (Cl^-, Br^-, I^-) or pseudohalides (SCN^-) (79). This reaction, known as the halogenation cycle, involves a direct two-electron reduction reaction that leads to the formation of hypohalous acids and the conversion of MPO-compound I back into its native ferric state (99).

Besides halides, MPO compound I can also oxidize various organic and inorganic substrates (79). This reaction, known as the peroxidase cycle, involves two sequential one-electron reduction reactions and includes the formation of the redox intermediate MPO compound II (99). MPO compound II contains an oxy-ferryl heme center but has lost the porphyrin π-cation radical and does not react with halides. One-electron reduction of MPO compound II results in the native, active form of MPO and is the rate-limiting step in the peroxidase cycle. However, physiological reductants such as $O_2^{\bullet}$ and ascorbic acid can accelerate this reaction. Besides MPO compounds I and II, an additional intermediate-termed compound III can be formed, either by reaction of the native enzyme with superoxide or through one-electron reduction reactions of compounds I and II with subsequent reaction with O_2. Compound III is, however, not stable and decays to native MPO and $O_2^{\cdot}$ within minutes.

Thus, catalytically active MPO can follow either a halogenation or a peroxidase cycle, which is determined by competition between peroxidase substrates and halides or pseudohalides for reaction with MPO compound I (79). The halogenation cycle results in the corresponding hypohalous acids. In the peroxidase cycle, various endogenous organic substrates are converted into radicals, including tyrosine, ascorbate, and steroid hormones (79).

2. MPO-derived oxidants. Depending on the local milieu, the MPO-H_2O_2 system is capable of generating a wide range of oxidant species, including HOCl, chloramines, hydroxyl radicals ($HO^{\bullet}$), singlet oxygen ($^1O_2$), and ozone (O_3) (79, 177) (Fig. 5). The conditions under which these oxidants are generated and their reactions with various endogenous and exogenous substrates have recently been reviewed in depth and are only briefly described here (79, 177).

HOCl is the product of the H_2O_2-catalyzed oxidation of chloride. At physiologic pH, HOCl exists as a mixture of the acid (HOCl) and the hypochlorite ion (OCl^-), whereas under conditions of low pH and high concentrations of chloride, it can react to form chlorine (Cl_2). All these products are short-lived but highly reactive compounds that can potentially oxidize any oxidizable group in any substrate, including thiols, thiol-esters, heme groups, and unsaturated fatty acids (79). Such oxidative modifications may alter protein activity and, as a consequence, may affect microbial and cellular functions and activity.

Cystein residues in proteins and in reduced glutathione are highly susceptible for HOCl-mediated oxidation. For enzymes containing cysteine in their active site, this may result in inactivation. Alternatively, such modifications may, through the creation of disulfide bonds, cause significant changes in tertiary protein structure, affecting protein function and interactions with other proteins (79). These effects may have important implications for cellular functions, because many important enzymes, transcription factors, and regulatory proteins contain cystein in their activated site. In addition, oxidation of reduced glutathione to oxidized glutathione would be expected to have a major impact on the cellular redox status, with significant effects on cellular signaling processes (79).

HOCl also can react with nitrogen-containing compounds, such as amines and amides, present in a wide variety

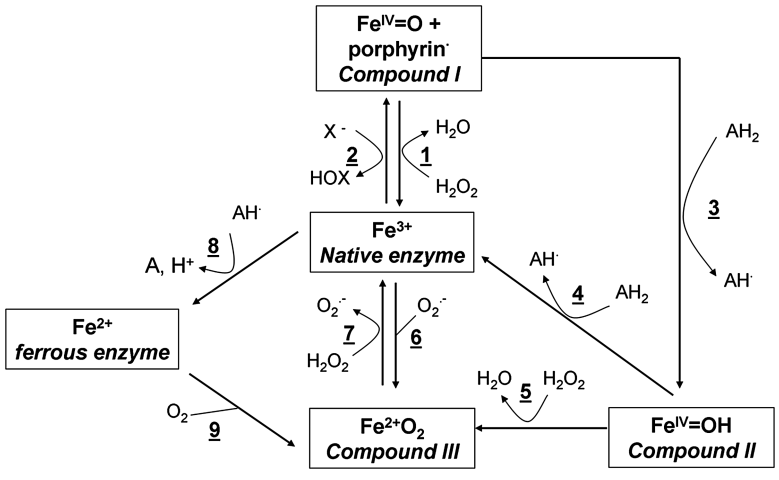

FIG. 4. Reactions of redox intermediates of MPO. H_2O_2 reacts with the native ferric form of MPO to generate compound I (reaction 1). Compound I can react with halides (X^-), reducing the enzyme back to its ferric state (reaction 2, the halogenation cycle). In the peroxidase cycle, compound I is transformed in a first one-electron reduction to compound II, which contains an oxoiron (IV) center (reaction 3). Compound II is then reduced back to ferric peroxidase in a second one-electron reduction (reaction 4). An additional intermediate, compound III (oxyperoxidase), is formed either from ferric MPO with superoxide anion (reaction 6), from ferrous MPO with O_2 (reaction 9), or from compound II with H_2O_2 (reaction 5). Reducing radicals generated in the peroxidase cycle can generate ferrous [Fe(II)]MPO through a one-electron reduction of ferric [Fe(III)]MPO (reaction 8). AH_2 indicates an organic peroxidase substrate (based on refs. 79 and 215).

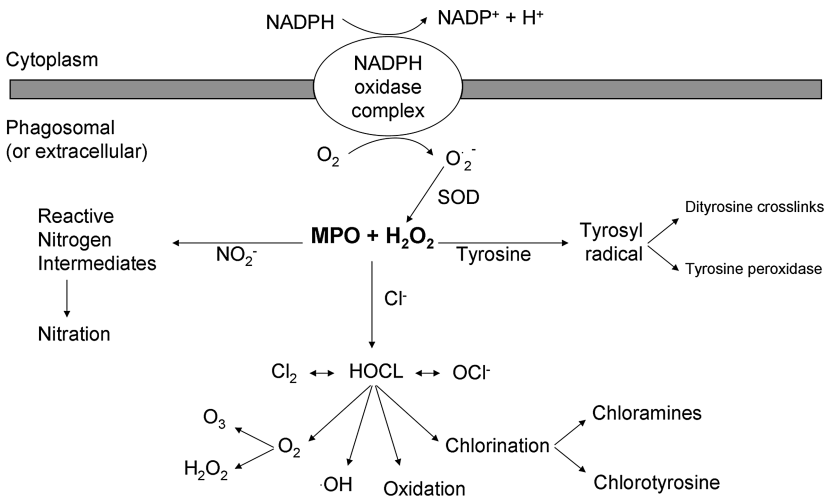

FIG. 5. Schematic representation of the MPO–H_2O_2 system and its products. See text for explanation.

of biologic substrates, including phospholipids, glycosaminoglycans, DNA, and RNA (79). This results in the formation of long-lived mono- or di-chloramines, of which some have been shown to retain oxidizing activity (338, 337). As such, the formation of chloramines has been viewed as a mechanism for prolonged peroxidase activity that importantly contributes to cellular injury. As an example, the sulfonic acid taurine, which is abundantly present in the cytoplasm of neutrophils, reacts rapidly with HOCl, resulting in the formation of taurine chloramine (195). Initially, this reaction was viewed as a mechanism to protect the neutrophil from the more-toxic effects of HOCl. However, taurine chloramine still has oxidating activity, is long lived, and thus may contribute to cellular damage (315). In this respect, recent data indicate that taurine chloramine is more selective than HOCl at targeting critical cysteines and inactivation of creatinine kinase and glyceraldehyde-3-phosphate dehydrogenase (274).

Tyrosine or tyrosine residues in proteins are targets for HOCl, resulting in the formation of 3-chlorotyrosine or 3,5-dichlorotyrosine (82). Detection of these chlorinated tyrosines has been used frequently as a tissue marker of (previous) MPO activity, but it is unclear whether formation of these compounds has any detrimental effects (177).

It has been postulated that $HO^{\bullet}$, $^1O_2$, and O_3 can be produced by activated neutrophils as well. Although certainly some indirect experimental evidence indicates that these ROS can be generated by activated leukocytes, the yields, conditions, and biologic significance of such reactions are controversial [for a detailed discussion, see (79)]. With respect to the production of $HO^{\bullet}$, some studies have provided indirect evidence for its generation in activated phagocytes, but whether peroxidase enzymes can form $HO^{\bullet}$ directly is questionable (71, 117). The main arguments for this contention include the low free iron concentrations in biologic fluids and the high levels of peroxidases in phagocytes that would be expected to consume the majority of H_2O_2 produced (71, 177, 368). Thus, the conditions required for $HO^{\bullet}$ production are unfavorable in activated phagocytes.

Another suggested source of $HO^{\bullet}$ is its indirect generation through the reaction of $O_2^{\bullet -}$ with HOCl (56). However, because HOCl is extremely reactive with various biologic targets, it is again unlikely that this reaction will result in significant amounts of $HO^{\bullet}$ (79).

The generation of $^1O_2$ by activated phagocytes also is controversial (79, 177). Initial studies aimed to detect $^1O_2$ production used probe compounds that were later questioned for their specificity (79). However, by using specific spin traps, several studies have now convincingly shown that activated phagocytes generate $^1O_2$, and that this is, at least in part, mediated by products of peroxidases (15, 158, 172). The formation of $^1O_2$ by the MPO-H_2O_2-Cl system has been demonstrated to peak at neutral pH, most likely through the reaction of HOCl with H_2O_2 (15, 158, 172). However, whether such reactions result in significant yields of $^1O_2$ remains questionable (367). Because $^1O_2$ is highly reactive and has a relatively long lifetime (milliseconds), its generation could have some quite detrimental consequences (78). Many amino acids in proteins can be targeted by $^1O_2$, including tryptophan, histidine, and tyrosine, which results in the formation of hydroperoxides and which can react further to form cross-links (78). In addition, $^1O_2$ can target DNA through the oxidation of guanine, and as such, could be genotoxic (54).

Generation of O_3 also has been suggested to occur during the respiratory burst in neutrophils, but this has been debated (177). Studies using dyes (*e.g.*, indigo carmine) or specific spin traps suggest that O_3 is produced by activated neutrophils, requiring $^1O_2$ generated by the MPO-H_2O_2-Cl for its formation (31, 166). However, the levels of $^1O_2$ needed for such reactions are considerable and are unlikely to occur under normal circumstances (367).

Nitric oxide (NO) and especially its major oxidized product nitrite (NO_2^-) have been identified as additional substrates for MPO under physiologic conditions (1, 84, 102). In tissues and biologic fluids, high levels of NO_2^- are normally present (1 to 5 μM), which increase even further during inflammation (>200 μM), and therefore, it may serve as an important reductant for MPO compound I (91, 101, 344). Oxidation of NO_2^- by MPO and H_2O_2 results in nitrogen dioxide radical ($NO_2^{\bullet}$) formation. $NO_2^{\bullet}$ is highly unstable but is very potent in promoting protein nitration and lipid peroxidation (23, 85, 125, 147, 171, 313, 344). Furthermore, the $NO_2^{\bullet}$-mediated oxidation of tyrosine, yielding nitrotyrosine (NO_2Tyr), is often used as a biologic fingerprint of oxidative stress (189, 286, 345) and has been shown to contribute to cellular dysfunction during inflammatory processes (85, 344).

III. MPO in Health

A. Microbicidal effects of MPO

In general, MPO is considered to contribute substantially to the microbicidal activity of neutrophils and monocytes through the generation of reactive oxidants, in particular HOCl, and radical species (117). The importance of MPO in killing of phagocytosed pathogens is supported by several observations (177).

First, it was convincingly demonstrated *in vitro* that the combination of MPO, its substrate H_2O_2, and a halide renders an effective and powerful antimicrobial system (175, 178). The dominant view is that under physiologic conditions, chloride is the preferred halide, as it is present at high concentrations in biologic fluids (>100 m*M* in plasma) (96). However, iodide, bromide, and the pseudohalide thiocyanate could serve this function as well (103, 174, 175, 336). More recent observations suggest that NO_2^- could substitute for the halide requirement in the system (84, 102). NO_2^- is a product of NO metabolism, and in neutrophils and especially in monocytes, the expression of nitric oxide synthase can be readily induced by inflammatory mediators, leading to substantial NO production.

Second, the contribution of the MPO-H_2O_2-halide system to the microbicidal activity of phagocytes is illustrated by experiments in which one of the components of the system is inhibited or lacking. For example, enzymes that scavenge H_2O_2, such as catalase, impair phagocyte killing of at least some pathogens, demonstrating the requirement for H_2O_2 for optimal bactericidal activity (7, 8, 218). Furthermore, patients with chronic granulomatous disease (CGD), a primary immunodeficiency syndrome characterized by a dysfunctional NADPH oxidase system, have severe, sometimes lethal, infections (323). Neutrophils from these patients are unable to mount a respiratory burst and are therefore impaired in their microbicidal activity. Interestingly, this defect can be restored *in vitro* by adding H_2O_2 extracellularly (105, 153).

Third, although not specific for MPO, inhibitors of peroxidases, such as azide and cyanide, significantly decrease the capacity of normal neutrophils to kill microbes (176, 179). Interestingly, these same inhibitors have little effect on the microbicidal activity of MPO-deficient neutrophils, suggesting that, in normal neutrophils, these inhibitors target MPO (177).

However, if the MPO-H_2O_2-halide system were to be critical in the microbicidal activity of phagocytes, one would expect MPO deficiency in humans to have severe consequences, but this does not appear to be the case. The first report of a case of MPO deficiency in neutrophils dates back to 1969 and described a diabetic patient with systemic candidiasis and whose neutrophils lacked MPO activity (201, 202). *In vitro*, neutrophils of this patient were impaired in their candidacidal activity and had a reduced capacity effectively to kill staphylococci (201). Although these observations support the contention that MPO contributes to the microbicidal activity of phagocytes, it should be noted that diabetes patients in general are more susceptible to infections (192). When automated and sensitive, peroxidase activity–based systems to differentiate leukocytes became routine practice in clinical laboratories, it also became apparent that partial or full MPO deficiency is not a rare event in the normal population. Studies in the United States and Europe have reported prevalences of hereditary MPO deficiencies ranging from 1:1,000 to 1:4,000 (44, 74, 173, 190, 269). Interestingly, MPO deficiency in Japan appears to be less common (157, 261). One large study reported only 26 complete MPO-deficient individuals of 1,223,427 individuals tested (estimated prevalence, 1:57,135) (261). Subtotal or total MPO deficiency is the consequence of gene mutations, and a number of such mutations have been described, including seven missense mutations (118). For four of these mutations, the functional consequences have been studied in more detail and were found to affect MPO biosynthesis. For example, a single-nucleotide mutation in the heavy subunit at codon 569 results in the replacement of an arginine with a tryptophan (R569W) and is the most common cause of MPO deficiency in Europe and the United States (251). Transfection studies of this mutated gene in hematopoietic cell lines showed that the mutation leads to a dysfunctional protein, because MPO biosynthesis stops at the apoproMPO stage without heme incorporation and proteolytic processing. Other mutations described include two mutations in the light subunit: a cysteine replacement of a tyrosine at codon 173 (Y173C) and a threonine replacement of methionine at codon 251 (M251T) (81, 302). More recently, two novel missense mutations, a glycine to serine at codon 501 (G501S) and an arginine to cystein at codon 499 (R499C), were identified in two Japanese adults with complete MPO deficiency (266, 273). These two mutations are close to the critical histidine 502 on the proximal side of the heme pocket in MPO. Transfection studies showed that both mutants incorporated heme but lacked proper proteolytic processing and targeting to granules (266, 273).

The direct clinical consequences of MPO deficiency have not been studied extensively, but Kutter *et al.* (187) compared a group of 100 totally or subtotally MPO-deficient individuals with a reference population of 118 individuals with normal MPO levels. In this study, a significantly higher incidence of severe infections and chronic inflammatory diseases was reported. However, in the majority of cases, partial or complete MPO-deficient individuals do not appear to be extremely more susceptible to infections, despite impaired microbicidal activity of MPO-deficient neutrophils *in vitro*. This contrasts with the severe infections observed in chronic granulomatous disease (CGD) patients that lack a functional NADPH oxidase (323). One explanation for this mild phenotype of MPO deficiency could be that MPO-independent microbicidal mechanisms increase their activity to compensate for the lack of MPO. The observation that the bactericidal activity of MPO-deficient neutrophils is greater than that of neutrophils treated with a peroxidase inhibitor supports this contention (176).

B. Lessons learned from MPO-deficient mice

Further to define the *in vivo* role of MPO in host defense, mice deficient in MPO have been generated by disruption of the *MPO* locus through homologous recombination by two independent research groups (11, 46). Both *MPO*-knockout strains are reported to be fertile and develop normally, but, as expected, their bone marrow cells lack MPO mRNA, whereas mature neutrophils and monocytes lack peroxidase activity and MPO protein. In addition, generation of HOCl by phorbol myristate acetate (PMA)-activated neutrophils is lacking, whereas $O_2^{\cdot}$ production is slightly increased. This latter observation is consistent with data obtained from human MPO-deficient neutrophils (254, 304). The MPO-deficient mice have

been extensively studied for their susceptibility to bacterial and fungal infections, showing increased susceptibility to some pathogens but not to others. Similar to neutrophil-depleted mice, *MPO*-knockout mice exhibited a high susceptibility to *Candida albicans* infections (11, 13, 47). Furthermore, these mice are considerably more susceptible to the development of pulmonary infections with intranasal administration of *Candida tropicalis*, *Trichosporon asahii*, and *Pseudomonas aeruginosa*, and, to a lesser degree, of *Aspergillus fumigatus*, *Klebsiella pneumoniae*, and *Cryptococcus neoformans* (12–14). In contrast, susceptibility to *Candida glabrata*, *S. aureus*, and *S. pneumoniae* is comparable to that of MPO-sufficient mice (14). The observation that MPO-deficient mice do not display an increased susceptibility to *S. aureus* infections is in line with the observation that humans deficient in MPO lack symptoms. Also, compared with normal neutrophils, MPO-deficient neutrophils are less effective in early killing of *S. aureus in vitro*, but a similar rate of killing is reached at later time points, suggesting that MPO contributes to but is not essential for killing of *S. aureus* (202).

Finally, *MPO*-knockout mice are more susceptible to sepsis induced by cecal ligation and puncture that cause intraabdominal infection (103). In contrast, reduced lung injury and mortality was recently reported in MPO-deficient mice intraperitoneally challenged with *Escherichia coli* (50). In this study, MPO-deficient mice were found to display an increased basal expression of inducible nitric oxide synthase (iNOS) in lungs and neutrophils, together with a two- to six-fold increase in NO production compared with wild-type mice. Thus, the augmented iNOS expression and NO production observed in MPO-deficient mice may compensate for the lack of HOCl-mediated bacterial killing (50).

When interpreting data obtained from mouse studies, including those involving MPO-deficient mice, it is important to note that the number of circulating neutrophils in mice is far less than that in humans (10–15% in mice *vs.* 60–70% in humans). In addition, the level of MPO in murine neutrophils has been estimated to be around 10–20% of that in human neutrophils (259, 291). Both these observations should be taken into account when translating results obtained in mice to the human situation (250). Nevertheless, MPO-deficient mice have been instrumental in dissecting the role of MPO and MPO-derived oxidants in various disease models, and this is discussed in more detail in the next sections.

IV. MPO in Disease

A. Properties of MPO in modulating immune responses and inflammation

Excessive generation of oxidants by MPO and other peroxidases has been linked to tissue damage in many diseases, especially those characterized by acute or chronic inflammation. This is evidenced by the fact that, in many cases, enzymatically active MPO in conjunction with 3-chlorotyrosine, a tissue marker for HOCl-mediated damage, or HOCl-modified proteins can be detected in diseased tissues. However, besides these direct toxic effects, it also is known that HOCl is able to modulate the function and activity of various immune cells (371). For example, *in vitro* studies showed that HOCl can activate nuclear factor-κB (NF-κB) and tyrosine phosphorylation in T and B cells, leading to increased calcium signaling and tumor necrosis factor α (TNF-α) production (311, 314). Furthermore, various other lymphocyte functions have been shown to be susceptible to MPO-derived oxidants, leading to reduced mitogen-induced proliferation and cytotoxic activity (86, 87). Thus, generation of MPO-derived oxidants may modulate immune responses and inflammatory reactions.

Interestingly, in recent years, it has become increasingly clear that MPO may also exert effects that are beyond its oxidative properties and that are, in many cases, independent of its enzymatic activity. For example, in a number of studies by Lefkowitz *et al.* (196–198), evidence has been provided that MPO can modulate immune responses through the activation of macrophages. In these studies, exposure of macrophages to MPO *in vitro* resulted in the release of TNF-α and low levels of interferon-γ (IFN-γ) in conjunction with enhanced macrophage-dependent cytotoxicity. Also, mice intravenously injected with MPO displayed increased circulating levels of TNF-α and IFN-γ (198). The significance of these MPO-mediated activities on macrophages is illustrated by the observations that macrophages exposed to MPO show both enhanced bacterial and fungal phagocytosis and killing (200, 205). Furthermore, such interactions between MPO and macrophages may play a role in the perpetuation of chronic inflammatory diseases. In an experimental rat model of arthritis, induced by intraarticular injection of streptococcus cell-wall fragments, local injection of enzymatically active or partially inactivated MPO was found to increase clinical symptoms, such as joint swelling and erythema (104, 196). These disease-aggravating effects of MPO were most likely mediated by interaction of MPO with mannose receptors on macrophages, because simultaneous administration of mannan abated these effects (104, 196).

MPO has also been shown to affect cell–cell interactions and cell adhesion of leukocytes. Johansson and colleagues (148) demonstrated that differentiated HL60 cells as well as peripheral human leukocytes adhered to MPO, an effect that was independent of its peroxidase activity and was not blocked by mannan (148). However, adhesion to MPO was inhibited by monoclonal antibodies to CD11b and CD18, suggesting a crucial role for integrins in mediating this effect. These results suggested that the adhesive properties of MPO may play a role in leukocyte migration and infiltration in inflammatory reactions. More recently, Lau and colleagues (193) extended these observations by demonstrating that MPO acts as an autocrine modulator of leukocyte function independent of its enzymatic activity (193). In this study, interaction of MPO with CD11b/CD18 integrins on neutrophils was characterized by increased tyrosine phosphorylation, p38 mitogen-activated protein kinase (p38 MAPK) activation, and enhanced nuclear translocation of NF-κB (193). Furthermore, MPO-exposed neutrophils had increased expression of surface CD11b, had augmented superoxide production, and were more prone to degranulation. In addition, a recent study suggests that the interaction of MPO with the CD11b/CD18 integrins also affects neutrophil life span. In this study, MPO delayed neutrophil apoptosis *in vitro* and prolonged the duration of carrageenan-induced lung injury, suggesting that MPO functions as a survival signal for neutrophils and, as such, may influence the duration of an inflammatory response (88).

Besides that in leukocytes, exposure to MPO has been found to modulate cellular functions in various other cell types. In human umbilical vein endothelial cells (HUVECs),

MPO is readily internalized, induces secretion of interleukin (IL)-6 and -8, and stimulates production of ROS (21, 199, 380). Furthermore, recent evidence suggests that endothelial internalization of MPO is facilitated by cytokeratin I, which also acts as a scaffolding protein for the vasoregulatory kallikrein-kinin system (21). Interestingly, MPO binding to cytokeratin I decreased the production of bradykinin and may affect vascular tone (21). Exposure of alveolar and bronchial epithelial cell lines to MPO also leads to internalization of the enzyme. In both cell types, MPO increased heme-oxygenase-1 (HO-1) expression and induced DNA damage (114). However, in primed cells, incubation with MPO inhibited IL-8 production in bronchial but not in alveolar epithelial cells (114).

Collectively, the data described here suggest that MPO, through its effects on signaling cascades in various cell types, especially endothelial and inflammatory cells, is an important modulator of inflammatory processes (summarized in Fig. 6). Interestingly, observations in a number of experimental models of inflammation using MPO-deficient mice appear to support this contention. In general, in experimental models characterized by acute neutrophil-dependent inflammation such as ischemia/reperfusion (I/R) injury, the absence of MPO ameliorates clinical and pathologic symptoms, including decreased neutrophil accumulation (19, 226). This effect is consistent with a role of MPO in neutrophil adhesion, activation, and survival, as described earlier. Conversely, in experimental models of inflammation that are dependent on the induction of an active T-cell–dependent immune response through, for example, active immunization, MPO deficiency generally aggravates disease symptoms (46, 265). A possible explanation for this effect could be that endogenous MPO downregulates antigen-specific adaptive immune responses in secondary lymphoid organs *in vivo*. In some of these studies, increased T-cell proliferation has been observed in antigen-challenged MPO-deficient mice (46, 265). Furthermore, studies *in vitro* demonstrated that taurine chloramine, a MPO-derived oxidant, can functionally inhibit both antigen-presenting cells and T-cells (219, 220).

The direct toxic effects of MPO-derived oxidants, together with its noncatalytic immune-modulating properties described earlier, have been implicated in the induction of malignancies, tissue injury, as well as in the progression of the inflammatory response in multiple diseases, and these are discussed next.

B. MPO and cardiovascular diseases

1. Clinical studies. Many clinical studies have implicated MPO in a broad range of aspects of cardiovascular disease (CVD) in human patients. First, several studies showed the link between MPO levels and the extent of coronary artery disease (CAD). In a case–control study, Zhang *et al.* (385) studied the association between circulating MPO levels and CAD. After correction for traditional risk factors for cardiovascular disease, both leukocyte- and blood-MPO levels were associated with the presence of CAD, indicating that MPO may be involved in the pathophysiology of atherosclerosis. In line with these findings, it was more recently shown that a clear correlation existed between MPO levels and the extent of plaque burden assessed with coronary angiography (255). MPO levels were increased in patients with CAD, and even more elevated levels of MPO were associated with the progression from stable CAD to disease with acute myocardial infarction (MI). In a recent nested case–control study among participants of the EPIC-Norfolk study (which contains 25,000 participants), 3,500 or more apparently healthy individuals, matched for age and gender, were analyzed for MPO (230). During an 8-year follow-up, part of this group developed fatal or nonfatal CAD, and part remained healthy. Baseline MPO levels were an excellent predictor of future risk of CAD, showing that this inflammatory marker precedes the presentation of clinical symptoms of CAD.

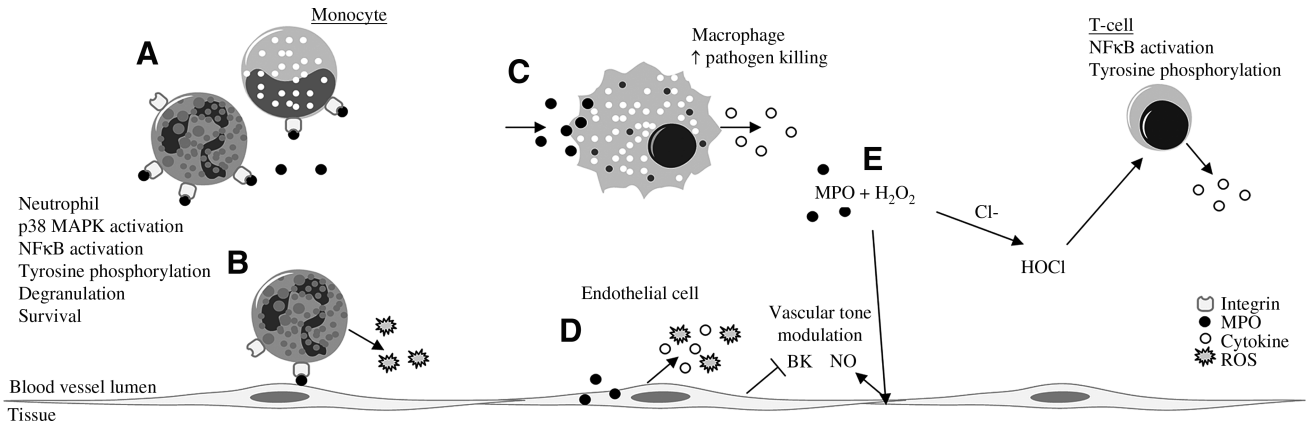

FIG. 6. Effects of MPO on inflammatory and endothelial cells. MPO can adhere to β_2-integrins (CD11b/CD18) on neutrophils and monocytes (**A**). In neutrophils, MPO binding leads to activation of signal-transduction pathways (p38 MAPK and NF-κB), tyrosine phosphorylation, production of superoxide, degranulation, prolonged survival, and increased integrin expression (**A**, **B**), which can enhance neutrophil–endothelium interactions (**B**). MPO is taken up by macrophages, which leads to production of cytokines (TNF-α and IFN-γ) and to increased pathogen killing (**C**). Endothelial cells internalize MPO, resulting in production of cytokines (IL-6 and IL-8) and ROS (**D**). In addition, MPO internalization by endothelial cells leads to inhibition of bradykinin (BK) (**D**), which, together with NO that is released as an indirect response to MPO (**E**), can modulate the vascular tone. In T cells, MPO-derived HOCl can induce NF-κB activation, tyrosine phosphorylation, and subsequent TNF-α production (**E**).

In addition, several articles described the relation between MPO level and adverse clinical outcome in patients with acute ischemic events. In a study examining a group of patients with chest pain, MPO levels were shown greatly to predict the risk of MI, both early after presentation in the clinic and in the next 6-month follow-up (48). In patients with established acute coronary syndromes, MPO serum levels also seemed a good predictor of subsequent death or MI in the 6-month follow-up period (33). More recently, a study was performed investigating the relation between plasma levels of MPO, carbonylation products as a marker for oxidation, and 5-year mortality in patients after acute MI (235). Both protein carbonylation and MPO levels were elevated in MI patients compared with controls. In a subgroup of patients, chlorotyrosine levels, as a marker of HOCl production, also were measured. However, despite elevated MPO levels, no increased levels of chlorotyrosine were found, suggesting that in these patients, protein carbonylation was independent of MPO activity. Finally, elevated MPO levels were associated with long-term mortality, and this may thus be a good predictive marker.

In line with these observations, some recent findings in an animal model also implicated MPO in ischemic heart disease (207). It was shown that hypercholesterolemia in rabbits induces cardiac MPO and neutrophil accumulation. On induction of I/R injury, MPO inhibition actually decreased postischemic apoptosis of cardiomyocytes, and treatment of the rabbits with rosiglitazone (a PPAR agonist) reduced MPO activation and cardiac infarct size.

MPO also has been linked to chronic heart failure (HF). In a group of 102 patients with chronic HF *versus* 105 healthy controls, plasma MPO levels were strongly associated with the prevalence of HF, even after adjustment for age and B-type natriuretic peptide, and MPO levels increased in parallel with worsening degrees of HF (331). The prognostic value of MPO for HF was further supported by a more recent study (332) and in a large study looking for markers that could improve the specificity of B-type natriuretic peptide as a marker for systolic HF (256).

Gene variations of MPO in relation to cardiovascular disease also were studied. A group of 100 individuals with inherited MPO deficiency were analyzed and, compared with controls, were protected against cardiovascular damage (187). However, they also had a higher occurrence of infections and chronic inflammatory processes. In line with this, the −463 G/A promoter polymorphism described earlier and that affects expression levels of MPO also has been linked to CVD. Individuals carrying the −463G allele, which leads to elevated MPO expression, had an increased risk for CAD (20, 258) and showed enhanced severity of atherosclerosis (211). Although certainly not complete, these data indicate that MPO can be associated with different aspects of cardiovascular disease in humans.

2. MPO in endothelial dysfunction. Proper endothelial function is essential for regulating vascular tone, reducing platelet aggregation, inhibiting endothelial inflammatory responses, and controlling intimal smooth muscle cell proliferation. Both systemic endothelial dysfunction and local coronary endothelial dysfunction were shown to be associated with increased risk for cardiovascular disease (333). A critical mediator of endothelial function is endothelium-derived NO. MPO has been demonstrated to affect endothelial function greatly by inhibiting NO. MPO, as well as other peroxidases, can act as a sink for NO at sites of inflammation (1). Because of its localization in the vasculature, subendothelial accumulated MPO can locally act as an NO oxidase, inhibiting NO function and causing endothelial dysfunction. In a model of endotoxemia, MPO accumulated subendothelially in response to lipopolysaccharide (LPS) treatment and inhibited vasorelaxation in response to acetylcholine, a known inducer of endothelial NO (84). In contrast, MPO-deficient mice showed normal vasorelaxation after LPS administration. Altered vascular responses were shown to be caused by the reaction of MPO-derived radicals with NO. Substantiating this direct role for MPO in regulating endothelial function was a study by Vita *et al*. (354). The authors demonstrated that serum MPO levels are excellent predictors of endothelial dysfunction in humans. Moreover, it was recently shown that acetylcholine-stimulated forearm blood flow in patients with symptomatic coronary artery disease was inversely correlated with plasma levels of MPO (34). Thus, MPO seems to act as a modulator of NO action, and thereby it directly contributes to endothelial dysfunction.

3. Lipoprotein modification mediated by MPO. The main cause of CVD is atherosclerosis, and oxidized lipoproteins (mainly oxidized low-density lipoproteins; oxLDLs) are considered crucial mediators of foam cell formation and atherogenesis. Several mechanisms have been implicated in the generation of oxLDL, and modification of lipoproteins by MPO is one of them. One of the early observations of MPO in CVD was the demonstration of the enzyme in human atherosclerotic lesions. Daugherty *et al.* (76) showed the presence of active MPO in extracts from human atherosclerotic arteries. Moreover, they demonstrated a clear colocalization of MPO with the macrophage marker CD68. Positivity was demonstrated mainly at the shoulder region of the lesions and in regions surrounding the lipid core of advanced atherosclerotic plaques. These were the first indications that MPO might contribute to the oxidative modification of lipoproteins. Subsequently, it was shown that 3-chlorotyrosine, a specific marker of MPO-mediated oxidation, was elevated in LDL isolated from human atherosclerotic lesions compared with LDL from the circulation (124). These data showed that MPO was actively modifying LDL in the atherosclerotic lesions. In follow-up of these studies, it was next shown that modification of LDL with MPO generates a high-uptake form of LDL that can induce foam cell formation (279). *In vitro* treatment of LDL with reactive nitrogen species generated by the MPO-H_2O_2-NO_2-system led to the nitration of apolipoprotein-B and the initiation of lipid peroxidation, hereby yielding an LDL particle (NO_2-LDL) that promotes intracellular cholesterol ester accumulation and foam cell formation by macrophages. NO_2-LDL did not bind to the macrophage LDL-receptor (LDLR) or scavenger-receptor class A, but was taken up by macrophages through CD36 (278). Additional studies demonstrated increased numbers of MPO-positive macrophages in human lesions, characteristic of acute coronary syndromes, such as eroded or ruptured plaques, whereas macrophages in stable early fatty streaks were negative for MPO (328). HOCl-modified proteins accumulated at these ruptured or eroded sites in the lesions, and several stimuli present in atherosclerotic lesions, such as CD40, lysophosphatidylcholine, or

cholesterol crystals were shown to induce MPO release from monocytes *in vitro*.

More recently, an additional protein modification generated by MPO was linked to atherosclerosis formation (355). It was shown that protein carbamylation can be catalyzed by MPO. Carbamyl-modified proteins were detected in human atherosclerotic lesions and colocalized with MPO. Moreover, carbamylation of LDL rendered this particle unable to bind to LDLR but generated an atherogenic particle that could bind with high affinity to the macrophage-scavenger receptor class A and could hereby induce foam cell formation. In the same article, it was shown that levels of homocitrulline-modified proteins, a product of carbamylation of protein lysine residues, predicted risk of CAD, MI, stroke, and death. Thus, MPO-catalyzed carbamylation of proteins may be an additional mechanism contributing to a proatherogenic role of MPO.

In addition to the modification of LDL, rendering it proatherogenic with the potential to induce foam cell formation, MPO also was shown to affect HDL function. HDL is the lipoprotein that is considered to be antiatherogenic and antiinflammatory, by mediating cholesterol efflux from macrophages and hereby relieving the lipid burden in macrophages. The main apolipoprotein component found on HDL is apolipoprotein-AI. HDL from atherosclerotic plaques showed strongly elevated levels of nitrotyrosine and chlorotyrosine modifications, as compared with circulating HDL, and, in addition, circulating HDL from patients with CAD showed more of these modifications than did circulating HDL isolated from healthy controls (271, 387). More specifically, modification of Tyr-192 of apolipoprotein AI by chlorination was shown to inhibit HDL function and to impair ABCA1-dependent cholesterol efflux (320). Thus, MPO modification of lipoproteins is a double-edged sword, not only mediating the formation of proatherogenic oxLDL particles but also inhibiting the function of antiatherogenic HDL particles.

4. Lessons learned from MPO mouse models. All these data made it very relevant to study the direct effect of MPO in mouse models of atherosclerosis. However, the outcome of these studies has not been completely conclusive. The earliest study generated MPO-deficient mice and analyzed the effect on atherosclerosis development (47). Unexpectedly, MPO deficiency worsened atherosclerosis development. Both by performing bone marrow transplantation to atherosclerosis-susceptible LDLR-deficient mice and by crossing the MPO-knockout mice with LDLR-deficient mice, they found that the lesions were significantly larger and contained more monocytes/macrophages when MPO was lacking. A clear explanation for this finding is difficult to give, but the authors speculate about several potential mechanisms in their discussion. First, mouse-human differences may explain part. As indicated earlier, human atherosclerotic lesions contain substantial amounts of MPO and its products. However, the authors were hardly able to detect chlorotyrosine levels in the mouse lesions. Moreover, they had difficulty in detecting MPO in the mouse lesions, because antibody staining of lesions from MPO-deficient mice still showed positivity. More recently, it was firmly established that neutrophils and MPO are integral parts of murine atherosclerotic lesions (349, 384). With newly generated antibodies, MPO is readily detectable in murine atherosclerotic lesions of both LDLR-deficient mice on a high-fat diet and apolipoprotein E (APOE)-deficient mice (349). In contrast to human lesions, MPO in these mice colocalizes not with macrophages but with neutrophils infiltrating the plaques (349) (Fig. 7). Furthermore, these studies also showed increased levels of circulating MPO in LDLR-deficient mice with high-fat feeding (Fig. 8). Interestingly, increased plasma levels of MPO in conjunction with increased levels of oxidized LDL were recently described in hypercholesterolemic children as well (276).

An alternative explanation for the adverse effect of MPO deficiency may lie in the fact that MPO may be regulating additional not-yet-identified processes in atherosclerosis. A study by Milla *et al.* (231) showed that MPO deficiency actually enhances lung injury–related inflammation induced by allogeneic bone marrow transplantation. They found that MPO-deficient mice show enhanced T-cell–dependent inflammation in their lungs, characterized by elevated levels of proinflammatory cytokines, such as TNF-α and monocyte chemoattractant protein 1 (MCP-1), and increased accumulation of T cells and monocytes in the lungs. This

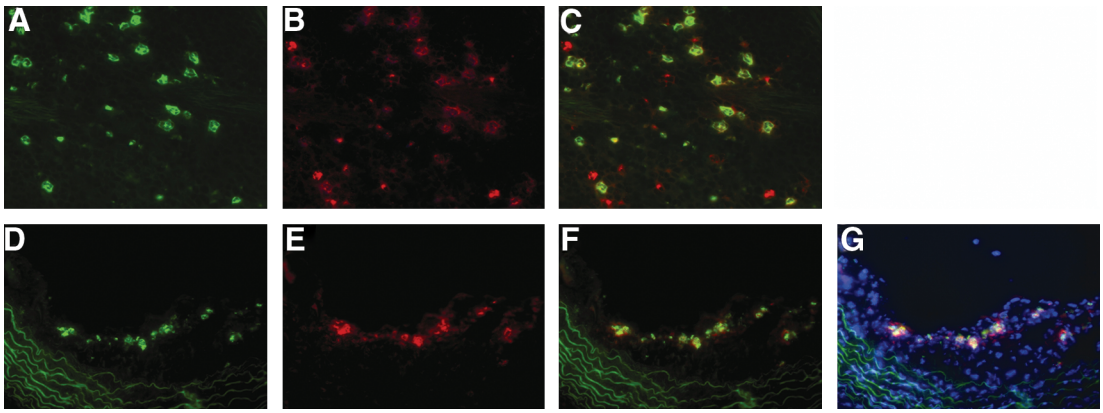

FIG. 7. Accumulation of MPO-positive neutrophils in atherosclerotic lesions of LDLR$^{-/-}$ mice. (**A–C**) Colocalization of MPO (**A**) and neutrophils (**B**) in murine spleen sections. (**C**) Overlay of **A** and **B**. (**D–G**) Colocalization of MPO (**D**) and neutrophils (**E**) in an atherosclerotic lesion from an LDLR$^{-/-}$ mouse fed a high-fat diet for 10 weeks. (**F**) Nuclear staining (DAPI). (**G**) Overlay of **D–F**. Reprinted with permission from (349). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

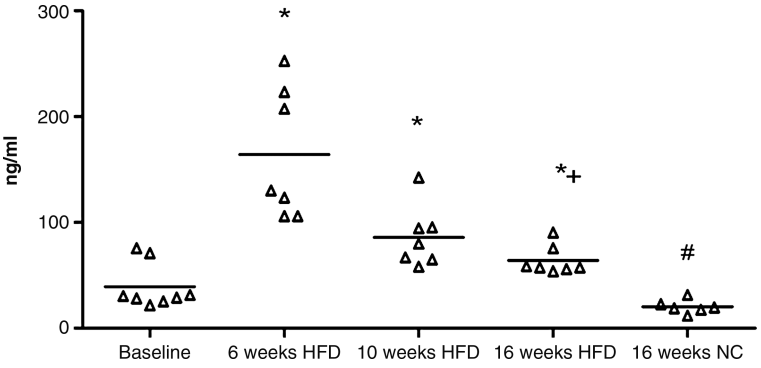

FIG. 8. Circulating plasma MPO levels in LDLR$^{-/-}$ mice after high-fat feeding. Circulating plasma MPO levels were measured with a capture ELISA at baseline and after 6, 10, and 16 weeks of high-fat diet. Compared with baseline values, plasma levels of MPO were significantly elevated in high fat–challenged LDLR$^{-/-}$ mice at 6 weeks. Reprinted with permission from (349).

proinflammatory state was probably caused by a suppression of apoptosis of inflammatory cells in the lungs that was observed in the MPO-deficient mice. MPO deficiency also worsened disease in an experimental autoimmune encephalomyelitis (EAE) model, linked to accelerated lymphocyte proliferation (46), but these results are discussed more extensively in the section on neurodegenerative diseases. Thus, the findings of enhanced atherosclerosis in the absence of MPO may very well be linked to altered T-cell responses or modified apoptosis regulation. However, studies investigating these processes in detail are lacking.

In contrast to the original MPO-knockout study, other mouse experiments confirmed a proatherogenic role of MPO. Transgenic mice expressing human MPO under the control of a Visna promoter were used in atherosclerosis studies similar to those described earlier (227). These animals showed strongly increased peroxidase activity in their macrophages, and bone marrow transplantation studies showed that overexpression of human MPO leads to increased atherosclerosis (227). Interestingly, in a parallel experiment, they also confirmed the previous finding that MPO deficiency enhanced atherosclerosis development. In addition, the group of Reynolds (185) generated transgenic mice expressing human MPO genes with two promoter variants, −463G/A, which have been associated with increased CAD and severity of atherosclerosis (59, 185). Both these transgenic lines developed increased atherosclerosis when crossed on an LDLR-deficient background, whereas the −463G male variants even showed increased hyperlipidemia and obesity. These data indicate significant differences between mouse and human MPO.

Thus, MPO is clearly an important player in cardiovascular diseases. It is associated with a broad spectrum of clinical manifestations of CVD. It may contribute by different forms of lipoprotein modification affecting atherogenesis and may affect many other cellular processes involved in CVD and atherosclerosis. The main clinical and experimental findings supporting a pathogenic role for MPO in several cardiovascular diseases are summarized in Table 1. With mouse models, a challenge still lies ahead in better understanding of which atherogenesis-related cellular processes are regulated by MPO and to what extent human–mouse differences are contributing to unexpected outcomes of animal experiments.

C. MPO and carcinogenesis

Chronic inflammation, caused by either infections or noninfectious agents (*e.g.*, asbestos), is associated with an increased incidence of malignancies (361). This has been linked to the continuous oxidative stress of the affected tissues due to accumulation of activated phagocytes, which may result in oxidative damage of genomic DNA of proliferating cells, causing mutations and the development of cancer (361). Some evidence strongly indicates that oxidants produced by activated phagocytes, including superoxide, $HO^{\bullet}$, H_2O_2, and MPO-derived oxidants, are genotoxic (222, 361). These ROS can induce carcinogenesis in several ways (*e.g.*, by modulating genes that regulate cell proliferation or by the induction of DNA damage, such as DNA strand breaks and mutations). The first observation that linked genotoxicity with phagocyte ROS production was described by Weitzman and Stossel (362), who showed that the ability of phagocytes to induce mutations in bacteria was markedly attenuated when cells from a patient with CGD were used. Subsequent *in vitro* studies demonstrated that phagocyte-derived ROS exert similar effects on genotoxicity in various mammalian cells, which in many instances could be attenuated by specific antioxidants, including catalase and SOD (359, 360, 363). Furthermore, *in vivo* studies have shown that exposure to asbestos and smoking causes an inflammatory response in the lungs and it also is associated with the onset and progression of carcinogenesis (318). In human bronchoalveolar lavage fluids (BALFs), elevated levels of MPO can be detected after smoking or exposure to particulates such as asbestos, suggesting that MPO may contribute to lung cancer development (138). Compared with normal tissues, elevated levels of MPO and oxidative-stress markers can be detected in malignant tissues, again suggesting a role for MPO-derived oxidants in carcinogenesis (288, 303). Mechanistically, MPO-catalyzed production of HOCl has been linked directly to DNA damage, causing oxidation of pyrimidine bases and chlorination of cytosine in bronchial epithelial cells (322). Furthermore, human neutrophils use MPO to halogenate uracil, as evidenced by the observation that exposure of uracil to either purified MPO or activated neutrophils causes uracil chlorination (132, 133). In human inflammatory tissues, both chlorinated and brominated uracils can be detected, suggesting that such reactions are pathogenetically relevant (133). It has been proposed that incorporation of such halogenated nucleosides into the host DNA may cause transition mutations in genes, resulting in alterations of DNA repair, DNA replication, and regulation of the cell cycle.

Besides direct damaging effects on DNA, evidence exists that MPO-derived oxidants are involved in the bioactivation of inhaled carcinogens such as polycyclic aromatic hydrocarbons (PAHs). For example, MPO has been demonstrated to

Table 1. Clinical and Experimental Evidence for a Pathogenic Role of MPO in Various Diseases

Disease	*Type of research*	*Findings*	*Supporting pathogenic role? (ref)*
Cardiovascular disease			
Cardiovascular disease	Clinical	Protection with inherited MPO deficiency	Yes (187)
Atherosclerosis	Experimental	Increased atherosclerosis with MPO gene deletion	No (47, 227)
		Increased atherosclerosis with human MPO overexpression	Yes (227, 59)
	Clinical	Increased MPO expression in affected tissue	Yes (76, 328)
		Increased presence of MPO-modified proteins/lipids in affected tissue	Yes (124, 328, 355)
		Correlation studies on −463G/A MPO promoter polymorphism with disease severity	Yes (211)
Coronary artery disease	Clinical	Correlation studies on MPO levels with disease presence/severity/risk	Yes (230, 255, 385)
		Correlation studies on −463G/A MPO promoter polymorphism with disease risk	Yes (20, 258)
Myocardial infarction	Experimental	Reduced myocardial cell death with MPO inhibition	Yes (207)
		Decreased left ventricle dilation associated with improved function with MPO gene deletion	Yes (19, 351)
	Clinical	Correlation studies on MPO levels with disease presence/severity/risk	Yes (33, 48, 235)
Heart failure	Clinical	Correlation studies on MPO levels with disease presence/severity/risk	Yes (256, 331, 332)
Cancer			
Colon cancer	Clinical	Increased MPO expression in affected tissue	Yes (288, 303)
Lung cancer	Clinical	Correlation studies on −463 G/A MPO promoter polymorphism with disease risk	Yes (58, 194, 209, 268, 309, 310, 329) No (68, 232, 373, 377)
Larynx cancer	Clinical	Correlation studies on −463 G/A MPO promoter polymorphism with disease risk	Yes (58)
Bladder cancer	Clinical	Correlation studies on −463 G/A MPO promoter polymorphism with disease risk	Yes (137)
Breast cancer	Clinical	Correlation studies on −463 G/A MPO promoter polymorphism with disease risk	Yes (4)
Pancreatic cancer	Clinical	Correlation studies on −463 G/A MPO promoter polymorphism with disease risk	Yes (365)
Myeloid leukemia	Clinical	Correlation studies on −463 G/A MPO promoter polymorphism with disease risk	Yes (295)
Neurodegenerative diseases			
Alzheimer's disease	Experimental	Increased MPO expression in affected tissue	Yes (213)
		Increased presence of MPO-modified proteins/lipids in affected tissue	Yes (213)
		Decreased memory function with human MPO-G transgene expression	Yes (213)
	Clinical	Increased MPO expression in affected tissue	Yes (107)
		Increased presence of MPO-modified proteins/lipids in affected tissue	Yes (107)
		Correlation studies on −463G/A MPO promoter polymorphism with disease presence	Yes (203, 299, 296)/ no (72, 327)

Multiple sclerosis	Experimental	Increased susceptibility to hindlimb paralysis with MPO gene deletion	No, protective (46)
	Clinical	Increased MPO expression in affected tissue	Yes (242)
		Increased presence of MPO-modified proteins/lipids in affected tissue	Yes (242)
		Correlation studies on −463G/A MPO promoter polymorphism with disease presence/severity	Yes (242)/no (159)
Parkinson's disease	Experimental	Increased MPO expression in affected tissue	Yes (70)
		Increased presence of MPO-modified proteins/lipids in affected tissue	Yes (70)
		Less neurotoxicity with MPO gene deletion	Yes (70)
	Clinical	Increased MPO expression in affected tissue	Yes (70)
Renal diseases			
Immune-complex glomerulonephritis	Experimental	Increased MPO expression in affected tissue	Yes (265)
		Decreased neutrophil-mediated injury with MPO gene deletion	Yes (265)
		Increased T-cell–induced crescentic glomerulonephritis with MPO gene deletion	No, protective (265)
Membranous glomerulonephritis	Clinical	Increased MPO expression in affected tissue	Yes (108)/no (306)
		Increased presence of MPO-modified proteins/lipids in affected tissue	Yes (108)
Ischemia/reperfusion injury	Experimental	Decreased neutrophil influx and improved renal function with MPO gene deletion	Yes (226)
Diabetic nephropathy	Clinical	Correlation studies on −463 G/A MPO promoter polymorphism with disease presence	Yes (52)
Lung diseases			
Asbestos-induced injury	Experimental	Increased MPO expression in broncheal alveolar fluid	Yes (113)
		Delayed lung inflammation with MPO gene deletion	Yes (113)
Cystic fibrosis	Clinical	Increased MPO expression in sputum	Yes (165, 346)
		Increased presence of MPO-modified proteins/lipids in sputum	Yes (165, 346)
		Correlation studies on MPO activity with disease presence/severity	Yes (293)
BM-transplantation–induced injury	Experimental	Increased lung inflammation and impaired lung function with MPO gene deletion	No, protective (231)

BM, bone marrow.

enhance the transformation of the prototype PAH benzo[a]-pyrene (B[a]P) into DNA-binding metabolites (*e.g.*, B[a]P-diol-epoxide) that may cause mutagenesis and carcinogenesis (163, 340, 341). Finally, HOCl has been found to inhibit DNA strand-break repair and nucleotide excision repair (112, 272). Collectively, the data described earlier strongly support a link between MPO activity and carcinogenesis and indicate that both direct and indirect DNA-damaging effects are involved.

As described earlier, various polymorphisms that occur in the MPO promoter region affect MPO levels. Given the link between MPO activity and cancer development, genetically determined MPO levels could influence cancer risk, and this has been investigated in an increasing number of epidemiologic studies. In this respect, the main focus has been on the MPO −463G/A promoter polymorphism in which the G allele increases MPO gene expression, whereas the A allele is associated with lower MPO gene-transcription rates (186, 275, 297). To date, a number of studies have reported an association between the variant −463A allele and a decreased risk of lung cancer, which would be consistent with a role of MPO in carcinogenesis (58, 194, 209, 268, 309, 310, 329). However, controversies exist, and not all studies are in agreement regarding additional effects of age, gender, and gene–environment interactions (68, 232, 373, 377). In addition to lung cancer, the −463A has been associated with a decreased risk in larynx (58), bladder (137), and breast (4) cancer, whereas an increased frequency of the −463G allele has been reported in acute promyelocitic leukemia (295) and pancreatic adenocarcinoma (365). In summary, the −463 G/A polymorphism in the MPO promoter region that has been reported to affect MPO transcription levels appears to contribute to the modulation of cancer risk. However, as discussed earlier, controversy exists on whether this polymorphism indeed leads to changes in MPO protein levels or enzyme activity, and this clearly needs further investigation. Table 1 summarizes the pathogenic potential of MPO in several forms of cancer, as analyzed in clinical studies.

D. MPO and neurodegenerative diseases

Mounting evidence points to an important role of oxidative injury, including MPO-derived oxidants, in the pathogenesis of neurodegenerative disorders (summarized in Table 1). The underlying mechanism is believed to involve oxidative damage to various cell components, including proteins, carbohydrates, lipids, and nucleic acids, because of overproduction of free radicals, leading to impairment of cellular functions and ultimately cell death. MPO, MPO-derived oxidants, and increased levels of lipid peroxidation have been demonstrated to be present in affected brain tissues of patients with AD (107), Parkinson's disease (PD) (70), and multiple sclerosis (MS) (242). For example, in contrast to normal brain tissue, in autopsy brain tissue of patients with AD, MPO protein is present in microglia and neurons, is enzymatically active, and colocalizes with β-amyloid (107). Furthermore, elevated levels of 3-chlorotyrosine have been detected in brain sections of AD, indicating HOCl-mediated tissue injury (107). All these observations suggest a pathogenic role for catalytically active MPO in neuronal dysfunction in AD, probably through the generation of lipid peroxides. However, results from epidemiologic studies investigating the relation between the −463G/A MPO promoter polymorphism and AD risk are inconclusive. Initial studies reported that the G allele, which is linked to increased MPO expression, was overrepresented in female AD patients, whereas an increased frequency of the A allele was observed in male patients (203, 299). In subsequent studies in a Finnish population, the presence of the MPO −463A allele together with the apolipoprotein E4 allele was found to be associated with increased AD risk in men but not in women (296). In contrast, studies in other cohorts could not confirm a role for the −463G/A MPO promoter polymorphism in the incidence of AD (72, 327).

Further to study the effects of human MPO in the development of AD, Maki *et al.* (213) recently crossed mice transgenic for APP23 with mice transgenic for either the human MPO −463G or the −463A allele (213). APP23 transgenic mice overexpress the human familial AD mutant APP751 transgene in neurons. These mice develop amyloid plaques and memory deficits, but, in contrast to humans, do not express murine MPO in their brain tissue. Maki *et al.* reasoned that this may be due to the absence of a primate-specific Alu element in the murine MPO gene, as described earlier, and therefore crossed the human MPO −463A or G transgenic mice to the APP23 mice (213). Interestingly, introduction of the −463G but not of the −463A allele in APP23 mice resulted in the development of abundant amyloid plaques in hippocampus and cerebral cortex, with strong expression of MPO in plaques and astrocytes in the cortex. In addition, in the brains of MPOG-APP23 double-transgenic mice, high levels of oxidized phospholipids could be detected. Finally, MPOG-APP23 mice had greater spatial-memory impairment in water-maze experiments compared with APP23 mice (213). Overall, these elegant studies support an important role for MPO-catalyzed oxidative injury in neuronal injury and dysfunction in AD.

MPO-mediated oxidative damage also has been linked to the progression of multiple sclerosis (MS), an inflammatory degenerative disorder of the central nervous system. In lesional tissue of MS patients, expression of MPO protein and mRNA has been detected in microglia and macrophages, although evidence for the presence of 3-chlorotyrosine or HOCl-modified proteins is lacking (242). Compared with healthy individuals, MPO activity in circulating leukocytes of MS patients has been reported to be lower. However, conflicting data exist on the association of the MPO −463G/A promoter polymorphism and MS incidence. In one study, an increased frequency of the variant −463G allele was reported in early-onset MS in female patients (242). However, in subsequent studies, no association between the MPO −463G/A polymorphism and MS disease susceptibility and severity was found (159). In contrast, studies in a mouse model of MS, known as experimental autoimmune encephalomyelitis, do suggest a role for MPO in disease development. On EAE induction, 90% of MPO-deficient mice developed hindlimb paralysis as compared with 33% in wild-type littermates, suggesting a protective role for MPO in EAE induction (46). The increase in EAE susceptibility in MPO-deficient mice was associated with an enhanced proliferation of antigen-specific T cells, suggesting a suppressive effect of MPO on adaptive immune responses (46).

Besides AD and MS, a role for MPO has been implicated in PD, although the evidence is less extensive. PD is characterized by the loss of dopaminergic neurons from the substantia

nigra pars compacta in the brain, and increased MPO expression in glial cells in this area in PD patients has been described (70). Furthermore, in an animal model of PD, induced by treatment with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), increased levels of 3-chlorotyrosine and HOCl-modified proteins in brain tissues have been reported, suggesting MPO-mediated tissue damage (70). Supporting this contention are observations in MPO-deficient mice treated with MPTP (70). Compared with wild-type littermates, MPO-deficient mice were found to be more resistant to MPTP-induced neurotoxicity, suggesting that MPO participates in the MPTP neurotoxic process.

E. MPO and renal diseases

The capacity of the MPO-H_2O_2 system to harm the kidney directly was initially demonstrated by renal perfusion studies in rats (49, 151, 152). On renal perfusion, MPO was shown to bind to the glomerular basement membrane. When MPO perfusion was immediately followed by perfusion of H_2O_2, this resulted in acute glomerular injury, characterized by platelet degranulation and endothelial cell swelling and lysis, leading to proteinuria. These perfusion studies showed that the MPO-H_2O_2-chloride system, most likely through the generation of HOCl, can cause injury to cells and structures implicated in renal physiology. Various clinical and experimental studies have been performed since, to explore the role of MPO in a number of renal diseases (summarized in Table 1). Here, we describe the possible contribution of MPO to kidney dysfunction in immune-complex glomerulonephritis, ischemia/reperfusion injury, diabetic nephropathy, and lipid-associated glomerulopathy.

Based on the perfusion studies in rats described earlier, MPO would be expected to play a role in inflammatory renal diseases characterized by acute neutrophilic inflammation, as observed in acute immune complex crescentic glomerulonephritis (GN). A well-characterized model of crescentic GN is autologous-phase anti–glomerular basement membrane (anti-GBM) globulin GN, in which heterologous anti-GBM antibody acts as a planted antigen in glomeruli. Renal injury in this model occurs in two phases. During the initial, or heterologous, phase of the disease, injury, characterized by abnormal proteinuria, is mediated by infiltrating neutrophils whose accumulation in glomeruli peaks 2 h after anti-GBM Ig administration and causes the extracellular release of MPO (131, 265). The autologous phase of the disease is mediated by a T-helper 1 cell (Th1)-polarized adaptive immune response against the immunizing antigen, which develops 1–3 weeks later. During this phase, severe glomerular injury, characterized by glomerular crescent formation, is mediated by infiltrating $CD4^+$ T cells and macrophages. By using MPO-deficient mice, Odobasic *et al.* (265) investigated the role of MPO in the heterologous (2 h) as well as the autologous (3 wk) phase of anti-GBM glomerulonephritis (265). Increased MPO activity in kidneys of wild-type mice was observed at both time points. In the heterologous phase, MPO-deficient mice showed increased glomerular neutrophil accumulation but significantly decreased proteinuria, consistent with a role of MPO-derived oxidants in tissue injury. In contrast, in the autologous phase of the disease, no effect of MPO deficiency was found on proteinuria and glomerular crescent formation. However, increased glomerular accumulation of $CD4^+$ T cells and macrophages was observed in conjunction with increased proliferation of splenic $CD4^+$ T cells and increased production of Th1 cytokines, suggesting that endogenous MPO downregulates antigen-specific T-cell responses. Overall, this study demonstrates a complex role of MPO in immune-mediated glomeronephritis with differential effects on the heterologous (neutrophil-mediated) and autologous (Th1/macrophage-mediated) phases of the disease.

Membranous glomerulonephritis (MGN) is a form of immune-complex glomerulonephritis that is characterized by subepithelial immune deposits in the glomerular basement membrane, leading to glomerular damage and proteinuria. A model that is commonly used to study MGN is passive Heymann nephritis. Passive Heymann nephritis is an immune-complex glomerulonephritis that is induced by administration of nephrotoxic antibodies to rats. Proteinuria in passive Heymann nephritis was shown to involve complement activation and ROS production (164, 283). Increased expression of H_2O_2 was found in glomeruli of rats with proteinuric passive Heymann nephritis, predominantly in podocytes and along the glomerular basement membrane (164), suggesting a role for H_2O_2 in immune-complex glomerulonephritides. Important evidence for a role of the MPO-H_2O_2-HOCl system in MGN came from expression studies in patients. Gröne *et al.* (108) showed presence of HOCl-modified proteins in glomeruli of MGN patients by using immunohistochemical detection. The HOCl-modified proteins were observed in podocytes, intracapillary cells (monocytes/macrophages), and the glomerular basement membrane. In addition, the authors could detect MPO in glomeruli of MGN patients. The expression of MPO colocalized with expression of HOCl-modified proteins along the glomerular basement membrane and in monocytes and macrophages, suggesting that the HOCl-mediated modifications were MPO dependent. Possibly, the HOCl-mediated modifications include HOCl-modified LDL. An increased antibody titer directed against HOCl-oxidized LDL was detected in patients, suggesting an increased LDL-oxidation contributing to glomerular damage in MGN (97). In line with this, HOCl-modified LDL was shown to affect tubular epithelial cells in MGN patients by inducing differential gene expression (280). The differential regulation of genes correlated with renal function and included genes involved in ROS metabolism, cell stress, tissue remodeling, and inflammation. Although one study could not detect glomerular MPO expression in human MGN (306), most findings point to activity of the MPO-H_2O_2-HOCl system in MGN.

Renal I/R injury is an important determinant in the pathogenesis of kidney-transplant rejection and sepsis. I/R injury is characterized by the extravasation of inflammatory cells, including neutrophils and monocytes/macrophages, of which neutrophils are the first cells that become activated and that migrate into the tissue (142). The use of adhesion-molecule knock-out mice (162) or chemokines-blocking antibodies (233) in experimental renal I/R led to a decrease in neutrophil migration and, as a consequence, reduced tissue damage. In addition, inhibition of neutrophil activation with an inflammatory cytokine synthesis inhibitor diminished renal damage after I/R in rats (18). Because of these observations, neutrophils are believed to be important effector cells in I/R injury, although a crucial role for neutrophils could not always be proven (80, 229). The increase in number of neutrophils in

I/R-subjected organs is accompanied by increased levels of MPO. On I/R, the infiltration of neutrophils and the release of MPO will trigger inflammatory processes such as the activation of complement and the production of proinflammatory cytokines (142). The increased MPO activity in I/R-injured kidneys can also be detrimental because of the generation of chlorinated and nitrated species (214). H_2O_2, the main substrate for MPO, is abundantly present in the kidney after I/R. It is not known which enzyme is mainly responsible for the increased presence of H_2O_2, but NADPH oxidases, xanthine oxidase, and monoamine oxidase are appropriate candidates (214). Furthermore, HOCl appears to be important in I/R injury because addition of taurine, an *in vivo* scavenger of HOCl, had an improved effect on I/R damage in isolated solid organs, including rat kidney (214). Nevertheless, renal microvascular injury on lower limb reperfusion in rats did not improve with systemic taurine application (37). It should be noted that, as discussed earlier, taurine reacts with HOCl to form taurine chloramines, which is less toxic than HOCl. However, taurine chloramine still has biologic activity and has been shown to be more selective than HOCl at targeting thiol groups on cysteines (274). As such, taurine chloramine-mediated oxidation of cysteines may affect the function of many proteins, including cellular receptors and transcription factors, and contribute to cellular damage.

The generation of nitrogen oxygen species may be involved in I/R injury. Inactivation of inducible nitric oxide synthase (iNOS) in experimental I/R resulted in less renal damage, suggesting a role for NO in renal I/R (63, 64). The reduced renal damage was accompanied by a decrease in neutrophil infiltration and MPO activity. As a consequence of iNOS inhibition, less nitrotyrosine was detected in the I/R kidney, probably because of reduced peroxynitrite formation (64). Nitrotyrosine also can be generated by the reaction of MPO with H_2O_2 or by the reaction of NO_2^- with HOCl (an indirect effect of MPO) (85). However, the biologic significance of the latter reaction is uncertain because of its slow kinetics (79, 366). Although these data suggest a role for MPO-derived chlorinated and nitrated species, the exact importance of these species in renal I/R injury is not known.

Besides a possible role for MPO through the formation of ROS, MPO is important for neutrophil extravasation in renal I/R injury. Matthijsen *et al.* (226) recently showed that $Mpo^{-/-}$ mice have less renal damage and function loss after experimental I/R compared with wild-type mice, which was characterized by a significant reduction in the number of infiltrating neutrophils 24 h after reperfusion (226) (Fig. 9). Interestingly, no differences were found in complement activation either after a short period of reperfusion (2 h) or after 24 h of reperfusion. These data suggest that MPO contributes to renal I/R damage rather through reducing neutrophil extravasation than by complement activation. As described earlier, MPO is able to activate neutrophils directly and control neutrophil adhesion to endothelial cells through binding to CD11b/CD18 (148, 193), which could explain the observed reduction in migrated neutrophils in the study of Matthijsen *et al.* (226). Taken together, MPO contributes to renal I/R injury by promoting migration of neutrophils, and possibly by the production of harmful chlorinated and nitrated species.

Recent findings suggest a role for MPO in diabetic nephropathy through promotion of HOCl production. Diabetic nephropathy is a progressive disease that occurs as a result of hyperglycemia. Diabetic nephropathy is characterized by a reduced glomerular filtration rate and proteinuria, which will eventually result in end-stage renal disease. ROS play a detrimental role in diabetic nephropathy, as evidenced by the observations that inhibition of ROS production reduces proliferative and fibrotic changes and improves renal function in experimental diabetic nephropathy (17). Normally, oxygen radicals can be cleared by SOD and catalase. SOD catalyzes the conversion of $O_2^{\bullet -}$ into H_2O_2, and catalase, the subsequent conversion into H_2O. In diabetic nephropathy, however, both SOD and catalase have decreased activity, leading to impaired clearance of ROS. Several groups tried to restore the clearance of radicals and to prevent concomitant renal damage in experimental diabetic nephropathy models by the use of tempol. Tempol is a nitroxide that is mainly referred to as a synthetic mimetic of SOD. Nitroxides, however, are antioxidants that act in various ways by reaction with oxidants and reductants (24, 352), and these mechanisms should be considered as well when interpreting the effects of treatment with tempol in experimental models. Interestingly, Rees *et al.* (292) recently reported that nitroxides are also potent inhibitors of

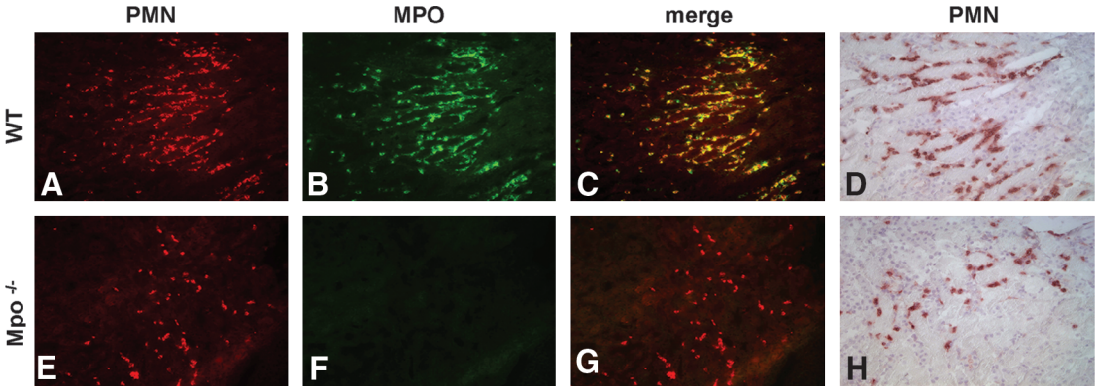

FIG. 9. Decreased accumulation of neutrophils in kidneys from MPO-deficient mice after renal ischemia/reperfusion (I/R) injury. Immunofluorescent staining of neutrophils (red) and MPO (green) in kidneys of wild-type (**A–C**) and $Mpo^{-/-}$ mice (**E–G**) 24 h after induction of I/R. (**D**, **H**) Immunohistochemical staining of neutrophils in renal tissue from wild-type (**D**) and $Mpo^{-/-}$ (**H**) mice 24 h after induction of I/R. Reprinted with permission from (226). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

MPO-mediated HOCl production. Asaba *et al.* (16) treated diabetic rats with tempol, and although this treatment inhibited mesangial matrix expansion, it did not reduce proteinuria. Indeed, tempol treatment increased the conversion of superoxide to H_2O_2, scavenging the harmful oxygen radical. However, catalase activity was not restored by tempol, leading to an accumulation of H_2O_2 in the kidney. Moreover, tempol treatment increased renal MPO activity, which accelerated the conversion of H_2O_2 into HOCl. These findings suggest that the beneficial effects of ROS scavenging were overcome by detrimental effects of excess HOCl. Additionally, the data imply a role for MPO and HOCl in protein-barrier loss rather than in glomerular matrix expansion in diabetic nephropathy. Similar results were found by Rafivoka *et al.* (287), who examined the effect of tempol treatment in obese diabetic hypertensive rats. Obese diabetic rats had proteinuria; increased renal cortical lipid peroxidation and protein oxidation; and increased glomerular and interstitial inflammation, proliferation, and fibrosis. Tempol treatment decreased lipid peroxidation; improved insulin resistance; and reduced inflammatory, proliferative, and fibrotic changes, most likely because of decreased superoxide levels. Conversely, tempol treatment had no effect on proteinuria and renal hemodynamics and did not reduce protein oxidation. Because catalase activity was not restored by tempol, protein oxidation and proteinuria were believed to be caused by excess amounts of H_2O_2. In contrast to Asaba *et al.* (16), no increased renal MPO activity was detected, but instead, a decrease in total tissue peroxidase activity was observed (287). It is, however, plausible that even reduced peroxidase activity is sufficient to exert protein damage in the presence of excess amounts of H_2O_2. The fact that Rafivoka *et al.* (287) did not observe reduced protein oxidation supports this hypothesis. Taken together, these data suggest a role for MPO, H_2O_2, and HOCl in diabetic nephropathy, particularly in renal hemodynamics and proteinuria. Furthermore, a role for MPO in diabetic nephropathy has been suggested through the production of advanced glycation end products (AGEs) (214). AGEs are biochemical end products of glycation reactions that can bind to and activate the receptor for AGE (RAGE), leading to the release of profibrogenic mediators and proinflammatory cytokines. Activation of the AGE-RAGE system is known to take part in the pathogenesis of diabetic nephropathy (41, 364). Furthermore, the MPO-H_2O_2-HOCl system is capable of forming AGEs *in vivo* (9). Hence, the MPO-mediated production of AGEs might considerably contribute to diabetic nephropathy. The pattern of MPO expression and activity in human diabetic nephropathy is still unresolved. Whereas in the vitreous and epiretinal membranes of patients with diabetic retinopathy, increased MPO expression could be detected (22), no reports exist on renal MPO expression in patients with diabetic nephropathy. In neutrophils from diabetes patients, MPO activity was shown to be decreased (307, 342), whereas an increased MPO plasma level was detected in diabetic nephropathy patients that received peritoneal hemodialysis treatment (52, 326). In addition, a difference in MPO −463G/A promoter genotype frequencies was found between patients with diabetic nephropathy and in patients with other renal diseases or control subjects, with GG and AA genotypes being more common in diabetic nephropathy patients (52). The MPO −463G/A genotype frequency difference did not correlate with MPO plasma levels, because MPO plasma levels were similar in patients with diabetic nephropathy and in patients with other renal diseases. The authors also observed a correlation between MPO −463G/A genotype and an earlier onset of disease within the diabetic nephropathy patient group. However, these results need confirmation before definitive conclusions can be drawn. Taken together, although MPO potentially contributes to diabetic nephropathy in both a direct (by HOCl) and an indirect (by production of AGEs) way, still little knowledge exists on MPO expression and activity during human diabetic nephropathy. The exact role of MPO in diabetic nephropathy must be explored more extensively.

A role for MPO has also been suggested in lipid-associated glomerulopathy. Guinea pigs that were fed a high-cholesterol diet were shown to have increased serum cholesterol levels and glomerular lesions, suggesting a role for lipids in glomerular diseases (6). The observed lipid-associated glomerular lesions were partially mediated by monocytes. Because lipid-association and monocyte involvement are characteristic in both lipid-associated glomerulopathy and atherosclerosis, these disorders are suggested to have a similar pathologic mechanism (214). The contribution of MPO to atherosclerosis is quite established and was discussed in more detail earlier. One effect of MPO likely to contribute to atherosclerosis development is HOCl modification of LDL and HDL. HOCl-modified LDL and HDL can exert effects on inflammatory and endothelial cells. HOCl-LDL stimulates IL-8 production by human monocytes and is chemotactic for neutrophils *in vitro* (372). HOCl-LDL and -HDL both reduce NO formation in endothelial cells *in vitro*, which may lead to endothelial cell dysfunction (224, 263). Moreover, HOCl-HDL binds more MPO than does unmodified HDL. At the same time, it decreases binding of MPO to endothelial cells and macrophages and internalization and degradation of MPO by macrophages (223). This implies that, in the presence of HOCl-HDL, an increased amount of MPO is available in the circulation and able to execute MPO-mediated protein modifications. Although HOCl-modified lipoproteins can affect cell types relevant in glomerulopathy, an involvement of MPO in lipid-associated glomerulopathy is still speculative. Further investigation is needed to confirm that the pathogenic mechanisms of lipid-associated glomerulopathy and atherosclerosis are comparable, and to find out whether MPO-mediated modification of lipoproteins is indeed involved in the occurrence of lipid-associated glomerulopathy.

F. MPO and lung diseases

Neutrophilia of the lungs is a phenomenon common to a variety of lung-related diseases, including chronic obstructive pulmonary disease (COPD), asbestosis, and acute respiratory distress syndrome (ARDS) (240, 270, 334). Hence, a contributory role for MPO-derived oxidants in the development of lung tissue injury has been proposed. This is supported by the detection of increased levels of MPO in BALF and MPO protein expression in lung epithelium in various lung diseases (113, 161). Furthermore, similar to observations in the kidney, as described earlier, perfusion of the lung with MPO and H_2O_2 or intratracheal infusion of a peroxidase, including MPO, and an H_2O_2-generating system in rats induces severe acute lung injury characterized by increased permeability and intraalveolar fibrin deposition that progresses to interstitial

fibrosis (98, 149). More recently, a direct effect of MPO was shown on alveolar and bronchial epithelial cell lines. On exposure to MPO, these cells are able to internalize MPO, resulting in increased HO-1 expression and DNA strand breakage and, in bronchial epithelial cells, in inhibition of LPS-stimulated IL-8 production (114). Further evidence for a role of MPO in the development of lung injury comes from studies in experimental asbestos- or LPS-induced lung inflammation. In mice, inhalation of chrysotile asbestos fibers causes increased MPO activity in BALF and MPO immune reactivity in epithelial cells lining distal bronchioles and alveolar ducts. Haegens and colleagues (113) recently demonstrated that asbestos-induced lung inflammation is modulated in MPO-deficient mice, as evidenced by a decreased accumulation of macrophages and lymphocytes and less epithelial cell proliferation in these mice compared to wild-type littermates. In addition, Haegens and colleagues (115) recently demonstrated that MPO-deficient mice are largely protected from the acute inflammatory effects of intratracheal LPS instillation. Compared with wild-type mice, MPO-deficient mice showed a dramatic reduction in neutrophil accumulation in the lungs on intratracheal instillation of LPS, which was associated with a decrease in mRNA and protein expression of neutrophil-specific chemoattractants. Interestingly, migration of MPO-deficient neutrophils toward a neutrophil chemoattractant *in vitro* was significantly decreased compared with wild-type neutrophils, suggesting a direct effect of MPO on neutrophil migration.

In cystic fibrosis (CF), a prominent role for MPO-derived oxidants in causing lung damage has been suggested. Large amounts of active MPO and high levels of 3-chlorotyrosine have been detected in sputum samples from patients with CF (165, 346). The peroxidase activity in such samples has been found to correlate with disease severity (293). Furthermore, in CF patients, MPO activity in circulating neutrophils correlates with airway obstruction and sputum production, suggesting a direct link between neutrophil-derived MPO oxidants and lung injury (100). Besides a direct damaging effect, MPO-derived oxidants may also indirectly contribute to lung injury. Evidence exists that in CF patients, the protease inhibitor α_1-antitrypsin is (partially) oxidized, resulting in inactivation of this protease inhibitor (57), an effect that may be mediated by MPO-derived oxidants (367).

In summary, the MPO-H_2O_2 system, either directly or indirectly, is an important contributor to tissue injury in various lung diseases, as summarized in Table 1.

G. MPO in other chronic inflammatory diseases

In various other chronic inflammatory diseases, a role for MPO-derived oxidants in the development of tissue injury has been suggested including rheumatoid arthritis (RA) and inflammatory bowel disease (IBD). In synovial fluids of RA patients, large numbers of activated neutrophils can be detected, and many of these neutrophils can be found in or near areas of cartilage erosion (116, 312). Hence, it has been suggested that these cells inflict tissue damage through the release of oxidants and proteases and contribute to the development of joint injury, as observed in RA. The importance of neutrophils in tissue injury in RA is further supported by animal studies. Sera from transgenic K/BXN mice contain immune complexes directed against glucose-6-phosphate isomerase. On transfer to wild-type mice, these immune complexes induce arthritis (225). Disease development in this passive-transfer model requires neutrophils as the main effector cells and is dependent on neutrophil Fc receptor expression (225, 370). A specific role for MPO in RA is suggested by the observations that catalytically active MPO can be detected in synovial fluids of RA patients (83, 262). Finally, a large number of *in vitro* studies have demonstrated that MPO-derived oxidants can degrade various joint components, including cartilage and hyaluronic acid [reviewed in (312)].

Similar to RA, considerable neutrophil accumulation in conjunction with increased MPO activity is seen in intestinal mucosal lesions of patients with IBD. Furthermore, MPO-positive neutrophils and monocytes/macrophages have been detected immunohistochemically in the lamina propria of patients with IBD (182, 183, 301). Results from animal models further support the contention that neutrophils contribute to tissue injury in IBD. Oral administration of dextran sulfate sodium in rats induces colitis that mimics human ulcerative colitis and is associated with abundant neutrophil infiltration. In this model, several lines of evidence indicate an important role for neutrophil-mediated inflammation in the development of colitis. These include the protective effect of selective neutrophil depletion (247) and the protective effects of scavengers or inhibitors of neutrophil oxidants (244) and proteases (239), respectively, on dextran sulfate sodium-induced colitis. Although not studied directly, these observations also suggest a role for MPO-derived oxidants in IBD pathogenesis.

H. MPO as autoantigen in systemic vasculitis

MPO can serve as an autoantigen in antineutrophil cytoplasmic autoantibodies-associated small-vessel vasculitides (ANCA-SVV) (144). ANCA-SVV are a group of autoimmune disorders, including Wegener's granulomatosis, microscopic polyangiitis, Churg Strauss syndrome, and renal limited vasculitis, in which inflammation and destruction of small blood vessels occurs (144). This group of disorders is characterized by the presence of circulating anti-neutrophil cytoplasmic autoantibodies (ANCAs) directed against lysosomal constituents of neutrophils and monocytes. Two major target antigens are known for ANCAs, one of which is MPO (90). The other antigen is Pr3, a serine protease that, like MPO, is stored in the azurophilic granules of neutrophils and peroxidase-positive lysosomes of monocytes (106, 145).

The original and still widely used method to screen patients' sera for the presence of ANCAs is indirect immunofluorescence on ethanol-fixed neutrophils. With this technique, two different fluorescence patterns can be distinguished produced by ANCA-containing sera; a cytoplasmic staining pattern termed c-ANCA and a perinuclear staining pattern termed p-ANCA (Fig. 10). Identification of the main target antigens of ANCAs showed that ANCAs producing a c-ANCA immunofluorescence pattern recognized Pr3, whereas the vast majority of ANCAs producing a p-ANCA staining pattern recognized MPO.

ANCA-SVV is relatively rare, but patients with ANCA-SVV have an extremely high mortality, which makes immediate and strong immunosuppressive treatment necessary. In addition, disease relapses are frequent in ANCA-SVV patients requiring long-term treatment, with severe side effects as a result.

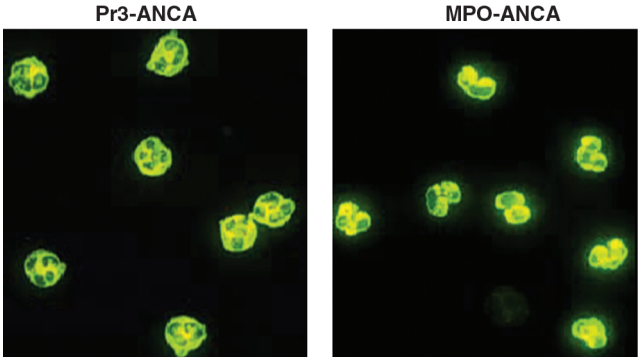

FIG. 10. Fluorescence patterns on ethanol-fixed human neutrophils produced by anti-neutrophil cytoplasmic autoantibodies (ANCAs) with specificity for proteinase 3 (Pr3-ANCA) or MPO (MPO-ANCA). A granular cytoplasmic pattern is observed for Pr3-ANCA, whereas a perinuclear pattern is produced by MPO-ANCA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

Although ANCA-SVV can affect multiple organs, the kidneys together with the lungs are the primary organs affected (144). ANCA-SVV manifests itself in the kidney predominantly as glomerulonephritis with segmental necrotic and fibrotic lesions in conjunction with inflammatory cell infiltrates. The lesions and infiltrates cause disruption of glomerular capillaries, which may eventually lead to the formation of crescent-shaped scars of proliferating epithelial cells and macrophages that compress the glomerulus (144). The glomerular abnormalities are occasionally accompanied by inflammatory infiltrates in the tubulo-interstitial area. ANCA-SVV is a pauci-immune disease because no or little deposits of immune products like IgG and complement factors are found in the vasculitic lesions. Besides histologic signs of glomerular damage, ANCA-SVV patients have urinary abnormalities such as hematuria and proteinuria.

Since the discovery of ANCAs by Davies *et al.* in 1982 (77), detection of ANCAs in patients with glomerulonephritis has become a valuable marker in diagnosis (155). It was uncertain, though, for a long time whether ANCAs are pathogenic and cause the underlying vasculitis or whether their presence is a consequence of the severe inflammatory response. Over the last decades, however, several clinical observations and extensive *in vitro* and experimental studies have revealed a pathogenic role for MPO-ANCA in ANCA-SVV (129, 146, 156).

1. Clinical observations. A strong correlation exists between the presence of ANCAs and SVVs. Ninety percent of patients with ANCA-SVV have ANCAs specific for either MPO or Pr3. MPO-ANCA is most common in microscopic polyangiitis, Churg-Strauss syndrome, and renal limited vasculitis, whereas Pr3-ANCA predominates in patients with Wegener's granulomatosis. In addition, ANCA titers follow disease activity, with low titers during remission and an increase in titer preceding relapses. Although the second correlation is not extremely strong, these observations suggest a pathogenic role for ANCAs. Furthermore, the MPO promoter −463G/A polymorphism was shown to influence disease development in a study on ANCA-SVV patients (300). In this study, the MPO A allele was associated with an increased incidence of relapses and an earlier age at diagnosis in MPO-ANCA–associated vasculitis patients. However, these associations could not be confirmed by others (94, 289). Additional evidence for MPO-ANCA pathogenicity comes from case reports in which patients treated with various drugs, including hydralazine and propylthiouracil, developed circulating ANCAs followed by vasculitic disease, suggesting causation (335).

However, the most compelling clinical evidence for ANCA pathogenicity comes from observations in a pregnant woman with a history of microscopic polyangiitis. Because of active disease during her pregnancy, MPO-ANCA from the mother was transferred across the placenta to the fetus, which caused neonatal pulmonary hemorrhage and renal vasculitis immediately after birth (36). When the neonate was treated with exchange transfusion and high doses of steroids, the MPO-ANCA level of the neonate declined below detection level, and the symptoms disappeared.

2. *In vitro* studies involving MPO-ANCA. Our knowledge on the pathogenic mechanisms involved in MPO-ANCA–mediated vascular injury is largely based on *in vitro* studies: MPO-ANCA can bind to and activate neutrophils *in vitro*, which leads to the release of toxic substances that destroy microvascular endothelium (156). This MPO-ANCA–mediated neutrophil activation is completely dependent on cellular MPO, as illustrated by the lack of activation of MPO-deficient neutrophils by these autoantibodies (294). The main aspects of this pathogenic mechanism are explained here.

For MPO-ANCA to activate neutrophils fully, these cells require priming with a low dose of a pro-inflammatory cytokine such as TNF-α (Fig. 11). TNF-α induces translocation of MPO to the neutrophil cell surface, making MPO accessible for binding to MPO-ANCA. In addition, MPO released from activated neutrophils can bind to unstimulated neutrophils, thereby sensitizing them for MPO-ANCA–mediated activation (135). Translocation of MPO to the cell surface with TNF-α priming has been shown to depend on p38 MAPK signaling (170). Furthermore, TNF-α priming increases β_2-integrin membrane expression, which is required for neutrophil–endothelium interactions. The need for neutrophil priming *in vitro* suggests a role for some kind of preactivation trigger in ANCA-SVV patients. Such a trigger could, for instance, be mediated by a bacterial infection. This idea is supported by studies on Wegener granulomatosis, showing that the chronic nasal presence of *Staphylococcus aureus* increases the risk of relapse (325), and that antibacterial (co-trimoxazole) treatment reduces the incidence of relapses in remissive patients (324).

The activation of primed neutrophils by MPO-ANCA requires both $F(ab')_2$-mediated antigen binding and Fc-mediated Fcγ-receptor ligation (290). Although binding of $F(ab')_2$ to neutrophil MPO initiates G protein signaling, it does not activate all signaling pathways involved in ANCA-IgG signaling (169). Ligation of the Fc portion of MPO-ANCA to Fc-receptors, especially FcγRIIa, is needed as well (241). FcγRIIa is an Fc-receptor with high affinity for immunoglobulins of the IgG3 subclass. Interestingly, ANCAs with subclass IgG3 are more abundantly present in ANCA-SVV patients with active disease (143). FcγR engagement by

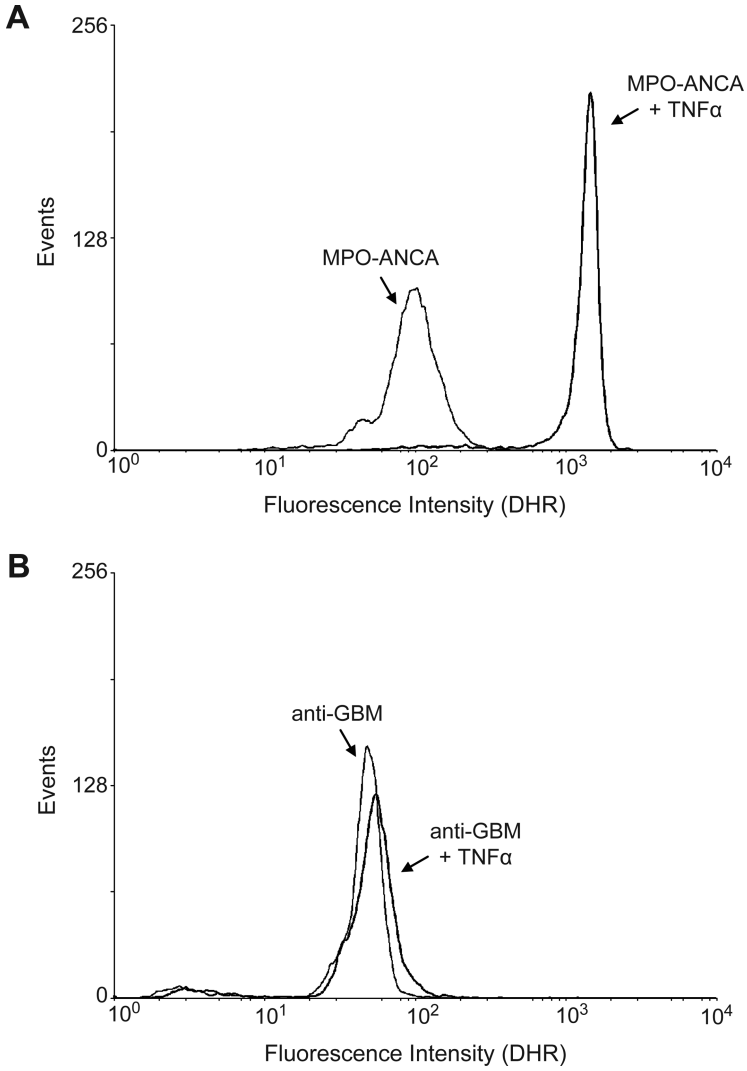

FIG. 11. Neutrophil-activating potential of MPO-ANCA. (**A**) MPO-ANCA IgG induces an oxidative burst in isolated human neutrophils, as determined by flow-cytometric analysis and the fluorescent probe dihydrorhodamine123 (DHR). MPO-ANCA–mediated neutrophil activation is dependent on prior priming of the cells with the pro-inflammatory cytokine TNF-α. (**B**) IgG isolated from a patient with anti–glomerular basement membrane (GBM) disease does not activate isolated human neutrophils with or without priming.

ANCAs leads to activation of protein kinase Cβ, protein kinase B, and phosphatidylinositol 3 kinase (PI3K) pathways (290). The signal-transduction pathways downstream of ANCA-mediated FcγR ligation are, however, different from conventional FcγR cross-linking, as activation of a different subtype of PI3K occurs with no phospholipase D activation (38). It is not clear how simultaneous binding of the $F(ab')_2$ and Fc portions of MPO-ANCA occurs *in vivo*. MPO-ANCA can bind either as a monomer to both MPO and Fc receptor or as an MPO-containing immune complex to the Fc receptor (*i.e.*, conventional FcR ligation). Although these mechanisms initiate different signaling pathways, they both result in neutrophil activation. It is conceivable that the two mechanisms occur alongside each other as soon as activation is initiated and MPO becomes released abundantly from already activated neutrophils.

When primed neutrophils become activated by MPO-ANCA, they are able to kill endothelial cells (308). MPO-ANCA–activated neutrophils produce reactive oxygen species (ROS) that cause oxidative stress. In addition, they degranulate, releasing enzymes like MPO, Pr3, and elastase, which can directly harm endothelial cells. Pr3 and elastase can induce cell detachment and apoptosis (380). MPO itself can be involved in endothelial cell death in at least three ways. First, MPO can catalyze the production of HOCl, which has cell-destructive activities. In addition to this, Guilpain *et al.* (110) showed that when anti-MPO antibodies bind to soluble MPO, the ability of MPO to catalyze HOCl production even increased. Second, because MPO is a highly cationic protein, it can bind to the endothelial cell membrane (32, 380). Endothelial cell–bound MPO can be recognized by MPO-ANCA, and binding may lead to antibody-mediated cellular cytotoxicity (89, 308). Third, MPO can be internalized on binding to endothelial cells, which was shown to induce production of ROS (380). ANCA-activated neutrophils can also directly interact with activated endothelium. *In vitro* flow assays have shown that MPO-ANCA directly enhance neutrophil adhesion to and migration through endothelial cell layers that are primed with a low dose of TNF-α (55, 284, 285). The neutrophil–endothelial cell interactions were shown to be FcγR and β_2-integrin dependent (55). The several roles of MPO in endothelial cell injury in MPO-ANCA–mediated SVV are illustrated in Fig. 12.

An additional effect of MPO-ANCA is that it accelerates apoptosis in neutrophils (120). Because the clearance of these apoptotic neutrophils by macrophages is impaired, apoptotic

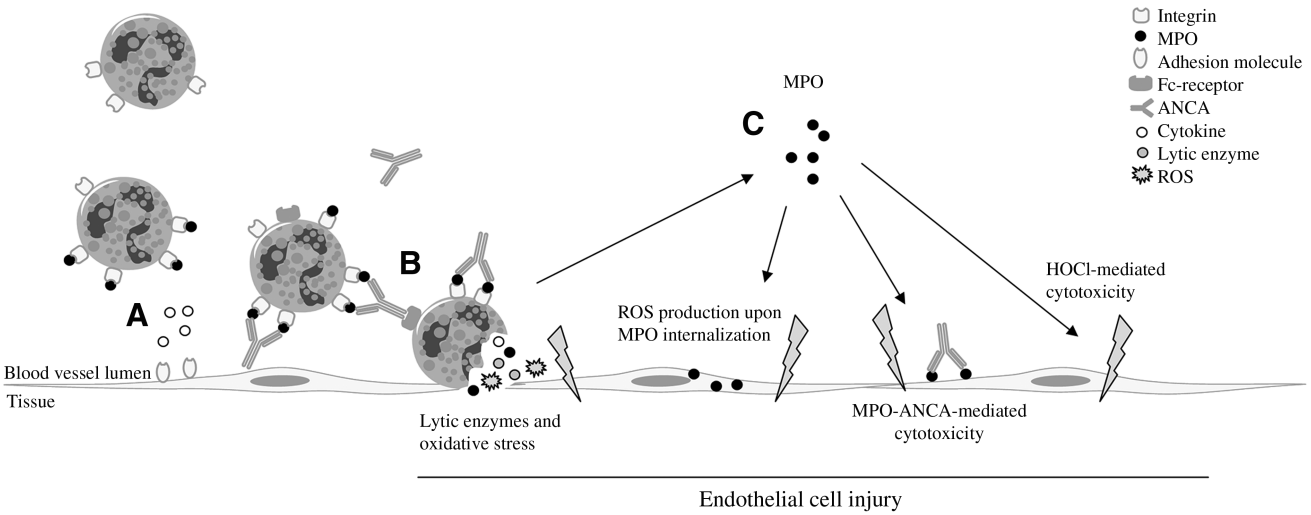

FIG. 12. Overview of the different contributions of MPO to endothelial cell injury in MPO-ANCA-SVV. (**A**) Pro-inflammatory cytokines prime neutrophils and activate endothelial cells. MPO binds to primed neutrophils. (**B**) MPO-ANCA activates neutrophils *via* MPO binding and Fc-receptor ligation. Activated neutrophils degranulate, causing release of proteolytic enzymes and ROS, which can injure endothelial cells. (**C**) In addition, more MPO becomes available on neutrophil degranulation. MPO can harm endothelial cells in three ways, by internalization, by binding and interaction with MPO-ANCA, and by production of HOCl.

neutrophils are probably more likely to release their proteolytic contents, which may lead to damage of surrounding cells. Furthermore, apoptotic neutrophils express more MPO on their membrane, which results in more MPO-ANCA binding (119). However, MPO-ANCA binding to apoptotic neutrophils does not lead to neutrophil activation, but instead, leads to increased uptake by macrophages, probably in a pro-inflammatory manner (119).

Besides neutrophils and endothelial cells, other cell types play a role in ANCA-SVV. As mentioned earlier, MPO also is expressed by monocytes, suggesting that monocytes can be activated by MPO-ANCA as well. Indeed, ANCAs were shown to activate monocytes to produce ROS and chemokines (*e.g.*, IL-8) *in vitro* (356). In this way, ANCA-activated monocytes contribute directly to the inflammatory process in ANCA-SVV. Because the expression of MPO decreases during the differentiation of monocytes to macrophages, MPO-ANCA does not affect mature macrophages (62). Mature macrophages and monocytes are, however, present in granulomatous lesions and glomerular crescents in ANCA-SVV. Autoreactive B and T cells are involved in ANCA-SVV as well, with B cells producing ANCAs, and T cells assisting in ANCA production and ANCA isotype switching.

3. Lessons learned from MPO-ANCA animal models. The first direct evidence for the pathogenicity of MPO-ANCA came from studies in a mouse model developed by Xiao *et al.* in 2002 (374). This model differed from previous models (49, 126–128), as it is an autologous model, using anti-MPO antibodies raised in mice to study their effects in mice (Fig. 13). Xiao *et al.* used purified murine MPO (mMPO) isolated from a murine myeloid cell line to immunize $Mpo^{-/-}$ mice. The immunization induced an immune response against mMPO in $Mpo^{-/-}$ mice, resulting in circulating anti-MPO antibodies. The authors isolated splenocytes from mMPO immunized and control immunized $Mpo^{-/-}$ mice, and they passively transferred different amounts of splenocytes to recombinase-activating gene-2–deficient ($Rag2^{-/-}$) mice, which lack functioning B and T cells. All $Rag2^{-/-}$ mice that received anti-MPO splenocytes developed circulating anti-MPO antibodies within 3 days, but mice that received 1×10^7 splenocytes showed a substantially lower anti-MPO antibody titer than did mice that received 5×10^7 or 1×10^8 splenocytes. Mice that received 5×10^7 or 1×10^8 splenocytes showed severe renal failure, as demonstrated by increased blood urea nitrogen and serum creatinine 13 days after receiving the splenocytes. In addition, these mice all developed severe necrotizing and crescentic glomerulonephritis. In contrast, in control mice and in mice that received 1×10^7 splenocytes, no renal failure or crescent formation was detected, although necrosis was seen occasionally. In all $Rag2^{-/-}$ mice receiving control or anti-MPO splenocytes, glomerular deposits of IgG were found. In some $Rag2^{-/-}$ mice that received 5×10^7 or 1×10^8 splenocytes, vasculitis or granulomatous inflammation in other organs (*e.g.*, spleen and lungs) was observed, whereas this was never observed in mice that received 1×10^7 anti-MPO splenocytes or control splenocytes. These results suggested that anti-MPO antibodies can cause systemic vasculitis and glomerulonephritis, provided that the antibody titer is high enough.

To confirm that the anti-MPO antibodies alone were indeed pathogenic, total IgG was isolated from mMPO-immunized $Mpo^{-/-}$ mice, and the purified IgG was passively transferred to both $Rag2^{-/-}$ and wild-type mice. With this strategy, the contribution of anti-MPO antibodies to the vasculitic lesions could be separated from potential effects of anti-MPO B and T cells. Circulating anti-MPO antibodies could still be detected in the mice several days after passive transfer, with titers gradually decreasing over time. Mice were killed after 6 days and, although the serum creatinine was not increased, they developed hematuria, proteinuria, and leukocyturia. In addition, they displayed focal glomerulonephritis with necrosis and crescent formation. Mice that had received control IgG did not demonstrate any abnormalities. Only limited amounts of immunoglobulins were detected in glomeruli,

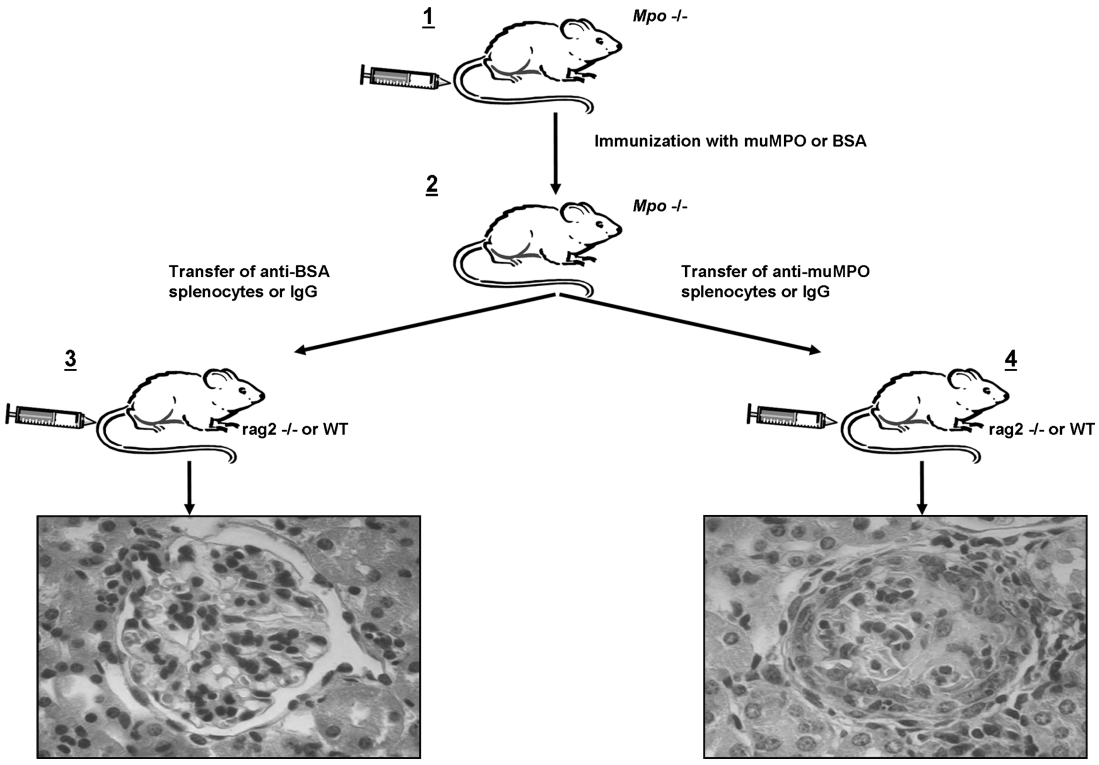

FIG. 13. Strategy for the development of a mouse model of anti-MPO–mediated glomerulonephritis/vasculitis. (**1**) MPO-deficient mice ($Mpo^{-/-}$) are immunized with murine MPO (mMPO) or control antigen [bovine serum albumin (BSA)]. (**2**) Splenocytes and IgG are obtained from immunized $Mpo^{-/-}$ mice. (**3**) Adoptive transfer of anti-BSA splenocytes into immune-deficient recombinase-activating gene–deficient ($Rag2^{-/-}$) or passive transfer of anti-BSA IgG into $Rag2^{-/-}$ or C57Bl/6 mice induces no disease. (**4**) Adoptive transfer of anti-mMPO splenocytes into $Rag2^{-/-}$ or passive transfer of anti-mMPO IgG into $Rag2^{-/-}$ or wild-type mice induces vasculitis and necrotizing crescentic glomerulonephritis. Glomerular lesions are presented as observed 6 days after passive transfer into C57Bl/6 mice of anti-BSA IgG and anti-MPO IgG, respectively. PAS staining, x400. Reproduced from (130).

which is in agreement with the pauci-immune nature of the glomerular capillary lesions observed in human ANCA-associated glomerulonephritis. In addition to renal abnormalities, vasculitic lesions with striking similarity to lesions in ANCA-SVV patients were occasionally found in lungs and ears. The passive-transfer studies showed that anti-MPO IgG can cause vasculitis and glomerulonephritis, not only in the absence of anti-MPO effector cells ($Rag2^{-/-}$ mice), but also in the presence of a fully functional immune system (wild-type mice). Additional studies on this passive-transfer model revealed essential roles for neutrophils and the alternative complement pathway in anti-MPO–mediated vasculitis and glomerulonephritis (139, 375, 376). Furthermore, the effect of an inflammatory stimulus (*i.e.*, LPS) on the pathogenic process was tested in this model (140). Administration of LPS, shortly after anti-MPO IgG, aggravated renal-function loss and pathologic features of glomerulonephritis considerably.

Another important *in vivo* study confirmed the *in vitro* finding that MPO-ANCAs directly enhance the interactions between primed neutrophils and activated endothelial cells. Little *et al.* (206) used an intravital microscopy technique to study the behavior of leukocytes in the mesenteric venules and found increased adherence and transmigration activities toward the endothelium. They immunized Wistar-Kyoto rats with human MPO, which caused the rats to produce anti-MPO antibodies that cross-reacted with rat MPO. The anti-MPO antibodies were able to recognize both human and rat MPO, as shown by indirect staining of neutrophils. Antibodies isolated from control immunized rats did not stain neutrophils of either species. As a result of the circulating anti-MPO antibodies, the rats developed focal necrotizing crescentic glomerulonephritis and pulmonary hemorrhage. The glomerulonephritis had a pauci-immune character, because only scanty IgG deposits were found. Besides histologic abnormalities, MPO-immunized rats also had impaired renal function, as shown by the occurrence of hematuria and albuminuria. The control immunized rats did not show any renal or pulmonary abnormalities. When Little *et al.* (206) investigated the number of adherent and transmigrated cells in these animals by using intravital microscopy, they found an increased number of adherent cells in rats that were immunized with MPO compared with rats immunized with albumin. After topical administration of the chemokine CXCL1, the increase in number of adherent cells was even higher, and moreover, an increased number of cells had transmigrated. Furthermore, Little *et al.* performed passive-transfer experiments comparable to the experiments of Xiao *et al.* described earlier, in which they transferred IgG purified from immunized rats to naive rats. Intravital microscopy analysis of these rats revealed that comparable leukocyte responses were induced on anti-MPO IgGs that were introduced by passive transfer and that were raised by active immunization. Passive

transfer of anti-MPO IgG into rats induced increased adhesion of leukocytes to the vessel wall, an effect that was increased on topical administration of CXCL1. CXCL1 also caused significantly more transmigrating cells in rats receiving anti-MPO IgG than in rats receiving control IgG. Besides direct leukocyte–endothelial interactions, vascular injury was observed, as evidenced by the increased occurrence of hemorrhage in the postcapillary venules of the mesenteric vasculature in the animals that had received anti-MPO IgG, by either active immunization or passive transfer. With this study, Little *et al.* (206) were the first to show that anti-MPO IgG induces increased leukocyte–vessel wall interactions and leukocyte-mediated vascular damage *in vivo*.

A recent study by Nolan *et al.* (260) confirmed the concept of increased leukocyte–vessel wall interactions in the autologous mouse model originally described by Xiao *et al.* (260). By following a procedure similar to that of Little *et al.*, this study used intravital microscopy to examine leukocyte–endothelium interactions in the cremasteric microvessels. The authors observed less rolling but more adhesive and transmigrating leukocytes with anti-MPO IgG perfusion. However, these effects of anti-MPO IgG were seen only when the cremaster muscle was pretreated with a cytokine (preferably TNF-α or CXCL1). Interestingly, increased recruitment of MPO-positive leukocytes was found in renal and pulmonary tissue, suggesting that primed neutrophils and anti-MPO antibodies together can exert systemic effects and affect specific vascular beds. In addition, the authors showed that the anti-MPO effects were completely lost when either β_2-integrins or Fcγ-receptors were blocked, demonstrating β_2-integrin and FcγR involvement in the pathogenesis of anti-MPO mediated SVV *in vivo* (260).

In conclusion, autoantibodies directed against MPO are pathogenic through activation of neutrophils in an FcγR- and β_2-integrin–dependent fashion, resulting in vasculitis in specific microvascular beds.

V. MPO Detection in Tissues and Biologic Fluids

From the data reviewed here, it is clear that MPO is an important player in the pathophysiology of many diseases characterized by acute and chronic inflammation. At least in some of these disorders, most notably in atherosclerosis, measurement of MPO levels appears to have diagnostic and even prognostic value, and an increased interest exists in tools and assays to monitor accurately MPO levels and activity in experimental and clinical settings.

In experimental inflammation research, detection of active MPO, often combined with immunohistochemical staining of the protein, is a widely used marker to monitor the extent of neutrophil and monocyte influx in tissues. In principle, any peroxidase assay can be used for this purpose, but, in most cases, colorimetric assays that rely on the enzymatic activity of MPO are used with substrates such as 3,3′,5,5′-tetramethylbenzidine (TMB) and guaiacol (215). However, such assays have their limitations with respect to sensitivity and specificity, mainly because the substrates are not MPO specific, and contamination of biologic samples with other peroxidases or peroxidase inhibitors frequently occurs.

An alternative approach to measure the activity and involvement of the MPO-H_2O_2-Cl^- system is to determine the consequences of its end product (*i.e.*, HOCl). For this purpose, monoclonal antibodies specific for HOCl-modified epitopes on proteins have been generated and applied as an indicator of HOCL-mediated tissue damage in various pathologies (216). The most prominent example is atherosclerosis, in which these antibodies revealed the abundant presence of HOCl-modified proteins in human and animal atherosclerotic lesions (123, 216, 217). Especially in combination with immunohistochemistry for MPO itself, staining of HOCl-modified proteins in tissues is a powerful approach, allowing the detection and localization not only of the protein but also of the consequences of HOCl-induced injury (217). Obviously, the application of antibodies directed against HOCl-modified epitopes requires rigorous testing of the specificity of the stainings and ideally should be combined with additional techniques to validate the presence of HOCl-modified proteins.

Another analysis technique that is frequently used to detect HOCl modifications on proteins and lipids is gas chromatography followed by mass spectrometry (277). With this technique, HOCl-induced oxidative modifications can be readily detected, but many of these are not HOCl specific and can also be formed by free radicals (277). One exception is the reaction of HOCl with tyrosine, leading to the formation of 3-chlorotyrosine, which is HOCl specific and relatively stable (82, 369). Free 3-chlorotyrosine is considered to be a biomarker for MPO activity, and increased levels have been detected in a number of inflammatory conditions (82, 237, 368). Besides methods based on enzyme activity, MPO protein levels in biologic fluids and tissues can be determined by enzyme-linked immunosorbent assays (ELISA) with MPO-specific monoclonal or polyclonal antibodies. The advantage of ELISA systems lies in the fact that these assays are highly specific for MPO and are not influenced by enzyme activity or inhibitors. Moreover, ELISA assays are very sensitive, usually allowing protein detection in the nanogram to picogram range, and at present, a number of ELISA kits specific for human, mouse, and rat MPO are commercially available.

More recent developments in MPO detection focused on imaging technologies to noninvasively image MPO activity as a means to monitor the course of inflammatory processes *in vivo*. To date, a variety of activatible agents have been used to visualize MPO by means of magnetic resonance imaging (MRI) (45, 65, 66, 243), single-photon emission computed tomography (282), fluorescence (321), and bioluminescence imaging (BLI) (109). Nahrendorf and colleagues (243) showed that an MPO-activatible gadolinium chelate, a clinically used MRI agent, can be used to image MPO activity directly in the hearts of mice subjected to I/R. Gadolinium chelate is activated by MPO through the oxidation of the hydroxytryptamide moieties on the chelate. These MPO-mediated modifications cause polymerization of the agent that can cross-link to matrix proteins promoting the accumulation of the probe in areas of high MPO activity. In these studies, the MPO-activatible probe was successfully applied to monitor the course of the inflammatory response after myocardial I/R injury and the antiinflammatory effects of statins. Furthermore, MRI imaging of MPO correlated well with immunohistochemical detection and tissue activity of MPO, whereas no signal was detected in MPO-deficient mice, confirming the specificity of the probe (243). In addition, these MRI MPO-imaging agents have also been used to image and track MPO activity in mouse models of stroke (66) and multiple sclerosis (65) demonstrating the broad applicability of this technique.

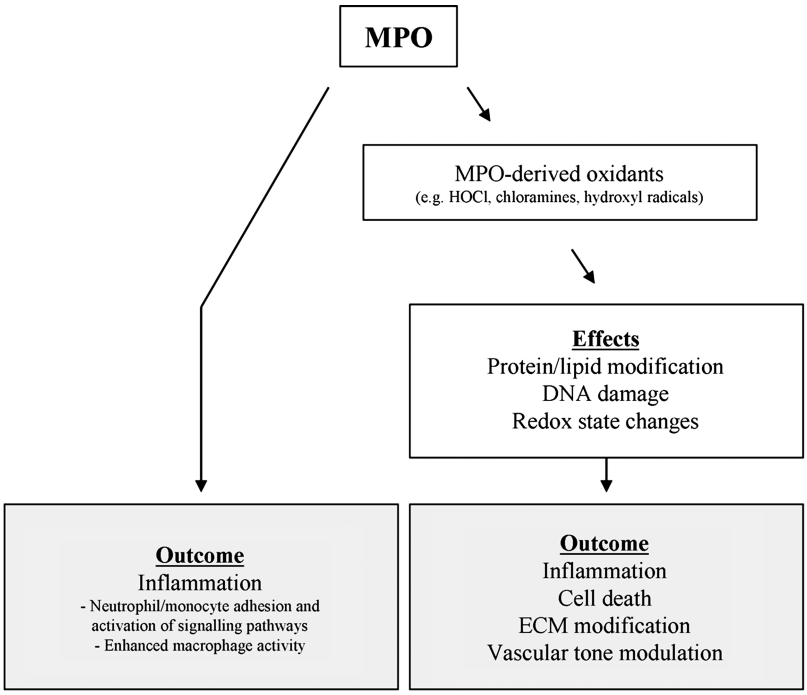

FIG. 14. Schematic representation of direct and indirect (*via* oxidants) effects of MPO, the consequences of which may contribute to diseases.

A recent study by Gross *et al.* (103) demonstrated the potential use of luminol for noninvasive BLI of MPO activity *in vivo* (109). The highly stable, redox-sensitive compound luminol is a widely used reagent to detect reactive oxygen intermediates, which, on oxidation, emits blue luminescence. Because luminol can react with a variety of ROS, it seems unlikely at first glance that this compound can be used as a specific imaging agent for myeloperoxidase. However, rigorous *in vitro* testing by Gross *et al.* by using blood samples, isolated neutrophils, and cell-free systems in combination with the MPO-specific inhibitor 4-aminobenzoic acid hydrazide (4-ABAH), convincingly demonstrated that luminol bioluminescence is dependent on MPO activity. Subsequently, two mouse models of acute inflammation were tested to determine whether luminol could be used noninvasively to monitor MPO activity. The results showed that systemic administration of luminol enabled BLI of MPO activity in inflammatory lesions in experimental acute dermatitis and mixed allergic contact hypersensitivity in a longitudinal and quantitative fashion. Furthermore, additional experiments in MPO-deficient mice demonstrated that the luminol-BLI is dependent on MPO activity.

Although it is still in the experimental stage, the obvious advantage of MPO-specific imaging approaches is that they provide us with imaging tools to detect MPO activity noninvasively at the actual sites of inflammation in time. When these methods become available for humans, these will be very helpful for diagnostic purposes and for determining the effects of antiinflammatory treatments.

VI. Conclusions

From the data reviewed, it is evident that MPO and its derived oxidant species contribute to both health and disease. In health, MPO-catalyzed formation of highly reactive halide-derived oxidants is an important component of the armament of neutrophils and monocytes needed to kill and digest phagocytosed pathogens. In diseases characterized by increased inflammation and oxidant stress, properties of MPO independent of its enzymatic activity, as well as MPO-derived oxidants, appear to participate in a range of events that contribute to the initiation and propagation of the inflammatory response and, as such, are involved in tissue pathology (schematically summarized in Fig. 14). These effects suggest that MPO-targeted strategies may be of therapeutic benefit (215). At present, 4-ABAH is the only known specific MPO inhibitor, as has been demonstrated mainly in *in vitro* studies, but the *in vivo* efficacy of this inhibitor is less clear (53, 167, 168). At first glance, the development of MPO-specific inhibitors for *in vivo* use is potentially difficult, given the beneficial role of the MPO system in pathogen killing. However, the rather large variation of MPO levels in the healthy population and the relatively mild phenotype of fully or partially MPO-deficient individuals suggest that a therapeutic window must exist in which MPO levels can be maintained for host defense. It will thus be interesting to see whether MPO-specific inhibitors can be designed and how these will affect the development of inflammatory diseases. Given our increased knowledge of MPO protein structure and reaction mechanisms, this research area is expected to advance rapidly.

Acknowledgments

P. Heeringa and M.P.J. de Winther are supported by the Dutch Organization of Scientific Research (NWO VIDI 917.66.341 (P.H.) and 917.66.329 (M.P.J.W). M.P.J.W. is an established investigator of the Netherlands Heart Foundation (2007T067).

References

1. Abu-Soud HM and Hazen SL. Nitric oxide modulates the catalytic activity of myeloperoxidase. *J Biol Chem* 275: 5425–5430, 2000.

2. Agner K. Verdoperoxidase: a ferment isolated from leucocytes. *Acta Chem Scand A* 2: 1–62, 1941.
3. Agner K. Crystalline myeloperoxidase. *Acta Chem Scand A* 12: 89–94, 1958.
4. Ahn J, Gammon MD, Santella RM, Gaudet MM, Britton JA, Teitelbaum SL, Terry MB, Neugut AI, Josephy PD, and Ambrosone CB. Myeloperoxidase genotype, fruit and vegetable consumption, and breast cancer risk. *Cancer Res* 64: 7634–7639, 2004.
5. Akin DT and Kinkade JM Jr. Processing of a newly identified intermediate of human myeloperoxidase in isolated granules occurs at neutral pH. *J Biol Chem* 261: 8370–8375, 1986.
6. Al-Shebeb T, Frohlich J, and Magil AB. Glomerular disease in hypercholesterolemic guinea pigs: a pathogenetic study. *Kidney Int* 33: 498–507, 1988.
7. Amin VM and Olson NF. Influence of catalase activity on resistance of coagulase-positive staphylococci to hydrogen peroxide. *Appl Microbiol* 16: 267–270, 1968.
8. Amin VM and Olson NF. Selective increase in hydrogen peroxide resistance of a coagulase-positive staphylococcus. *J Bacteriol* 95: 1604–1607, 1968.
9. Anderson MM, Requena JR, Crowley JR, Thorpe SR, and Heinecke JW. The myeloperoxidase system of human phagocytes generates N-epsilon-(carboxymethyl)lysine on proteins: a mechanism for producing advanced glycation end products at sites of inflammation. *J Clin Invest* 104: 103–113, 1999.
10. Andrews PC and Krinsky NI. The reductive cleavage of myeloperoxidase in half, producing enzymically active hemi-myeloperoxidase. *J Biol Chem* 256: 4211–4218, 1981.
11. Aratani Y, Koyama H, Nyui S, Suzuki K, Kura F, and Maeda N. Severe impairment in early host defense against *Candida albicans* in mice deficient in myeloperoxidase. *Infect Immun* 67: 1828–1836, 1999.
12. Aratani Y, Kura F, Watanabe H, Akagawa H, Takano Y, Ishida-Okawara A, Suzuki K, Maeda N, and Koyama H. Contribution of the myeloperoxidase-dependent oxidative system to host defence against *Cryptococcus neoformans*. *J Med Microbiol* 55: 1291–1299, 2006.
13. Aratani Y, Kura F, Watanabe H, Akagawa H, Takano Y, Suzuki K, Dinauer MC, Maeda N, and Koyama H. Critical role of myeloperoxidase and nicotinamide adenine dinucleotide phosphate-oxidase in high-burden systemic infection of mice with *Candida albicans*. *J Infect Dis* 185: 1833–1837, 2002.
14. Aratani Y, Kura F, Watanabe H, Akagawa H, Takano Y, Suzuki K, Maeda N, and Koyama H. Differential host susceptibility to pulmonary infections with bacteria and fungi in mice deficient in myeloperoxidase. *J Infect Dis* 182: 1276–1279, 2000.
15. Arisawa F, Tatsuzawa H, Kambayashi Y, Kuwano H, Fujimori K, and Nakano M. MCLA-dependent chemiluminescence suggests that singlet oxygen plays a pivotal role in myeloperoxidase-catalysed bactericidal action in neutrophil phagosomes. *Luminescence* 18: 229–238, 2003.
16. Asaba K, Tojo A, Onozato ML, Goto A, and Fujita T. Double-edged action of SOD mimetic in diabetic nephropathy. *J Cardiovasc Pharmacol* 49: 13–19, 2007.
17. Asaba K, Tojo A, Onozato ML, Goto A, Quinn MT, Fujita T, and Wilcox CS. Effects of NADPH oxidase inhibitor in diabetic nephropathy. *Kidney Int* 67: 1890–1898, 2005.
18. Asaga T, Ueki M, Chujo K, and Taie S. JTE-607, an inflammatory cytokine synthesis inhibitor, attenuates ischemia/reperfusion-induced renal injury by reducing neutrophil activation in rats. *J Biosci Bioeng* 106: 22–26, 2008.
19. Askari AT, Brennan ML, Zhou X, Drinko J, Morehead A, Thomas JD, Topol EJ, Hazen SL, and Penn MS. Myeloperoxidase and plasminogen activator inhibitor 1 play a central role in ventricular remodeling after myocardial infarction. *J Exp Med* 197: 615–624, 2003.
20. Asselbergs FW, Reynolds WF, Cohen-Tervaert JW, Jessurun GA, and Tio RA. Myeloperoxidase polymorphism related to cardiovascular events in coronary artery disease. *Am J Med* 116: 429–430, 2004.
21. Astern JM, Pendergraft WF 3rd, Falk RJ, Jennette JC, Schmaier AH, Mahdi F, and Preston GA. Myeloperoxidase interacts with endothelial cell-surface cytokeratin 1 and modulates bradykinin production by the plasma kallikrein-kinin system. *Am J Pathol* 171: 349–360, 2007.
22. Augustin AJ, Breipohl W, Boker T, Lutz J, and Spitznas M. Increased lipid peroxide levels and myeloperoxidase activity in the vitreous of patients suffering from proliferative diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol* 231: 647–650, 1993.
23. Augusto O, Bonini MG, Amanso AM, Linares E, Santos CC, and De Menezes SL. Nitrogen dioxide and carbonate radical anion: two emerging radicals in biology. *Free Radic Biol Med* 32: 841–859, 2002.
24. Augusto O, Trindade DF, Linares E, and Vaz SM. Cyclic nitroxides inhibit the toxicity of nitric oxide-derived oxidants: mechanisms and implications. *An Acad Bras Cienc* 80: 179–189, 2008.
25. Austin GE, Chan WC, Zhao W, and Racine M. Myeloperoxidase gene expression in normal granulopoiesis and acute leukemias. *Leuk Lymphoma* 15: 209–226, 1994.
26. Austin GE, Lam L, Zaki SR, Chan WC, Hodge T, Hou J, Swan D, Zhang W, Racine M, Whitsett C, and Brown T. Sequence comparison of putative regulatory DNA of the 5′ flanking region of the myeloperoxidase gene in normal and leukemic bone marrow cells. *Leukemia* 7: 1445–1450, 1993.
27. Austin GE, Zhao WG, Regmi A, Lu JP, and Braun J. Identification of an upstream enhancer containing an AML1 site in the human myeloperoxidase (MPO) gene. *Leuk Res* 22: 1037–1048, 1998.
28. Austin GE, Zhao WG, Zhang W, Austin ED, Findley HW, and Murtagh JJ Jr. Identification and characterization of the human myeloperoxidase promoter. *Leukemia* 9: 848–857, 1995.
29. Babior BM. NADPH oxidase. *Curr Opin Immunol* 16: 42–47, 2004.
30. Babior BM, Lambeth JD, and Nauseef W. The neutrophil NADPH oxidase. *Arch Biochem Biophys* 397: 342–344, 2002.
31. Babior BM, Takeuchi C, Ruedi J, Gutierrez A, and Wentworth P, Jr. Investigating antibody-catalyzed ozone generation by human neutrophils. *Proc Natl Acad Sci U S A* 100: 3031–3034, 2003.
32. Baldus S, Eiserich JP, Mani A, Castro L, Figueroa M, Chumley P, Ma W, Tousson A, White CR, Bullard DC, Brennan ML, Lusis AJ, Moore KP, and Freeman BA. Endothelial transcytosis of myeloperoxidase confers specificity to vascular ECM proteins as targets of tyrosine nitration. *J Clin Invest* 108: 1759–1770, 2001.
33. Baldus S, Heeschen C, Meinertz T, Zeiher AM, Eiserich JP, Munzel T, Simoons ML, and Hamm CW. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. *Circulation* 108: 1440–1445, 2003.

34. Baldus S, Heitzer T, Eiserich JP, Lau D, Mollnau H, Ortak M, Petri S, Goldmann B, Duchstein HJ, Berger J, Helmchen U, Freeman BA, Meinertz T, and Munzel T. Myeloperoxidase enhances nitric oxide catabolism during myocardial ischemia and reperfusion. *Free Radic Biol Med* 37: 902–911, 2004.
35. Ballieux BE, Zondervan KT, Kievit P, Hagen EC, van Es LA, van der Woude FJ, and Daha MR. Binding of proteinase 3 and myeloperoxidase to endothelial cells: ANCA-mediated endothelial damage through ADCC? *Clin Exp Immunol* 97: 52–60, 1994.
36. Bansal PJ and Tobin MC. Neonatal microscopic polyangiitis secondary to transfer of maternal myeloperoxidase-antineutrophil cytoplasmic antibody resulting in neonatal pulmonary hemorrhage and renal involvement. *Ann Allergy Asthma Immunol* 93: 398–401, 2004.
37. Barry MC, Kelly CJ, Abdih H, Watson RW, Stapleton P, Sheehan SJ, Redmond HP, and Hayes DB. Differential effects of lower limb revascularisation on organ injury and the role of the amino acid taurine. *Eur J Vasc Endovasc Surg* 13: 193–201, 1997.
38. Ben Smith A, Dove SK, Martin A, Wakelam MJ, and Savage CO. Antineutrophil cytoplasm autoantibodies from patients with systemic vasculitis activate neutrophils through distinct signaling cascades: comparison with conventional Fcgamma receptor ligation. *Blood* 98: 1448–1455, 2001.
39. Bennouna S, Bliss SK, Curiel TJ, and Denkers EY. Cross-talk in the innate immune system: neutrophils instruct recruitment and activation of dendritic cells during microbial infection. *J Immunol* 171: 6052–6058, 2003.
40. Blair-Johnson M, Fiedler T, and Fenna R. Human myeloperoxidase: structure of a cyanide complex and its interaction with bromide and thiocyanate substrates at 1.9 A resolution. *Biochemistry* 40: 13990–13997, 2001.
41. Bohlender JM, Franke S, Stein G, and Wolf G. Advanced glycation end products and the kidney. *Am J Physiol Renal Physiol* 289: F645–F659, 2005.
42. Borregaard N and Cowland JB. Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood* 89: 3503–3521, 1997.
43. Borregaard N, Sorensen OE, and Theilgaard-Monch K. Neutrophil granules: a library of innate immunity proteins. *Trends Immunol* 28: 340–345, 2007.
44. Bos AJ, Weening RS, Hamers MN, Wever R, Behrendt H, and Roos D. Characterization of hereditary partial myeloperoxidase deficiency. *J Lab Clin Med* 99: 589–600, 1982.
45. Breckwoldt MO, Chen JW, Stangenberg L, Aikawa E, Rodriguez E, Qiu S, Moskowitz MA, and Weissleder R. Tracking the inflammatory response in stroke in vivo by sensing the enzyme myeloperoxidase. *Proc Natl Acad Sci U S A* 105: 18584–18589, 2008.
46. Brennan M, Gaur A, Pahuja A, Lusis AJ, and Reynolds WF. Mice lacking myeloperoxidase are more susceptible to experimental autoimmune encephalomyelitis. *J Neuroimmunol* 112: 97–105, 2001.
47. Brennan ML, Anderson MM, Shih DM, Qu XD, Wang X, Mehta AC, Lim LL, Shi W, Hazen SL, Jacob JS, Crowley JR, Heinecke JW, and Lusis AJ. Increased atherosclerosis in myeloperoxidase-deficient mice. *J Clin Invest* 107: 419–430, 2001.
48. Brennan ML, Penn MS, Van Lente F, Nambi V, Shishehbor MH, Aviles RJ, Goormastic M, Pepoy ML, McErlean ES, Topol EJ, Nissen SE, and Hazen SL. Prognostic value of myeloperoxidase in patients with chest pain. *N Engl J Med* 349: 1595–1604, 2003.
49. Brouwer E, Huitema MG, Klok PA, de Weerd H, Tervaert JW, Weening JJ, and Kallenberg CG. Antimyeloperoxidase-associated proliferative glomerulonephritis: an animal model. *J Exp Med* 177: 905–914, 1993.
50. Brovkovych V, Gao XP, Ong E, Brovkovych S, Brennan ML, Su X, Hazen SL, Malik AB, and Skidgel RA. Augmented inducible nitric oxide synthase expression and increased NO production reduce sepsis-induced lung injury and mortality in myeloperoxidase-null mice. *Am J Physiol Lung Cell Mol Physiol* 295: L96–L103, 2008.
51. Brown KE, Brunt EM, and Heinecke JW. Immunohistochemical detection of myeloperoxidase and its oxidation products in Kupffer cells of human liver. *Am J Pathol* 159: 2081–2088, 2001.
52. Buraczynska K, Koziol-Montewka M, Majdan M, Tokarz A, and Ksiazek A. Genetic determination of TNF and myeloperoxidase production in dialyzed patients with diabetic nephropathy. *Ren Fail* 26: 633–639, 2004.
53. Burner U, Obinger C, Paumann M, Furtmuller PG, and Kettle AJ. Transient and steady-state kinetics of the oxidation of substituted benzoic acid hydrazides by myeloperoxidase. *J Biol Chem* 274: 9494–9502, 1999.
54. Cadet J, Sage E, and Douki T. Ultraviolet radiation-mediated damage to cellular DNA. *Mutat Res* 571: 3–17, 2005.
55. Calderwood JW, Williams JM, Morgan MD, Nash GB, and Savage CO. ANCA induces beta2 integrin and CXC chemokine-dependent neutrophil-endothelial cell interactions that mimic those of highly cytokine-activated endothelium. *J Leukoc Biol* 77: 33–43, 2005.
56. Candeias LP, Patel KB, Stratford MR, and Wardman P. Free hydroxyl radicals are formed on reaction between the neutrophil-derived species superoxide anion and hypochlorous acid. *FEBS Lett* 333: 151–153, 1993.
57. Cantin A, Bilodeau G, and Begin R. Granulocyte elastase-mediated proteolysis of alpha 1-antitrypsin in cystic fibrosis bronchopulmonary secretions. *Pediatr Pulmonol* 7: 12–17, 1989.
58. Cascorbi I, Henning S, Brockmoller J, Gephart J, Meisel C, Muller JM, Loddenkemper R, and Roots I. Substantially reduced risk of cancer of the aerodigestive tract in subjects with variant −463A of the myeloperoxidase gene. *Cancer Res* 60: 644–649, 2000.
59. Castellani LW, Chang JJ, Wang X, Lusis AJ, and Reynolds WF. Transgenic mice express human MPO −463G/A alleles at atherosclerotic lesions, developing hyperlipidemia and obesity in −463G males. *J Lipid Res* 47: 1366–1377, 2006.
60. Castro R, Piazzon MC, Noya M, Leiro JM, and Lamas J. Isolation and molecular cloning of a fish myeloperoxidase. *Mol Immunol* 45: 428–437, 2008.
61. Chang KS, Trujillo JM, Cook RG, and Stass SA. Human myeloperoxidase gene: molecular cloning and expression in leukemic cells. *Blood* 68: 1411–1414, 1986.
62. Charles LA, Falk RJ, and Jennette JC. Reactivity of antineutrophil cytoplasmic autoantibodies with mononuclear phagocytes. *J Leukoc Biol* 51: 65–68, 1992.
63. Chatterjee PK, Kvale EO, Patel NS, and Thiemermann C. GW274150 inhibits nitric oxide production by primary cultures of rat proximal tubular cells. *Med Sci Monit* 9: BR357–BR362, 2003.
64. Chatterjee PK, Patel NS, Kvale EO, Cuzzocrea S, Brown PA, Stewart KN, Mota-Filipe H, and Thiemermann C.

Inhibition of inducible nitric oxide synthase reduces renal ischemia/reperfusion injury. *Kidney Int* 61: 862–871, 2002.
65. Chen JW, Breckwoldt MO, Aikawa E, Chiang G, and Weissleder R. Myeloperoxidase-targeted imaging of active inflammatory lesions in murine experimental autoimmune encephalomyelitis. *Brain* 131: 1123–1133, 2008.
66. Chen JW, Querol Sans M, Bogdanov A Jr, and Weissleder R. Imaging of myeloperoxidase in mice by using novel amplifiable paramagnetic substrates. *Radiology* 240: 473–481, 2006.
67. Chertov O, Ueda H, Xu LL, Tani K, Murphy WJ, Wang JM, Howard OM, Sayers TJ, and Oppenheim JJ. Identification of human neutrophil-derived cathepsin G and azurocidin/CAP37 as chemoattractants for mononuclear cells and neutrophils. *J Exp Med* 186: 739–747, 1997.
68. Chevrier I, Stucker I, Houllier AM, Cenee S, Beaune P, Laurent-Puig P, and Loriot MA. Myeloperoxidase: new polymorphisms and relation with lung cancer risk. *Pharmacogenetics* 13: 729–739, 2003.
69. Chevrier I, Tregouet DA, Massonnet-Castel S, Beaune P, and Loriot MA. Myeloperoxidase genetic polymorphisms modulate human neutrophil enzyme activity: genetic determinants for atherosclerosis? *Atherosclerosis* 188: 150–154, 2006.
70. Choi DK, Pennathur S, Perier C, Tieu K, Teismann P, Wu DC, Jackson-Lewis V, Vila M, Vonsattel JP, Heinecke JW, and Przedborski S. Ablation of the inflammatory enzyme myeloperoxidase mitigates features of Parkinson's disease in mice. *J Neurosci* 25: 6594–6600, 2005.
71. Cohen MS, Britigan BE, Hassett DJ, and Rosen GM. Do human neutrophils form hydroxyl radical? Evaluation of an unresolved controversy. *Free Radic Biol Med* 5: 81–88, 1988.
72. Combarros O, Infante J, Llorca J, Pena N, Fernandez-Viadero C, and Berciano J. The myeloperoxidase gene in Alzheimer's disease: a case-control study and meta-analysis. *Neurosci Lett* 326: 33–36, 2002.
73. Cowland JB and Borregaard N. The individual regulation of granule protein mRNA levels during neutrophil maturation explains the heterogeneity of neutrophil granules. *J Leukoc Biol* 66: 989–995, 1999.
74. Cramer R, Soranzo MR, Dri P, Rottini GD, Bramezza M, Cirielli S, and Patriarca P. Incidence of myeloperoxidase deficiency in an area of northern Italy: histochemical, biochemical and functional studies. *Br J Haematol* 51: 81–87, 1982.
75. Dale DC, Boxer L, and Liles WC. The phagocytes: neutrophils and monocytes. *Blood* 112: 935–945, 2008.
76. Daugherty A, Dunn JL, Rateri DL, and Heinecke JW. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J Clin Invest* 94: 437–444, 1994.
77. Davies DJ, Moran JE, Niall JF, and Ryan GB. Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *Br Med J (Clin Res Ed)* 285: 606, 1982.
78. Davies MJ. Reactive species formed on proteins exposed to singlet oxygen. *Photochem Photobiol Sci* 3: 17–25, 2004.
79. Davies MJ, Hawkins CL, Pattison DI, and Rees MD. Mammalian heme peroxidases: from molecular mechanisms to health implications. *Antioxid Redox Signal* 10: 1199–1234, 2008.
80. de Vries B, Kohl J, Leclercq WK, Wolfs TG, van Bijnen AA, Heeringa P, and Buurman WA. Complement factor C5a mediates renal ischemia-reperfusion injury independent from neutrophils. *J Immunol* 170: 3883–3889, 2003.
81. DeLeo FR, Goedken M, McCormick SJ, and Nauseef WM. A novel form of hereditary myeloperoxidase deficiency linked to endoplasmic reticulum/proteasome degradation. *J Clin Invest* 101: 2900–2909, 1998.
82. Domigan NM, Charlton TS, Duncan MW, Winterbourn CC, and Kettle AJ. Chlorination of tyrosyl residues in peptides by myeloperoxidase and human neutrophils. *J Biol Chem* 270: 16542–16558, 1995.
83. Edwards SW, Hughes V, Barlow J, and Bucknall R. Immunological detection of myeloperoxidase in synovial fluid from patients with rheumatoid arthritis. *Biochem J* 250: 81–85, 1988.
84. Eiserich JP, Baldus S, Brennan ML, Ma W, Zhang C, Tousson A, Castro L, Lusis AJ, Nauseef WM, White CR, and Freeman BA. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. *Science* 296: 2391–2394, 2002.
85. Eiserich JP, Hristova M, Cross CE, Jones AD, Freeman BA, Halliwell B, and van der Vliet A. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature* 391: 393–397, 1998.
86. el-Hag A and Clark RA. Immunosuppression by activated human neutrophils: dependence on the myeloperoxidase system. *J Immunol* 139: 2406–2413, 1987.
87. el-Hag A, Lipsky PE, Bennett M, and Clark RA. Immunomodulation by neutrophil myeloperoxidase and hydrogen peroxide: differential susceptibility of human lymphocyte functions. *J Immunol* 136: 3420–3426, 1986.
88. El Kebir D, Jozsef L, Pan W, and Filep JG. Myeloperoxidase delays neutrophil apoptosis through CD11b/CD18 integrins and prolongs inflammation. *Circ Res* 103: 352–359, 2008.
89. Ewert BH, Jennette JC, and Falk RJ. Anti-myeloperoxidase antibodies stimulate neutrophils to damage human endothelial cells. *Kidney Int* 41: 375–383, 1992.
90. Falk RJ and Jennette JC. Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N Engl J Med* 318: 1651–1657, 1988.
91. Farrell AJ, Blake DR, Palmer RM, and Moncada S. Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. *Ann Rheum Dis* 51: 1219–1222, 1992.
92. Faurschou M and Borregaard N. Neutrophil granules and secretory vesicles in inflammation. *Microbes Infect* 5: 1317–1327, 2003.
93. Fenna RE. Crystallization and subunit structure of canine myeloperoxidase. *J Mol Biol* 196: 919–925, 1987.
94. Fiebeler A, Borgmann S, Woywodt A, Haller H, and Haubitz M. No association of G-463A myeloperoxidase gene polymorphism with MPO-ANCA-associated vasculitis. *Nephrol Dial Transplant* 19: 969–971, 2004.
95. Fiedler TJ, Davey CA, and Fenna RE. X-ray crystal structure and characterization of halide-binding sites of human myeloperoxidase at 1.8 A resolution. *J Biol Chem* 275: 11964–11971, 2000.
96. Fliss H. Oxidation of proteins in rat heart and lungs by polymorphonuclear leukocyte oxidants. *Mol Cell Biochem* 84: 177–188, 1988.
97. Fornasieri A, Perugini C, Seccia M, Napodano P, D'Amico G, and Bellomo G. Circulating antibodies recognising oxidatively modified low-density lipoproteins in patients with

IgA nephropathy, membranous glomerulonephritis and focal glomerulosclerosis. *J Nephrol* 15: 349–357, 2002.

98. Foucher P, Heeringa P, Petersen AH, Huitema MG, Brouwer E, Tervaert JW, Prop J, Camus P, Weening JJ, and Kallenberg CG. Antimyeloperoxidase-associated lung disease: an experimental model. *Am J Respir Crit Care Med* 160: 987–994, 1999.
99. Furtmuller PG, Zederbauer M, Jantschko W, Helm J, Bogner M, Jakopitsch C, and Obinger C. Active site structure and catalytic mechanisms of human peroxidases. *Arch Biochem Biophys* 445: 199–213, 2006.
100. Garner HP, Phillips JR, Herron JG, Severson SJ, Milla CE, and Regelmann WE. Peroxidase activity within circulating neutrophils correlates with pulmonary phenotype in cystic fibrosis. *J Lab Clin Med* 144: 127–133, 2004.
101. Gaston B, Reilly J, Drazen JM, Fackler J, Ramdev P, Arnelle D, Mullins ME, Sugarbaker DJ, Chee C, Singel DJ, Loscalzo J, and Stamler JS. Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways. *Proc Natl Acad Sci U S A* 90: 10957–10961, 1993.
102. Gaut JP, Byun J, Tran HD, Lauber WM, Carroll JA, Hotchkiss RS, Belaaouaj A, and Heinecke JW. Myeloperoxidase produces nitrating oxidants in vivo. *J Clin Invest* 109: 1311–1319, 2002.
103. Gaut JP, Yeh GC, Tran HD, Byun J, Henderson JP, Richter GM, Brennan ML, Lusis AJ, Belaaouaj A, Hotchkiss RS, and Heinecke JW. Neutrophils employ the myeloperoxidase system to generate antimicrobial brominating and chlorinating oxidants during sepsis. *Proc Natl Acad Sci U S A* 98: 11961–11966, 2001.
104. Gelderman MP, Stuart R, Vigerust D, Fuhrmann S, Lefkowitz DL, Allen RC, Lefkowitz SS, and Graham S. Perpetuation of inflammation associated with experimental arthritis: the role of macrophage activation by neutrophilic myeloperoxidase. *Mediators Inflamm* 7: 381–389, 1998.
105. Gerber LM, Schwartz JE, Cedeno-Mero C, Warren K, and Pickering TG. Association of urinary albumin concentration with casual and ambulatory blood pressure: a similar relationship in normotensive and hypertensive subjects. *Blood Press Monit* 6: 245–251, 2001.
106. Goldschmeding R, van der Schoot CE, Bokkel Huinink D, Hack CE, van den Ende ME, Kallenberg CG, and dem Borne AE. Wegener's granulomatosis autoantibodies identify a novel diisopropylfluorophosphate-binding protein in the lysosomes of normal human neutrophils. *J Clin Invest* 84: 1577–1587, 1989.
107. Green PS, Mendez AJ, Jacob JS, Crowley JR, Growdon W, Hyman BT, and Heinecke JW. Neuronal expression of myeloperoxidase is increased in Alzheimer's disease. *J Neurochem* 90: 724–733, 2004.
108. Grone HJ, Grone EF, and Malle E. Immunohistochemical detection of hypochlorite-modified proteins in glomeruli of human membranous glomerulonephritis. *Lab Invest* 82: 5–14, 2002.
109. Gross S, Gammon ST, Moss BL, Rauch D, Harding J, Heinecke JW, Ratner L, and Piwnica-Worms D. Bioluminescence imaging of myeloperoxidase activity in vivo. *Nat Med* 15: 455–461, 2009.
110. Guilpain P, Servettaz A, Batteux F, Guillevin L, and Mouthon L. Natural and disease associated anti-myeloperoxidase (MPO) autoantibodies. *Autoimmun Rev* 7: 421–425, 2008.
111. Gullberg U, Bengtsson N, Bulow E, Garwicz D, Lindmark A, and Olsson I. Processing and targeting of granule proteins in human neutrophils. *J Immunol Methods* 232: 201–210, 1999.
112. Gungor N, Godschalk RW, Pachen DM, Van Schooten FJ, and Knaapen AM. Activated neutrophils inhibit nucleotide excision repair in human pulmonary epithelial cells: role of myeloperoxidase. *FASEB J* 21: 2359–2367, 2007.
113. Haegens A, van der Vliet A, Butnor KJ, Heintz N, Taatjes D, Hemenway D, Vacek P, Freeman BA, Hazen SL, Brennan ML, and Mossman BT. Asbestos-induced lung inflammation and epithelial cell proliferation are altered in myeloperoxidase-null mice. *Cancer Res* 65: 9670–9677, 2005.
114. Haegens A, Vernooy JH, Heeringa P, Mossman BT, and Wouters EF. Myeloperoxidase modulates lung epithelial responses to pro-inflammatory agents. *Eur Respir J* 31: 252–260, 2008.
115. Haegens AH, Heeringa P, van Suylen RJ, Steele C, Aratani Y, O'Donoghue RJJ, Mutsaers SE, Mossman BT, and Wouters EF. Myeloperoxidase deficiency attenuates lipopolysaccharide-induced acute lung inflammation and subsequent cytokine and chemokine production *J Immunol* 182: 7990–7996, 2009.
116. Hallett MB and Williams AS. Stopping the traffic: a route to arthritis therapy. *Eur J Immunol* 38: 2650–2653, 2008.
117. Hampton MB, Kettle AJ, and Winterbourn CC. Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. *Blood* 92: 3007–3017, 1998.
118. Hansson M, Olsson I, and Nauseef WM. Biosynthesis, processing, and sorting of human myeloperoxidase. *Arch Biochem Biophys* 445: 214–224, 2006.
119. Harper L, Cockwell P, Adu D, and Savage CO. Neutrophil priming and apoptosis in anti-neutrophil cytoplasmic autoantibody-associated vasculitis. *Kidney Int* 59: 1729–1738, 2001.
120. Harper L, Ren Y, Savill J, Adu D, and Savage CO. Antineutrophil cytoplasmic antibodies induce reactive oxygen-dependent dysregulation of primed neutrophil apoptosis and clearance by macrophages. *Am J Pathol* 157: 211–220, 2000.
121. Harrison JE, Pabalan S, and Schultz J. The subunit structure of crystalline canine myeloperoxidase. *Biochim Biophys Acta* 493: 247–259, 1977.
122. Hashinaka K and Yamada M. Undermethylation and DNase I hypersensitivity of myeloperoxidase gene in HL-60 cells before and after differentiation. *Arch Biochem Biophys* 293: 40–45, 1992.
123. Hazell LJ, Arnold L, Flowers D, Waeg G, Malle E, and Stocker R. Presence of hypochlorite-modified proteins in human atherosclerotic lesions. *J Clin Invest* 97: 1535–1544, 1996.
124. Hazen SL and Heinecke JW. 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. *J Clin Invest* 99: 2075–2081, 1997.
125. Hazen SL, Zhang R, Shen Z, Wu W, Podrez EA, MacPherson JC, Schmitt D, Mitra SN, Mukhopadhyay C, Chen Y, Cohen PA, Hoff HF, and Abu-Soud HM. Formation of nitric oxide-derived oxidants by myeloperoxidase in monocytes: pathways for monocyte-mediated protein nitration and lipid peroxidation in vivo. *Circ Res* 85: 950–958, 1999.
126. Heeringa P, Brouwer E, Klok PA, Huitema MG, van den Born J, Weening JJ, and Kallenberg CG. Autoantibodies to myeloperoxidase aggravate mild anti-glomerular-

basement-membrane-mediated glomerular injury in the rat. *Am J Pathol* 149: 1695–706, 1996.
127. Heeringa P, Brouwer E, Tervaert JW, Weening JJ, and Kallenberg CG. Animal models of anti-neutrophil cytoplasmic antibody associated vasculitis. *Kidney Int* 53: 253–263, 1998.
128. Heeringa P, Foucher P, Klok PA, Huitema MG, Tervaert JW, Weening JJ, and Kallenberg CG. Systemic injection of products of activated neutrophils and H_2O_2 in myeloperoxidase-immunized rats leads to necrotizing vasculitis in the lungs and gut. *Am J Pathol* 151: 131–140, 1997.
129. Heeringa P, Huugen D, and Tervaert JW. Anti-neutrophil cytoplasmic autoantibodies and leukocyte-endothelial interactions: a sticky connection? *Trends Immunol* 26: 561–564, 2005.
130. Heeringa P and Tervaert JW. Pathophysiology of ANCA-associated vasculitides: are ANCAs really pathogenic? *Kidney Int* 65: 1564–1567, 2004.
131. Heeringa P, van Goor H, Itoh-Lindstrom Y, Maeda N, Falk RJ, Assmann KJ, Kallenberg CG, and Jennette JC. Lack of endothelial nitric oxide synthase aggravates murine accelerated anti-glomerular basement membrane glomerulonephritis. *Am J Pathol* 156: 879–888, 2000.
132. Henderson JP, Byun J, and Heinecke JW. Chlorination of nucleobases, RNA and DNA by myeloperoxidase: a pathway for cytotoxicity and mutagenesis by activated phagocytes. *Redox Rep* 4: 319–320, 1999.
133. Henderson JP, Byun J, Takeshita J, and Heinecke JW. Phagocytes produce 5-chlorouracil and 5-bromouracil, two mutagenic products of myeloperoxidase, in human inflammatory tissue. *J Biol Chem* 278: 23522–23528, 2003.
134. Henson PM and Johnston RB Jr. Tissue injury in inflammation: oxidants, proteinases, and cationic proteins. *J Clin Invest* 79: 669–674, 1987.
135. Hess C, Sadallah S, and Schifferli JA. Induction of neutrophil responsiveness to myeloperoxidase antibodies by their exposure to supernatant of degranulated autologous neutrophils. *Blood* 96: 2822–2827, 2000.
136. Hoy A, Tregouet D, Leininger-Muller B, Poirier O, Maurice M, Sass C, Siest G, Tiret L, and Visvikis S. Serum myeloperoxidase concentration in a healthy population: biological variations, familial resemblance and new genetic polymorphisms. *Eur J Hum Genet* 9: 780–786, 2001.
137. Hung RJ, Boffetta P, Brennan P, Malaveille C, Gelatti U, Placidi D, Carta A, Hautefeuille A, and Porru S. Genetic polymorphisms of MPO, COMT, MnSOD, NQO1, interactions with environmental exposures and bladder cancer risk. *Carcinogenesis* 25: 973–978, 2004.
138. Hunninghake GW, Gadek JE, Kawanami O, Ferrans VJ, and Crystal RG. Inflammatory and immune processes in the human lung in health and disease: evaluation by bronchoalveolar lavage. *Am J Pathol* 97: 149–206, 1979.
139. Huugen D, van Esch A, Xiao H, Peutz-Kootstra CJ, Buurman WA, Tervaert JW, Jennette JC, and Heeringa P. Inhibition of complement factor C5 protects against anti-myeloperoxidase antibody-mediated glomerulonephritis in mice. *Kidney Int* 71: 646–654, 2007.
140. Huugen D, Xiao H, van Esch A, Falk RJ, Peutz-Kootstra CJ, Buurman WA, Tervaert JW, Jennette JC, and Heeringa P. Aggravation of anti-myeloperoxidase antibody-induced glomerulonephritis by bacterial lipopolysaccharide: role of tumor necrosis factor-alpha. *Am J Pathol* 167: 47–58, 2005.
141. Inazawa J, Inoue K, Nishigaki H, Tsuda S, Taniwaki M, Misawa S, and Abe T. Assignment of the human myeloperoxidase gene (MPO) to bands q21.3-q23 of chromosome 17. *Cytogenet Cell Genet* 50: 135–136, 1989.
142. Jang HR and Rabb H. The innate immune response in ischemic acute kidney injury. *Clin Immunol* 130: 41–50, 2009.
143. Jayne DR, Weetman AP, and Lockwood CM. IgG subclass distribution of autoantibodies to neutrophil cytoplasmic antigens in systemic vasculitis. *Clin Exp Immunol* 84: 476–481, 1991.
144. Jennette JC and Falk RJ. Small-vessel vasculitis. *N Engl J Med* 337: 1512–1523, 1997.
145. Jennette JC, Hoidal JR, and Falk RJ. Specificity of anti-neutrophil cytoplasmic autoantibodies for proteinase 3. *Blood* 75: 2263–2264, 1990.
146. Jennette JC, Xiao H, and Falk RJ. Pathogenesis of vascular inflammation by anti-neutrophil cytoplasmic antibodies. *J Am Soc Nephrol* 17: 1235–1242, 2006.
147. Jiang Q and Hurst JK. Relative chlorinating, nitrating, and oxidizing capabilities of neutrophils determined with phagocytosable probes. *J Biol Chem* 272: 32767–3272, 1997.
148. Johansson MW, Patarroyo M, Oberg F, Siegbahn A, and Nilsson K. Myeloperoxidase mediates cell adhesion via the alpha M beta 2 integrin (Mac-1, CD11b/CD18). *J Cell Sci* 110: 1133–1139, 1997.
149. Johnson KJ, Fantone JC 3rd, Kaplan J, and Ward PA. In vivo damage of rat lungs by oxygen metabolites. *J Clin Invest* 67: 983–993, 1981.
150. Johnson KR, Nauseef WM, Care A, Wheelock MJ, Shane S, Hudson S, Koeffler HP, Selsted M, Miller C, and Rovera G. Characterization of cDNA clones for human myeloperoxidase: predicted amino acid sequence and evidence for multiple mRNA species. *Nucleic Acids Res* 15: 2013–2028, 1987.
151. Johnson RJ, Couser WG, Chi EY, Adler S, and Klebanoff SJ. New mechanism for glomerular injury: myeloperoxidase-hydrogen peroxide-halide system. *J Clin Invest* 79: 1379–1387, 1987.
152. Johnson RJ, Guggenheim SJ, Klebanoff SJ, Ochi RF, Wass A, Baker P, Schulze M, and Couser WG. Morphologic correlates of glomerular oxidant injury induced by the myeloperoxidase-hydrogen peroxide-halide system of the neutrophil. *Lab Invest* 58: 294–301, 1988.
153. Johnston RB Jr and Baehner RL. Improvement of leukocyte bactericidal activity in chronic granulomatous disease. *Blood* 35: 350–355, 1970.
154. Kabutomori O, Yanagihara T, Iwatani Y, Kawarazaki A, and Kabutomori M. Sex difference in myeloperoxidase activity of neutrophils. *Am J Hematol* 60: 312–313, 1999.
155. Kallenberg CG, Brouwer E, Weening JJ, and Tervaert JW. Anti-neutrophil cytoplasmic antibodies: current diagnostic and pathophysiological potential. *Kidney Int* 46: 1–15, 1994.
156. Kallenberg CG, Heeringa P, and Stegeman CA. Mechanisms of disease: pathogenesis and treatment of ANCA-associated vasculitides. *Nat Clin Pract Rheumatol* 2: 661–670, 2006.
157. Kameoka Y, Persad AS, and Suzuki K. Genomic variations in myeloperoxidase gene in the Japanese population. *Jpn J Infect Dis* 57: S12–S13, 2004.
158. Kanofsky JR, Wright J, Miles-Richardson GE, and Tauber AI. Biochemical requirements for singlet oxygen production by purified human myeloperoxidase. *J Clin Invest* 74: 1489–1495, 1984.
159. Kantarci OH, Atkinson EJ, Hebrink DD, McMurray CT, and Weinshenker BG. Association of a myeloperoxidase

promoter polymorphism with multiple sclerosis. *J Neuroimmunol* 105: 189–194, 2000.
160. Kantari C, Pederzoli-Ribeil M, and Witko-Sarsat V. The role of neutrophils and monocytes in innate immunity. *Contrib Microbiol* 15: 118–146, 2008.
161. Keatings VM and Barnes PJ. Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. *Am J Respir Crit Care Med* 155: 449–453, 1997.
162. Kelly KJ, Williams WW Jr, Colvin RB, Meehan SM, Springer TA, Gutierrez-Ramos JC, and Bonventre JV. Intercellular adhesion molecule-1-deficient mice are protected against ischemic renal injury. *J Clin Invest* 97: 1056–1063, 1996.
163. Kensler TW, Egner PA, Moore KG, Taffe BG, Twerdok LE, and Trush MA. Role of inflammatory cells in the metabolic activation of polycyclic aromatic hydrocarbons in mouse skin. *Toxicol Appl Pharmacol* 90: 337–346, 1987.
164. Kerjaschki D and Neale TJ. Molecular mechanisms of glomerular injury in rat experimental membranous nephropathy (Heymann nephritis). *J Am Soc Nephrol* 7: 2518–2526, 1996.
165. Kettle AJ, Chan T, Osberg I, Senthilmohan R, Chapman AL, Mocatta TJ, and Wagener JS. Myeloperoxidase and protein oxidation in the airways of young children with cystic fibrosis. *Am J Respir Crit Care Med* 170: 1317–1323, 2004.
166. Kettle AJ, Clark BM, and Winterbourn CC. Superoxide converts indigo carmine to isatin sulfonic acid: implications for the hypothesis that neutrophils produce ozone. *J Biol Chem* 279: 18521–18525, 2004.
167. Kettle AJ, Gedye CA, Hampton MB, and Winterbourn CC. Inhibition of myeloperoxidase by benzoic acid hydrazides. *Biochem J* 308: 559–563, 1995.
168. Kettle AJ, Gedye CA, and Winterbourn CC. Mechanism of inactivation of myeloperoxidase by 4-aminobenzoic acid hydrazide. *Biochem J* 321: 503–508, 1997.
169. Kettritz R, Jennette JC, and Falk RJ. Crosslinking of ANCA-antigens stimulates superoxide release by human neutrophils. *J Am Soc Nephrol* 8: 386–394, 1997.
170. Kettritz R, Schreiber A, Luft FC, and Haller H. Role of mitogen-activated protein kinases in activation of human neutrophils by antineutrophil cytoplasmic antibodies. *J Am Soc Nephrol* 12: 37–46, 2001.
171. Kirsch M, Korth HG, Sustmann R, and de Groot H. The pathobiochemistry of nitrogen dioxide. *Biol Chem* 383: 389–399, 2002.
172. Kiryu C, Makiuchi M, Miyazaki J, Fujinaga T, and Kakinuma K. Physiological production of singlet molecular oxygen in the myeloperoxidase-H_2O_2-chloride system. *FEBS Lett* 443: 154–158, 1999.
173. Kitahara M, Eyre HJ, Simonian Y, Atkin CL, and Hasstedt SJ. Hereditary myeloperoxidase deficiency. *Blood* 57: 888–893, 1981.
174. Klebanoff SJ. Iodination of bacteria: a bactericidal mechanism. *J Exp Med* 126: 1063–1078, 1967.
175. Klebanoff SJ. Myeloperoxidase-halide-hydrogen peroxide antibacterial system. *J Bacteriol* 95: 2131–2138, 1968.
176. Klebanoff SJ. Myeloperoxidase: contribution to the microbicidal activity of intact leukocytes. *Science* 169: 1095–1097, 1970.
177. Klebanoff SJ. Myeloperoxidase: friend and foe. *J Leukoc Biol* 77: 598–625, 2005.
178. Klebanoff SJ and Hamon CB. Role of myeloperoxidase-mediated antimicrobial systems in intact leukocytes. *J Reticuloendothel Soc* 12: 170–196, 1972.
179. Koch C. Effect of sodium azide upon normal and pathological granulocyte function. *Acta Pathol Microbiol Scand [B] Microbiol Immunol* 82: 136–142, 1974.
180. Koeffler HP, Ranyard J, and Pertcheck M. Myeloperoxidase: its structure and expression during myeloid differentiation. *Blood* 65: 484–491, 1985.
181. Komatsu J, Koyama H, Maeda N, and Aratani Y. Earlier onset of neutrophil-mediated inflammation in the ultraviolet-exposed skin of mice deficient in myeloperoxidase and NADPH oxidase. *Inflamm Res* 55: 200–206, 2006.
182. Kruidenier L, Kuiper I, Lamers CB, and Verspaget HW. Intestinal oxidative damage in inflammatory bowel disease: semi-quantification, localization, and association with mucosal antioxidants. *J Pathol* 201: 28–36, 2003.
183. Kruidenier L, Kuiper I, Van Duijn W, Mieremet-Ooms MA, van Hogezand RA, Lamers CB, and Verspaget HW. Imbalanced secondary mucosal antioxidant response in inflammatory bowel disease. *J Pathol* 201: 17–27, 2003.
184. Kubala L, Baldus S, and Eiserich JP. Glycosaminoglycan-dependent sequestration of myeloperoxidase within extracellular matrix *Free Radic Biol Med* 37(suppl 1): 52, 2004.
185. Kumar AP, Piedrafita FJ, and Reynolds WF. Peroxisome proliferator-activated receptor gamma ligands regulate myeloperoxidase expression in macrophages by an estrogen-dependent mechanism involving the −463GA promoter polymorphism. *J Biol Chem* 279: 8300–8315, 2004.
186. Kumar AP and Reynolds WF. Statins downregulate myeloperoxidase gene expression in macrophages. *Biochem Biophys Res Commun* 331: 442–451, 2005.
187. Kutter D, Devaquet P, Vanderstocken G, Paulus JM, Marchal V, and Gothot A. Consequences of total and subtotal myeloperoxidase deficiency: risk or benefit? *Acta Haematol* 104: 10–15, 2000.
188. Lam KM. Myeloperoxidase activity in chicken heterophils and adherent cells. *Vet Immunol Immunopathol* 57: 327–335, 1997.
189. Lancaster JR Jr. Nitroxidative, nitrosative, and nitrative stress: kinetic predictions of reactive nitrogen species chemistry under biological conditions. *Chem Res Toxicol* 19: 1160–1174, 2006.
190. Larrocha C, Fernandez de Castro M, Fontan G, Viloria A, Fernandez-Chacon JL, and Jimenez C. Hereditary myeloperoxidase deficiency: study of 12 cases. *Scand J Haematol* 29: 389–397, 1982.
191. Latos-Bielenska A, Klett C, Just W, and Hameister H. Refinement of localization of the human genes for myeloperoxidase (MPO), protein kinase C, alpha polypeptide, PRKCA, and the DNA fragment D17S21 on chromosome 17q. *Hereditas* 115: 69–72, 1991.
192. Lau D and Baldus S. Myeloperoxidase and its contributory role in inflammatory vascular disease. *Pharmacol Ther* 111: 16–26, 2006.
193. Lau D, Mollnau H, Eiserich JP, Freeman BA, Daiber A, Gehling UM, Brummer J, Rudolph V, Munzel T, Heitzer T, Meinertz T, and Baldus S. Myeloperoxidase mediates neutrophil activation by association with CD11b/CD18 integrins. *Proc Natl Acad Sci U S A* 102: 431–436, 2005.
194. Le Marchand L, Seifried A, Lum A, and Wilkens LR. Association of the myeloperoxidase-463G-->a polymorphism with lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 9: 181–184, 2000.
195. Learn DB, Fried VA, and Thomas EL. Taurine and hypotaurine content of human leukocytes. *J Leukoc Biol* 48: 174–182, 1990.

196. Lefkowitz DL, Gelderman MP, Fuhrmann SR, Graham S, Starnes JD 3rd, Lefkowitz SS, Bollen A, and Moguilevsky N. Neutrophilic myeloperoxidase-macrophage interactions perpetuate chronic inflammation associated with experimental arthritis. *Clin Immunol* 91: 145–155, 1999.
197. Lefkowitz DL, Mills K, Morgan D, and Lefkowitz SS. Macrophage activation and immunomodulation by myeloperoxidase. *Proc Soc Exp Biol Med* 199: 204–210, 1992.
198. Lefkowitz DL, Mills KC, Moguilevsky N, Bollen A, Vaz A, and Lefkowitz SS. Regulation of macrophage function by human recombinant myeloperoxidase. *Immunol Lett* 36: 43–49, 1993.
199. Lefkowitz DL, Roberts E, Grattendick K, Schwab C, Stuart R, Lincoln J, Allen RC, Moguilevsky N, Bollen A, and Lefkowitz SS. The endothelium and cytokine secretion: the role of peroxidases as immunoregulators. *Cell Immunol* 202: 23–30, 2000.
200. Lefkowitz SS, Gelderman MP, Lefkowitz DL, Moguilevsky N, and Bollen A. Phagocytosis and intracellular killing of *Candida albicans* by macrophages exposed to myeloperoxidase. *J Infect Dis* 173: 1202–1207, 1996.
201. Lehrer RI and Cline MJ. Leukocyte myeloperoxidase deficiency and disseminated candidiasis: the role of myeloperoxidase in resistance to *Candida* infection. *J Clin Invest* 48: 1478–1488, 1969.
202. Lehrer RI, Hanifin J, and Cline MJ. Defective bactericidal activity in myeloperoxidase-deficient human neutrophils. *Nature* 223: 78–79, 1969.
203. Leininger-Muller B, Hoy A, Herbeth B, Pfister M, Serot JM, Stavljenic-Rukavina M, Massana L, Passmore P, Siest G, and Visvikis S. Myeloperoxidase G-463A polymorphism and Alzheimer's disease in the ApoEurope study. *Neurosci Lett* 349: 95–98, 2003.
204. Lin KM and Austin GE. Functional activity of three distinct myeloperoxidase (MPO) promoters in human myeloid cells. *Leukemia* 16: 1143–1153, 2002.
205. Lincoln JA, Lefkowitz DL, Cain T, Castro A, Mills KC, Lefkowitz SS, Moguilevsky N, and Bollen A. Exogenous myeloperoxidase enhances bacterial phagocytosis and intracellular killing by macrophages. *Infect Immun* 63: 3042–3047, 1995.
206. Little MA, Smyth CL, Yadav R, Ambrose L, Cook HT, Nourshargh S, and Pusey CD. Antineutrophil cytoplasm antibodies directed against myeloperoxidase augment leukocyte-microvascular interactions in vivo. *Blood* 106: 2050–2058, 2005.
207. Liu HR, Tao L, Gao E, Qu Y, Lau WB, Lopez BL, Christopher TA, Koch W, Yue TL, and Ma XL. Rosiglitazone inhibits hypercholesterolaemia-induced myeloperoxidase upregulation: a novel mechanism for the cardioprotective effects of PPAR agonists. *Cardiovasc Res* 81: 344–352, 2009.
208. Loeb HG and Doniger R. Myeloperoxidase activity in bone marrow extracts from tumor-bearing mice. *Br J Cancer* 12: 669–676, 1958.
209. London SJ, Lehman TA, and Taylor JA. Myeloperoxidase genetic polymorphism and lung cancer risk. *Cancer Res* 57: 5001–5003, 1997.
210. Lubbert M, Miller CW, and Koeffler HP. Changes of DNA methylation and chromatin structure in the human myeloperoxidase gene during myeloid differentiation. *Blood* 78: 345–356, 1991.
211. Makela R, Dastidar P, Jokela H, Saarela M, Punnonen R, and Lehtimaki T. Effect of long-term hormone replacement therapy on atherosclerosis progression in postmenopausal women relates to myeloperoxidase promoter polymorphism. *J Clin Endocrinol Metab* 88: 3823–3828, 2003.
212. Makela R, Karhunen PJ, Kunnas TA, Ilveskoski E, Kajander OA, Mikkelsson J, Perola M, Penttila A, and Lehtimaki T. Myeloperoxidase gene variation as a determinant of atherosclerosis progression in the abdominal and thoracic aorta: an autopsy study. *Lab Invest* 83: 919–925, 2003.
213. Maki RA, Tyurin VA, Lyon RC, Hamilton RL, Dekosky ST, Kagan VE, and Reynolds WF. Aberrant expression of myeloperoxidase in astrocytes promotes phospholipid oxidation and memory deficits in a mouse model of Alzheimer's disease. *J Biol Chem* 284: 3158–3169, 2008.
214. Malle E, Buch T, and Grone HJ. Myeloperoxidase in kidney disease. *Kidney Int* 64: 1956–1967, 2003.
215. Malle E, Furtmuller PG, Sattler W, and Obinger C. Myeloperoxidase: a target for new drug development? *Br J Pharmacol* 152: 838–854, 2007.
216. Malle E, Hazell L, Stocker R, Sattler W, Esterbauer H, and Waeg G. Immunologic detection and measurement of hypochlorite-modified LDL with specific monoclonal antibodies. *Arterioscler Thromb Vasc Biol* 15: 982–989, 1995.
217. Malle E, Waeg G, Schreiber R, Grone EF, Sattler W, and Grone HJ. Immunohistochemical evidence for the myeloperoxidase/H_2O_2/halide system in human atherosclerotic lesions: colocalization of myeloperoxidase and hypochlorite-modified proteins. *Eur J Biochem* 267: 4495–4503, 2000.
218. Mandell GL. Catalase, superoxide dismutase, and virulence of *Staphylococcus aureus*: in vitro and in vivo studies with emphasis on staphylococcal-leukocyte interaction. *J Clin Invest* 55: 561–566, 1975.
219. Marcinkiewicz J, Grabowska A, and Chain BM. Modulation of antigen-specific T-cell activation in vitro by taurine chloramine. *Immunology* 94: 325–330, 1998.
220. Marcinkiewicz J, Nowak B, Grabowska A, Bobek M, Petrovska L, and Chain B. Regulation of murine dendritic cell functions in vitro by taurine chloramine: a major product of the neutrophil myeloperoxidase-halide system. *Immunology* 98: 371–378, 1999.
221. Marklund L, Kaul S, and Tuggle CK. Rapid communication: mapping of the myeloperoxidase (MPO) gene to pig chromosome 12. *J Anim Sci* 78: 3189–3190, 2000.
222. Marnett LJ. Oxyradicals and DNA damage. *Carcinogenesis* 21: 361–370, 2000.
223. Marsche G, Furtmuller PG, Obinger C, Sattler W, and Malle E. Hypochlorite-modified high-density lipoprotein acts as a sink for myeloperoxidase in vitro. *Cardiovasc Res* 79: 187–194, 2008.
224. Marsche G, Heller R, Fauler G, Kovacevic A, Nuszkowski A, Graier W, Sattler W, and Malle E. 2-Chlorohexadecanal derived from hypochlorite-modified high-density lipoprotein-associated plasmalogen is a natural inhibitor of endothelial nitric oxide biosynthesis. *Arterioscler Thromb Vasc Biol* 24: 2302–2306, 2004.
225. Matsumoto I, Maccioni M, Lee DM, Maurice M, Simmons B, Brenner M, Mathis D, and Benoist C. How antibodies to a ubiquitous cytoplasmic enzyme may provoke joint-specific autoimmune disease. *Nat Immunol* 3: 360–365, 2002.
226. Matthijsen RA, Huugen D, Hoebers NT, de Vries B, Peutz-Kootstra CJ, Aratani Y, Daha MR, Tervaert JW, Buurman WA, and Heeringa P. Myeloperoxidase is critically involved in the induction of organ damage after renal ischemia reperfusion. *Am J Pathol* 171: 1743–1752, 2007.

227. McMillen TS, Heinecke JW, and LeBoeuf RC. Expression of human myeloperoxidase by macrophages promotes atherosclerosis in mice. *Circulation* 111: 2798–2804, 2005.
228. Meisel P, Krause T, Cascorbi I, Schroeder W, Herrmann F, John U, and Kocher T. Gender and smoking-related risk reduction of periodontal disease with variant myeloperoxidase alleles. *Genes Immun* 3: 102–106, 2002.
229. Melnikov VY, Faubel S, Siegmund B, Lucia MS, Ljubanovic D, and Edelstein CL. Neutrophil-independent mechanisms of caspase-1- and IL-18-mediated ischemic acute tubular necrosis in mice. *J Clin Invest* 110: 1083–1091, 2002.
230. Meuwese MC, Stroes ES, Hazen SL, van Miert JN, Kuivenhoven JA, Schaub RG, Wareham NJ, Luben R, Kastelein JJ, Khaw KT, and Boekholdt SM. Serum myeloperoxidase levels are associated with the future risk of coronary artery disease in apparently healthy individuals: the EPIC-Norfolk Prospective Population Study. *J Am Coll Cardiol* 50: 159–165, 2007.
231. Milla C, Yang S, Cornfield DN, Brennan ML, Hazen SL, Panoskaltsis-Mortari A, Blazar BR, and Haddad IY. Myeloperoxidase deficiency enhances inflammation after allogeneic marrow transplantation. *Am J Physiol Lung Cell Mol Physiol* 287: L706–L714, 2004.
232. Misra RR, Tangrea JA, Virtamo J, Ratnasinghe D, Andersen MR, Barrett M, Taylor PR, and Albanes D. Variation in the promoter region of the myeloperoxidase gene is not directly related to lung cancer risk among male smokers in Finland. *Cancer Lett* 164: 161–167, 2001.
233. Miura M, Fu X, Zhang QW, Remick DG, and Fairchild RL. Neutralization of Gro alpha and macrophage inflammatory protein-2 attenuates renal ischemia/reperfusion injury. *Am J Pathol* 159: 2137–2145, 2001.
234. Miyasaki KT, Zambon JJ, Jones CA, and Wilson ME. Role of high-avidity binding of human neutrophil myeloperoxidase in the killing of *Actinobacillus actinomycetemcomitans*. *Infect Immun* 55: 1029–1036, 1987.
235. Mocatta TJ, Pilbrow AP, Cameron VA, Senthilmohan R, Frampton CM, Richards AM, and Winterbourn CC. Plasma concentrations of myeloperoxidase predict mortality after myocardial infarction. *J Am Coll Cardiol* 49: 1993–2000, 2007.
236. Moguilevsky N, Garcia-Quintana L, Jacquet A, Tournay C, Fabry L, Pierard L, and Bollen A. Structural and biological properties of human recombinant myeloperoxidase produced by Chinese hamster ovary cell lines. *Eur J Biochem* 197: 605–614, 1991.
237. Mohiuddin I, Chai H, Lin PH, Lumsden AB, Yao Q, and Chen C. Nitrotyrosine and chlorotyrosine: clinical significance and biological functions in the vascular system. *J Surg Res* 133: 143–149, 2006.
238. Morishita K, Kubota N, Asano S, Kaziro Y, and Nagata S. Molecular cloning and characterization of cDNA for human myeloperoxidase. *J Biol Chem* 262: 3844–3651, 1987.
239. Morohoshi Y, Matsuoka K, Chinen H, Kamada N, Sato T, Hisamatsu T, Okamoto S, Inoue N, Takaishi H, Ogata H, Iwao Y, and Hibi T. Inhibition of neutrophil elastase prevents the development of murine dextran sulfate sodium-induced colitis. *J Gastroenterol* 41: 318–324, 2006.
240. Mossman BT and Churg A. Mechanisms in the pathogenesis of asbestosis and silicosis. *Am J Respir Crit Care Med* 157: 1666–1680, 1998.
241. Mulder AH, Stegeman CA, and Kallenberg CG. Activation of granulocytes by anti-neutrophil cytoplasmic antibodies (ANCA) in Wegener's granulomatosis: a predominant role for the IgG3 subclass of ANCA. *Clin Exp Immunol* 101: 227–232, 1995.
242. Nagra RM, Becher B, Tourtellotte WW, Antel JP, Gold D, Paladino T, Smith RA, Nelson JR, and Reynolds WF. Immunohistochemical and genetic evidence of myeloperoxidase involvement in multiple sclerosis. *J Neuroimmunol* 78: 97–107, 1997.
243. Nahrendorf M, Sosnovik D, Chen JW, Panizzi P, Figueiredo JL, Aikawa E, Libby P, Swirski FK, and Weissleder R. Activatable magnetic resonance imaging agent reports myeloperoxidase activity in healing infarcts and noninvasively detects the antiinflammatory effects of atorvastatin on ischemia-reperfusion injury. *Circulation* 117: 1153–1160, 2008.
244. Naito Y, Takagi T, and Yoshikawa T. Molecular fingerprints of neutrophil-dependent oxidative stress in inflammatory bowel disease. *J Gastroenterol* 42: 787–798, 2007.
245. Nathan C. Specificity of a third kind: reactive oxygen and nitrogen intermediates in cell signaling. *J Clin Invest* 111: 769–778, 2003.
246. Nathan C. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol* 6: 173–182, 2006.
247. Natsui M, Kawasaki K, Takizawa H, Hayashi SI, Matsuda Y, Sugimura K, Seki K, Narisawa R, Sendo F, and Asakura H. Selective depletion of neutrophils by a monoclonal antibody, RP-3, suppresses dextran sulphate sodium-induced colitis in rats. *J Gastroenterol Hepatol* 12: 801–808, 1997.
248. Nauseef WM. Myeloperoxidase biosynthesis by a human promyelocytic leukemia cell line: insight into myeloperoxidase deficiency. *Blood* 67: 865–872, 1986.
249. Nauseef WM. Posttranslational processing of a human myeloid lysosomal protein, myeloperoxidase. *Blood* 70: 1143–1150, 1987.
250. Nauseef WM. The proper study of mankind. *J Clin Invest* 107: 401–403, 2001.
251. Nauseef WM, Brigham S, and Cogley M. Hereditary myeloperoxidase deficiency due to a missense mutation of arginine 569 to tryptophan. *J Biol Chem* 269: 1212–1216, 1994.
252. Nauseef WM, McCormick SJ, and Clark RA. Calreticulin functions as a molecular chaperone in the biosynthesis of myeloperoxidase. *J Biol Chem* 270: 4741–4747, 1995.
253. Nauseef WM, McCormick SJ, and Goedken M. Coordinated participation of calreticulin and calnexin in the biosynthesis of myeloperoxidase. *J Biol Chem* 273: 7107–7111, 1998.
254. Nauseef WM, Metcalf JA, and Root RK. Role of myeloperoxidase in the respiratory burst of human neutrophils. *Blood* 61: 483–492, 1983.
255. Ndrepepa G, Braun S, Mehilli J, von Beckerath N, Schomig A, and Kastrati A. Myeloperoxidase level in patients with stable coronary artery disease and acute coronary syndromes. *Eur J Clin Invest* 38: 90–96, 2008.
256. Ng LL, Pathik B, Loke IW, Squire IB, and Davies JE. Myeloperoxidase and C-reactive protein augment the specificity of B-type natriuretic peptide in community screening for systolic heart failure. *Am Heart J* 152: 94–101, 2006.
257. Nichols BA and Bainton DF. Differentiation of human monocytes in bone marrow and blood: sequential formation of two granule populations. *Lab Invest* 29: 27–40, 1973.
258. Nikpoor B, Turecki G, Fournier C, Theroux P, and Rouleau GA. A functional myeloperoxidase polymorphic variant is associated with coronary artery disease in French-Canadians. *Am Heart J* 142: 336–339, 2001.
259. Noguchi N, Nakano K, Aratani Y, Koyama H, Kodama T, and Niki E. Role of myeloperoxidase in the neutrophil-induced oxidation of low density lipoprotein as studied by

myeloperoxidase-knockout mouse. *J Biochem* 127: 971–976, 2000.
260. Nolan SL, Kalia N, Nash GB, Kamel D, Heeringa P, and Savage CO. Mechanisms of ANCA-mediated leukocyte-endothelial cell interactions in vivo. *J Am Soc Nephrol* 19: 973–984, 2008.
261. Nunoi H, Kohi F, Kajiwara H, and Suzuki K. Prevalence of inherited myeloperoxidase deficiency in Japan. *Microbiol Immunol* 47: 527–531, 2003.
262. Nurcombe HL, Bucknall RC, and Edwards SW. Activation of the neutrophil myeloperoxidase-H_2O_2 system by synovial fluid isolated from patients with rheumatoid arthritis. *Ann Rheum Dis* 50: 237–242, 1991.
263. Nuszkowski A, Grabner R, Marsche G, Unbehaun A, Malle E, and Heller R. Hypochlorite-modified low density lipoprotein inhibits nitric oxide synthesis in endothelial cells via an intracellular dislocalization of endothelial nitric-oxide synthase. *J Biol Chem* 276: 14212–14221, 2001.
264. O'Brien PJ. Peroxidases. *Chem Biol Interact* 129: 113–139, 2000.
265. Odobasic D, Kitching AR, Semple TJ, and Holdsworth SR. Endogenous myeloperoxidase promotes neutrophil-mediated renal injury, but attenuates T cell immunity inducing crescentic glomerulonephritis. *J Am Soc Nephrol* 18: 760–770, 2007.
266. Ohashi YY, Kameoka Y, Persad AS, Koi F, Yamagoe S, Hashimoto K, and Suzuki K. Novel missense mutation found in a Japanese patient with myeloperoxidase deficiency. *Gene* 327: 195–200, 2004.
267. Olsson I, Persson AM, and Stromberg K. Biosynthesis, transport and processing of myeloperoxidase in the human leukaemic promyelocytic cell line HL-60 and normal marrow cells. *Biochem J* 223: 911–920, 1984.
268. Park JH, Park JM, Kim EJ, Cha SI, Lee EB, Kim CH, Kam S, Jung TH, and Park JY. Myeloperoxidase −463G>A polymorphism and risk of primary lung cancer in a Korean population. *Cancer Detect Prev* 30: 257–261, 2006.
269. Parry MF, Root RK, Metcalf JA, Delaney KK, Kaplow LS, and Richar WJ. Myeloperoxidase deficiency: prevalence and clinical significance. *Ann Intern Med* 95: 293–301, 1981.
270. Peleman RA, Rytila PH, Kips JC, Joos GF, and Pauwels RA. The cellular composition of induced sputum in chronic obstructive pulmonary disease. *Eur Respir J* 13: 839–843, 1999.
271. Pennathur S, Bergt C, Shao B, Byun J, Kassim SY, Singh P, Green PS, McDonald TO, Brunzell J, Chait A, Oram JF, O'Brien K, Geary RL, and Heinecke JW. Human atherosclerotic intima and blood of patients with established coronary artery disease contain high density lipoprotein damaged by reactive nitrogen species. *J Biol Chem* 279: 42977–42983, 2004.
272. Pero RW, Sheng Y, Olsson A, Bryngelsson C, and Lund-Pero M. Hypochlorous acid/N-chloramines are naturally produced DNA repair inhibitors. *Carcinogenesis* 17: 13–18, 1996.
273. Persad AS, Kameoka Y, Kanda S, Niho Y, and Suzuki K. Arginine to cysteine mutation (R499C) found in a Japanese patient with complete myeloperoxidase deficiency. *Gene Expr* 13: 67–71, 2006.
274. Peskin AV and Winterbourn CC. Taurine chloramine is more selective than hypochlorous acid at targeting critical cysteines and inactivating creatine kinase and glyceraldehyde-3-phosphate dehydrogenase. *Free Radic Biol Med* 40: 45–53, 2006.
275. Piedrafita FJ, Molander RB, Vansant G, Orlova EA, Pfahl M, and Reynolds WF. An Alu element in the myeloperoxidase promoter contains a composite SP1-thyroid hormone-retinoic acid response element. *J Biol Chem* 271: 14412–14420, 1996.
276. Pignatelli P, Loffredo L, Martino F, Catasca E, Carnevale R, Zanoni C, Del Ben M, Antonini R, Basili S, and Violi F. Myeloperoxidase overexpression in children with hypercholesterolemia. *Atherosclerosis* 205: 239–243, 2009.
277. Pitt AR and Spickett CM. Mass spectrometric analysis of HOCl- and free-radical-induced damage to lipids and proteins. *Biochem Soc Trans* 36: 1077–1082, 2008.
278. Podrez EA, Febbraio M, Sheibani N, Schmitt D, Silverstein RL, Hajjar DP, Cohen PA, Frazier WA, Hoff HF, and Hazen SL. Macrophage scavenger receptor CD36 is the major receptor for LDL modified by monocyte-generated reactive nitrogen species. *J Clin Invest* 105: 1095–1108, 2000.
279. Podrez EA, Schmitt D, Hoff HF, and Hazen SL. Myeloperoxidase-generated reactive nitrogen species convert LDL into an atherogenic form in vitro. *J Clin Invest* 103: 1547–1560, 1999.
280. Porubsky S, Schmid H, Bonrouhi M, Kretzler M, Malle E, Nelson PJ, and Grone HJ. Influence of native and hypochlorite-modified low-density lipoprotein on gene expression in human proximal tubular epithelium. *Am J Pathol* 164: 2175–2187, 2004.
281. Potapova SG and Martynova VA. [Myeloperoxidase activity and the digestive capacity of the neutrophils in acute myeloblastic leukemia]. *Probl Gematol Pereliv Krovi* 24: 39–44, 1979.
282. Querol Sans M, Chen JW, Weissleder R, and Bogdanov AA Jr. Myeloperoxidase activity imaging using (67)Ga labeled substrate. *Mol Imaging Biol* 7: 403–410, 2005.
283. Raats CJ, Luca ME, Bakker MA, Van Der Wal A, Heeringa P, van Goor H, Van Den Born J, de Heer E, and Berden JH. Reduction in glomerular heparan sulfate correlates with complement deposition and albuminuria in active Heymann nephritis. *J Am Soc Nephrol* 10: 1689–1699, 1999.
284. Radford DJ, Luu NT, Hewins P, Nash GB, and Savage CO. Antineutrophil cytoplasmic antibodies stabilize adhesion and promote migration of flowing neutrophils on endothelial cells. *Arthritis Rheum* 44: 2851–2861, 2001.
285. Radford DJ, Savage CO, and Nash GB. Treatment of rolling neutrophils with antineutrophil cytoplasmic antibodies causes conversion to firm integrin-mediated adhesion. *Arthritis Rheum* 43: 1337–1345, 2000.
286. Radi R. Nitric oxide, oxidants, and protein tyrosine nitration. *Proc Natl Acad Sci U S A* 101: 4003–4008, 2004.
287. Rafikova O, Salah EM, and Tofovic SP. Renal and metabolic effects of tempol in obese ZSF1 rats: distinct role for superoxide and hydrogen peroxide in diabetic renal injury. *Metabolism* 57: 1434–1444, 2008.
288. Rainis T, Maor I, Lanir A, Shnizer S, and Lavy A. Enhanced oxidative stress and leucocyte activation in neoplastic tissues of the colon. *Dig Dis Sci* 52: 526–530, 2007.
289. Rajp A, Adu D, and Savage CO. Meta-analysis of myeloperoxidase G-463/A polymorphism in anti-neutrophil cytoplasmic autoantibody-positive vasculitis. *Clin Exp Immunol* 149: 251–256, 2007.
290. Rarok AA, Limburg PC, and Kallenberg CG. Neutrophil-activating potential of antineutrophil cytoplasm autoantibodies. *J Leukoc Biol* 74: 3–15, 2003.
291. Rausch PG and Moore TG. Granule enzymes of polymorphonuclear neutrophils: a phylogenetic comparison. *Blood* 46: 913–919, 1975.

292. Rees MD, Bottle SE, Fairfull-Smith KE, Malle E, Whitelock JM, and Davies MJ. Inhibition of myeloperoxidase-mediated hypochlorous acid production by nitroxides. *Biochem J* 421: 79–86, 2009.
293. Regelmann WE, Siefferman CM, Herron JM, Elliott GR, Clawson CC, and Gray BH. Sputum peroxidase activity correlates with the severity of lung disease in cystic fibrosis. *Pediatr Pulmonol* 19: 1–9, 1995.
294. Reumaux D, de Boer M, Meijer AB, Duthilleul P, and Roos D. Expression of myeloperoxidase (MPO) by neutrophils is necessary for their activation by anti-neutrophil cytoplasm autoantibodies (ANCA) against MPO. *J Leukoc Biol* 73: 841–849, 2003.
295. Reynolds WF, Chang E, Douer D, Ball ED, and Kanda V. An allelic association implicates myeloperoxidase in the etiology of acute promyelocytic leukemia. *Blood* 90: 2730–2737, 1997.
296. Reynolds WF, Hiltunen M, Pirskanen M, Mannermaa A, Helisalmi S, Lehtovirta M, Alafuzoff I, and Soininen H. MPO and APOEepsilon4 polymorphisms interact to increase risk for AD in Finnish males. *Neurology* 55: 1284–1290, 2000.
297. Reynolds WF, Kumar AP, and Piedrafita FJ. The human myeloperoxidase gene is regulated by LXR and PPARalpha ligands. *Biochem Biophys Res Commun* 349: 846–854, 2006.
298. Reynolds WF, Patel K, Pianko S, Blatt LM, Nicholas JJ, and McHutchison JG. A genotypic association implicates myeloperoxidase in the progression of hepatic fibrosis in chronic hepatitis C virus infection. *Genes Immun* 3: 345–349, 2002.
299. Reynolds WF, Rhees J, Maciejewski D, Paladino T, Sieburg H, Maki RA, and Masliah E. Myeloperoxidase polymorphism is associated with gender specific risk for Alzheimer's disease. *Exp Neurol* 155: 31–41, 1999.
300. Reynolds WF, Stegeman CA, and Tervaert JW. −463 G/A myeloperoxidase promoter polymorphism is associated with clinical manifestations and the course of disease in MPO-ANCA-associated vasculitis. *Clin Immunol* 103: 154–160, 2002.
301. Robinson CE, Kottapalli V, D'Astice M, Fields JZ, Winship D, and Keshavarzian A. Regulation of neutrophils in ulcerative colitis by colonic factors: a possible mechanism of neutrophil activation and tissue damage. *J Lab Clin Med* 130: 590–602, 1997.
302. Romano M, Dri P, Dadalt L, Patriarca P, and Baralle FE. Biochemical and molecular characterization of hereditary myeloperoxidase deficiency. *Blood* 90: 4126–4134, 1997.
303. Roncucci L, Mora E, Mariani F, Bursi S, Pezzi A, Rossi G, Pedroni M, Luppi D, Santoro L, Monni S, Manenti A, Bertani A, Merighi A, Benatti P, Di Gregorio C, and de Leon PM. Myeloperoxidase-positive cell infiltration in colorectal carcinogenesis as indicator of colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev* 17: 2291–2297, 2008.
304. Rosen H and Klebanoff SJ. Chemiluminescence and superoxide production by myeloperoxidase-deficient leukocytes. *J Clin Invest* 58: 50–60, 1976.
305. Rutgers A, Heeringa P, Giesen JE, Theunissen RT, Jacobs H, and Tervaert JW. Neutrophil myeloperoxidase activity and the influence of two single-nucleotide promoter polymorphisms. *Br J Haematol* 123: 536–538, 2003.
306. Saeki T, Kuroda T, Morita T, Suzuki K, Arakawa M, and Kawasaki K. Significance of myeloperoxidase in rapidly progressive glomerulonephritis. *Am J Kidney Dis* 26: 13–21, 1995.
307. Sato N, Kashima K, Tanaka Y, Shimizu H, and Mori M. Effect of granulocyte-colony stimulating factor on generation of oxygen-derived free radicals and myeloperoxidase activity in neutrophils from poorly controlled NIDDM patients. *Diabetes* 46: 133–137, 1997.
308. Savage CO, Pottinger BE, Gaskin G, Pusey CD, and Pearson JD. Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity toward cultured endothelial cells. *Am J Pathol* 141: 335–342, 1992.
309. Schabath MB, Spitz MR, Hong WK, Delclos GL, Reynolds WF, Gunn GB, Whitehead LW, and Wu X. A myeloperoxidase polymorphism associated with reduced risk of lung cancer. *Lung Cancer* 37: 35–40, 2002.
310. Schabath MB, Spitz MR, Zhang X, Delclos GL, and Wu X. Genetic variants of myeloperoxidase and lung cancer risk. *Carcinogenesis* 21: 1163–1166, 2000.
311. Schieven GL, de Fex H, and Stephenson L. Hypochlorous acid activates tyrosine phosphorylation signal pathways leading to calcium signaling and TNFalpha production. *Antioxid Redox Signal* 4: 501–507, 2002.
312. Schiller J, Fuchs B, Arnhold J, and Arnold K. Contribution of reactive oxygen species to cartilage degradation in rheumatic diseases: molecular pathways, diagnosis and potential therapeutic strategies. *Curr Med Chem* 10: 2123–2145, 2003.
313. Schmitt D, Shen Z, Zhang R, Colles SM, Wu W, Salomon RG, Chen Y, Chisolm GM, and Hazen SL. Leukocytes utilize myeloperoxidase-generated nitrating intermediates as physiological catalysts for the generation of biologically active oxidized lipids and sterols in serum. *Biochemistry* 38: 16904–16915, 1999.
314. Schoonbroodt S, Legrand-Poels S, Best-Belpomme M, and Piette J. Activation of the NF-kappaB transcription factor in a T-lymphocytic cell line by hypochlorous acid. *Biochem J* 321: 777–785, 1997.
315. Schuller-Levis GB and Park E. Taurine: new implications for an old amino acid. *FEMS Microbiol Lett* 226: 195–202, 2003.
316. Schultz J. Myeloperoxidase. *Ann N Y Acad Sci* 75: 22–30, 1958.
317. Schultz J and Kaminker K. Myeloperoxidase of the leucocyte of normal human blood, I: content and localization. *Arch Biochem Biophys* 96: 465–467, 1962.
318. Schwartz AG, Prysak GM, Bock CH, and Cote ML. The molecular epidemiology of lung cancer. *Carcinogenesis* 28: 507–518, 2007.
319. Selvaraj RJ, Zgliczynski JM, Paul BB, and Sbarra AJ. Enhanced killing of myeloperoxidase-coated bacteria in the myeloperoxidase-H_2O_2-Cl^- system. *J Infect Dis* 137: 481–485, 1978.
320. Shao B, Bergt C, Fu X, Green P, Voss JC, Oda MN, Oram JF, and Heinecke JW. Tyrosine 192 in apolipoprotein A-I is the major site of nitration and chlorination by myeloperoxidase, but only chlorination markedly impairs ABCA1-dependent cholesterol transport. *J Biol Chem* 280: 5983–5993, 2005.
321. Shepherd J, Hilderbrand SA, Waterman P, Heinecke JW, Weissleder R, and Libby P. A fluorescent probe for the detection of myeloperoxidase activity in atherosclerosis-associated macrophages. *Chem Biol* 14: 1221–1231, 2007.
322. Spencer JP, Whiteman M, Jenner A, and Halliwell B. Nitrite-induced deamination and hypochlorite-induced oxidation of DNA in intact human respiratory tract epithelial cells. *Free Radic Biol Med* 28: 1039–1050, 2000.

323. Stasia MJ and Li XJ. Genetics and immunopathology of chronic granulomatous disease. *Semin Immunopathol* 30: 209–235, 2008.
324. Stegeman CA, Tervaert JW, De Jong PE, and Kallenberg CG. Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis: Dutch Co-Trimoxazole Wegener Study Group. *N Engl J Med* 335: 16–20, 1996.
325. Stegeman CA, Tervaert JW, Sluiter WJ, Manson WL, De Jong PE, and Kallenberg CG. Association of chronic nasal carriage of *Staphylococcus aureus* and higher relapse rates in Wegener granulomatosis. *Ann Intern Med* 120: 12–17, 1994.
326. Stenvinkel P, Rodriguez-Ayala E, Massy ZA, Qureshi AR, Barany P, Fellstrom B, Heimburger O, Lindholm B, and Alvestrand A. Statin treatment and diabetes affect myeloperoxidase activity in maintenance hemodialysis patients. *Clin J Am Soc Nephrol* 1: 281–287, 2006.
327. Styczynska M, Religa D, Pfeffer A, Luczywek E, Wasiak B, Styczynski G, Peplonska B, Gabryelewicz T, Golebiowski M, Kobrys M, and Barcikowska M. Simultaneous analysis of five genetic risk factors in Polish patients with Alzheimer's disease. *Neurosci Lett* 344: 99–102, 2003.
328. Sugiyama S, Okada Y, Sukhova GK, Virmani R, Heinecke JW, and Libby P. Macrophage myeloperoxidase regulation by granulocyte macrophage colony-stimulating factor in human atherosclerosis and implications in acute coronary syndromes. *Am J Pathol* 158: 879–891, 2001.
329. Taioli E, Benhamou S, Bouchardy C, Cascorbi I, Cajas-Salazar N, Dally H, Fong KM, Larsen JE, Le Marchand L, London SJ, Risch A, Spitz MR, Stucker I, Weinshenker B, Wu X, and Yang P. Myeloperoxidase G-463A polymorphism and lung cancer: a HuGE genetic susceptibility to environmental carcinogens pooled analysis. *Genet Med* 9: 67–73, 2007.
330. Takizawa S, Aratani Y, Fukuyama N, Maeda N, Hirabayashi H, Koyama H, Shinohara Y, and Nakazawa H. Deficiency of myeloperoxidase increases infarct volume and nitrotyrosine formation in mouse brain. *J Cereb Blood Flow Metab* 22: 50–54, 2002.
331. Tang WH, Brennan ML, Philip K, Tong W, Mann S, Van Lente F, and Hazen SL. Plasma myeloperoxidase levels in patients with chronic heart failure. *Am J Cardiol* 98: 796–799, 2006.
332. Tang WH, Tong W, Troughton RW, Martin MG, Shrestha K, Borowski A, Jasper S, Hazen SL, and Klein AL. Prognostic value and echocardiographic determinants of plasma myeloperoxidase levels in chronic heart failure. *J Am Coll Cardiol* 49: 2364–2370, 2007.
333. Targonski PV, Bonetti PO, Pumper GM, Higano ST, Holmes DR Jr, and Lerman A. Coronary endothelial dysfunction is associated with an increased risk of cerebrovascular events. *Circulation* 107: 2805–289, 2003.
334. Tate RM and Repine JE. Neutrophils and the adult respiratory distress syndrome. *Am Rev Respir Dis* 128: 552–559, 1983.
335. ten Holder SM, Joy MS, and Falk RJ. Cutaneous and systemic manifestations of drug-induced vasculitis. *Ann Pharmacother* 36: 130–147, 2002.
336. Thomas EL and Fishman M. Oxidation of chloride and thiocyanate by isolated leukocytes. *J Biol Chem* 261: 9694–702, 1986.
337. Thomas EL, Grisham MB, and Jefferson MM. Cytotoxicity of chloramines. *Methods Enzymol* 132: 585–593, 1986.
338. Thomas EL, Grisham MB, and Jefferson MM. Preparation and characterization of chloramines. *Methods Enzymol* 132: 569–585, 1986.
339. Tonks NK. Redox redux: revisiting PTPs and the control of cell signaling. *Cell* 121: 667–670, 2005.
340. Trush A, Esterline RL, Mallet WG, Mosebrook DR, and Twerdok LE. Further evidence for the role of myeloperoxidase in the activation of benzo[a]pyrene-7,8-dihydrodiol by polymorphonuclear leukocytes. *Adv Exp Med Biol* 283: 399–401, 1991.
341. Trush MA, Seed JL, and Kensler TW. Reactive oxygen dependent activation of polycyclic hydrocarbons by phorbol ester-stimulated human polymorphonuclear leukocytes. *Carcinog Compr Surv* 8: 293–303, 1985.
342. Uchimura K, Nagasaka A, Hayashi R, Makino M, Nagata M, Kakizawa H, Kobayashi T, Fujiwara K, Kato T, Iwase K, Shinohara R, Kato K, and Itoh M. Changes in superoxide dismutase activities and concentrations and myeloperoxidase activities in leukocytes from patients with diabetes mellitus. *J Diabetes Complications* 13: 264–270, 1999.
343. Ueda T, Sakamaki K, Kuroki T, Yano I, and Nagata S. Molecular cloning and characterization of the chromosomal gene for human lactoperoxidase. *Eur J Biochem* 243: 32–41, 1997.
344. van der Vliet A, Eiserich JP, Halliwell B, and Cross CE. Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite: a potential additional mechanism of nitric oxide-dependent toxicity. *J Biol Chem* 272: 7617–7625, 1997.
345. van der Vliet A, Eiserich JP, Kaur H, Cross CE, and Halliwell B. Nitrotyrosine as biomarker for reactive nitrogen species. *Methods Enzymol* 269: 175–184, 1996.
346. Van Der Vliet A, Nguyen MN, Shigenaga MK, Eiserich JP, Marelich GP, and Cross CE. Myeloperoxidase and protein oxidation in cystic fibrosis. *Am J Physiol Lung Cell Mol Physiol* 279: L537–L546, 2000.
347. van Gisbergen KP, Geijtenbeek TB, and van Kooyk Y. Close encounters of neutrophils and DCs. *Trends Immunol* 26: 626–631, 2005.
348. van Gisbergen KP, Sanchez-Hernandez M, Geijtenbeek TB, and van Kooyk Y. Neutrophils mediate immune modulation of dendritic cells through glycosylation-dependent interactions between Mac-1 and DC-SIGN. *J Exp Med* 201: 1281–1292, 2005.
349. van Leeuwen M, Gijbels MJ, Duijvestijn A, Smook M, van de Gaar MJ, Heeringa P, de Winther MP, and Tervaert JW. Accumulation of myeloperoxidase-positive neutrophils in atherosclerotic lesions in LDLR−/− mice. *Arterioscler Thromb Vasc Biol* 28: 84–89, 2008.
350. van Tuinen P, Johnson KR, Ledbetter SA, Nussbaum RL, Rovera G, and Ledbetter DH. Localization of myeloperoxidase to the long arm of human chromosome 17: relationship to the 15; 17 translocation of acute promyelocytic leukemia. *Oncogene* 1: 319–322, 1987.
351. Vasilyev N, Williams T, Brennan ML, Unzek S, Zhou X, Heinecke JW, Spitz DR, Topol EJ, Hazen SL, and Penn MS. Myeloperoxidase-generated oxidants modulate left ventricular remodeling but not infarct size after myocardial infarction. *Circulation* 112: 2812–2820, 2005.
352. Vaz SM and Augusto O. Inhibition of myeloperoxidase-mediated protein nitration by tempol: kinetics, mechanism, and implications. *Proc Natl Acad Sci U S A* 105: 8191–8196, 2008.

353. Venturelli D, Bittenbender S, and Rovera G. Sequence of the murine myeloperoxidase (MPO) gene. *Nucleic Acids Res* 17: 7987–7988, 1989.
354. Vita JA, Brennan ML, Gokce N, Mann SA, Goormastic M, Shishehbor MH, Penn MS, Keaney JF Jr, and Hazen SL. Serum myeloperoxidase levels independently predict endothelial dysfunction in humans. *Circulation* 110: 1134–1139, 2004.
355. Wang Z, Nicholls SJ, Rodriguez ER, Kummu O, Horkko S, Barnard J, Reynolds WF, Topol EJ, DiDonato JA, and Hazen SL. Protein carbamylation links inflammation, smoking, uremia and atherogenesis. *Nat Med* 13: 1176–1184, 2007.
356. Weidner S, Neupert W, Goppelt-Struebe M, and Rupprecht HD. Antineutrophil cytoplasmic antibodies induce human monocytes to produce oxygen radicals in vitro. *Arthritis Rheum* 44: 1698–1706, 2001.
357. Weil SC, Rosner GL, Reid MS, Chisholm RL, Farber NM, Spitznagel JK, and Swanson MS. cDNA cloning of human myeloperoxidase: decrease in myeloperoxidase mRNA upon induction of HL-60 cells. *Proc Natl Acad Sci U S A* 84: 2057–2061, 1987.
358. Weiss SJ. Tissue destruction by neutrophils. *N Engl J Med* 320: 365–376, 1989.
359. Weitberg AB, Weitzman SA, Clark EP, and Stossel TP. Effects of antioxidants on oxidant-induced sister chromatid exchange formation. *J Clin Invest* 75: 1835–1841, 1985.
360. Weitberg AB, Weitzman SA, Destrempes M, Latt SA, and Stossel TP. Stimulated human phagocytes produce cytogenetic changes in cultured mammalian cells. *N Engl J Med* 308: 26–30, 1983.
361. Weitzman SA and Gordon LI. Inflammation and cancer: role of phagocyte-generated oxidants in carcinogenesis. *Blood* 76: 655–663, 1990.
362. Weitzman SA and Stossel TP. Mutation caused by human phagocytes. *Science* 212: 546–547, 1981.
363. Weitzman SA, Weitberg AB, Clark EP, and Stossel TP. Phagocytes as carcinogens: malignant transformation produced by human neutrophils. *Science* 227: 1231–1233, 1985.
364. Wendt TM, Tanji N, Guo J, Kislinger TR, Qu W, Lu Y, Bucciarelli LG, Rong LL, Moser B, Markowitz GS, Stein G, Bierhaus A, Liliensiek B, Arnold B, Nawroth PP, Stern DM, D'Agati VD, and Schmidt AM. RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy. *Am J Pathol* 162: 1123–1137, 2003.
365. Wheatley-Price P, Asomaning K, Reid A, Zhai R, Su L, Zhou W, Zhu A, Ryan DP, Christiani DC, and Liu G. Myeloperoxidase and superoxide dismutase polymorphisms are associated with an increased risk of developing pancreatic adenocarcinoma. *Cancer* 112: 1037–1042, 2008.
366. Whiteman M, Siau JL, and Halliwell B. Lack of tyrosine nitration by hypochlorous acid in the presence of physiological concentrations of nitrite: implications for the role of nitryl chloride in tyrosine nitration in vivo. *J Biol Chem* 278: 2380–2384, 2003.
367. Winterbourn CC. Comparative reactivities of various biological compounds with myeloperoxidase-hydrogen peroxide-chloride, and similarity of the oxidant to hypochlorite. *Biochim Biophys Acta* 840: 204–210, 1985.
368. Winterbourn CC, Hampton MB, Livesey JH, and Kettle AJ. Modeling the reactions of superoxide and myeloperoxidase in the neutrophil phagosome: implications for microbial killing. *J Biol Chem* 281: 39860–39869, 2006.
369. Winterbourn CC and Kettle AJ. Biomarkers of myeloperoxidase-derived hypochlorous acid. *Free Radic Biol Med* 29: 403–409, 2000.
370. Wipke BT and Allen PM. Essential role of neutrophils in the initiation and progression of a murine model of rheumatoid arthritis. *J Immunol* 167: 1601–1608, 2001.
371. Witko-Sarsat V and Descamps-Latscha B. Neutrophil-derived oxidants and proteinases as immunomodulatory mediators in inflammation. *Mediators Inflamm* 3: 257–273, 1994.
372. Woenckhaus C, Kaufmann A, Bussfeld D, Gemsa D, Sprenger H, and Grone HJ. Hypochlorite-modified LDL: chemotactic potential and chemokine induction in human monocytes. *Clin Immunol Immunopathol* 86: 27–33, 1998.
373. Wu X, Schabath MB, and Spitz MR. Myeloperoxidase promoter region polymorphism and lung cancer risk. *Methods Mol Med* 75: 121–133, 2003.
374. Xiao H, Heeringa P, Hu P, Liu Z, Zhao M, Aratani Y, Maeda N, Falk RJ, and Jennette JC. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest* 110: 955–963, 2002.
375. Xiao H, Heeringa P, Liu Z, Huugen D, Hu P, Maeda N, Falk RJ, and Jennette JC. The role of neutrophils in the induction of glomerulonephritis by anti-myeloperoxidase antibodies. *Am J Pathol* 167: 39–45, 2005.
376. Xiao H, Schreiber A, Heeringa P, Falk RJ, and Jennette JC. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am J Pathol* 170: 52–64, 2007.
377. Xu LL, Liu G, Miller DP, Zhou W, Lynch TJ, Wain JC, Su L, and Christiani DC. Counterpoint: the myeloperoxidase −463G->a polymorphism does not decrease lung cancer susceptibility in Caucasians. *Cancer Epidemiol Biomarkers Prev* 11: 1555–1559, 2002.
378. Yamada M. Myeloperoxidase precursors in human myeloid leukemia HL-60 cells. *J Biol Chem* 257: 5980–5982, 1982.
379. Yamada M, Hur SJ, Hashinaka K, Tsuneoka K, Saeki T, Nishio C, Sakiyama F, and Tsunasawa S. Isolation and characterization of a cDNA coding for human myeloperoxidase. *Arch Biochem Biophys* 255: 147–155, 1987.
380. Yang JJ, Preston GA, Pendergraft WF, Segelmark M, Heeringa P, Hogan SL, Jennette JC, and Falk RJ. Internalization of proteinase 3 is concomitant with endothelial cell apoptosis and internalization of myeloperoxidase with generation of intracellular oxidants. *Am J Pathol* 158: 581–592, 2001.
381. Yao C, Qin Z, Works KN, Austin GE, and Young AN. C/EBP and C-Myb sites are important for the functional activity of the human myeloperoxidase upstream enhancer. *Biochem Biophys Res Commun* 371: 309–314, 2008.
382. Zakrzewska-Pniewska B, Styczynska M, Podlecka A, Samocka R, Peplonska B, Barcikowska M, and Kwiecinski H. Association of apolipoprotein E and myeloperoxidase genotypes to clinical course of familial and sporadic multiple sclerosis. *Mult Scler* 10: 266–271, 2004.
383. Zeng J and Fenna RE. X-ray crystal structure of canine myeloperoxidase at 3 A resolution. *J Mol Biol* 226: 185–207, 1992.
384. Zernecke A, Bot I, Djalali-Talab Y, Shagdarsuren E, Bidzhekov K, Meiler S, Krohn R, Schober A, Sperandio M, Soehnlein O, Bornemann J, Tacke F, Biessen EA, and Weber C. Protective role of CXC receptor 4/CXC ligand 12 unveils

the importance of neutrophils in atherosclerosis. *Circ Res* 102: 209–217, 2008.
385. Zhang R, Brennan ML, Fu X, Aviles RJ, Pearce GL, Penn MS, Topol EJ, Sprecher DL, and Hazen SL. Association between myeloperoxidase levels and risk of coronary artery disease. *JAMA* 286: 2136–2142, 2001.
386. Zhao WG, Regmi A, Austin ED, Braun JE, Racine M, and Austin GE. Cis-elements in the promoter region of the human myeloperoxidase (MPO) gene. *Leukemia* 10: 1089–103, 1996.
387. Zheng L, Nukuna B, Brennan ML, Sun M, Goormastic M, Settle M, Schmitt D, Fu X, Thomson L, Fox PL, Ischiropoulos H, Smith JD, Kinter M, and Hazen SL. Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. *J Clin Invest* 114: 529–541, 2004.

Address correspondence to:
Dr. Peter Heeringa
Department of Pathology and Medical Biology
EA11
University Medical Center Groningen
Hanzeplein 1
9713 GZ Groningen
The Netherlands

E-mail: p.heeringa@med.umcg.nl

Date of first submission to ARS Central, February 25, 2009; date of final revised submission, July 1, 2009; date of acceptance, July 21, 2009.

Abbreviations Used

4-ABAH = 4-amino benzoic acid hydrazide
AD = Alzheimer's disease
AGE = advanced glycation end products
ANCA = antineutrophil cytoplasmic autoantibodies
BALF = bronchoalveolar lavage fluid
BLI = bioluminescence imaging
CAD = coronary artery disease
CF = cystic fibrosis
CGD = chronic granulomatous disease
CVD = cardiovascular disease
EAE = experimental autoimmune encephalomyelitis
ELISA = enzyme-linked immunosorbent assay
ER = estrogen receptor
GBM = glomerular basement membrane
GN = glomerulonephritis
HDL = high-density lipoprotein
HF = heart failure
H_2O_2 = hydrogen peroxide
HO-1 = heme-oxygenase-1
HOCl = hypochlorous acid
IBD = inflammatory bowel disease
IFN-γ = interferon-γ
IL = interleukin
iNOS = inducible nitric oxide synthase
I/R = ischemia/reperfusion
LDL = low-density lipoprotein
LDLR = LDL receptor
LPS = lipopolysaccharide
MAPK = mitogen-activated protein kinase
MGN = membranous glomerulonephritis
MI = myocardial infarction
mMPO = murine MPO
MPO = myeloperoxidase
MRI = magnetic resonance imaging
MS = multiple sclerosis
NF-κB = nuclear factor κB
NO = nitric oxide
PD = Parkinson's disease
PDB = Protein Data Bank
PPAR = peroxisome proliferation-activating receptor
Pr3 = proteinase 3
RA = rheumatoid arthritis
Rag2 = recombinase-activating gene-2
ROS = reactive oxygen species
SCN^- = thiocyanate
SNP = single-nucleotide polymorphism
SOD = superoxide dismutase
SVV = small-vessel vasculitis
TNF-α = tumor necrosis factor-α