Myeloperoxidase in kidney disease

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In glomerular and tubulointerstitial disease, polymorphonuclear- and monocyte-derived reactive oxygen species may contribute to oxidative modification of proteins, lipids, and nucleic acids. In part, the processes instigated by reactive oxygen species parallel events that lead to the development of atherosclerosis. Myeloperoxidase (MPO), a heme protein and catalyst for (lipo)protein oxidation is present in these mononuclear cells. The ability of MPO to generate hypochlorous acid/hypochlorite (HOCl/OCl\(^{-}\)) from hydrogen peroxide in the presence of chloride ions is a unique and defining activity for this enzyme. The MPO-hydrogen peroxide-chloride system leads to a variety of chlorinated protein and lipid adducts that in turn may cause dysfunction of cells in different compartments of the kidney. The aim of this article is to cover and interpret some experimental and clinical aspects in glomerular and tubulointerstitial diseases in which the MPO-hydrogen peroxide-chloride system has been considered an important pathophysiologic factor in the progression but also the attenuation of experimental renal disease. The colocalization of MPO and HOCl-modified proteins in glomerular peripheral basement membranes and podocytes in human membranous glomerulonephritis, the presence of HOCl-modified proteins in mononuclear cells of the interstitium and in damaged human tubular epithelia, the inflammation induced and exacerbated by MPO antibody complexes in necrotizing glomerulonephritis, and the presence of HOCl-modified epitopes in urine following hyperlipidemia-induced renal damage in rodents suggest that MPO is an important pathogenic factor in glomerular and tubulointerstitial diseases. Specifically, the interaction of MPO with nitric oxide metabolism adds to the complexity of actions of oxidants and may help to explain bimodal partly detrimental partly beneficial effects of the MPO-hydrogen peroxide-chloride system in redox-modulated renal diseases.

Neutrophilic granulocytes are one of the most professional phagocytes that represent the first line of defense against invading bacteria, viruses, and fungi. Neutrophils ingest microorganisms into intracellular compartments called phagosomes, to which they direct an arsenal of cytotoxic agents. Phagocytosing neutrophils undergo a burst of oxygen consumption via the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex leading to generation of superoxide anions plus a range of other reactive oxygen (hydroxyl radicals, hydrogen peroxide, hypochlorous acid) and reactive nitrogen species (nitric oxide radicals, peroxynitrite) (for review see [1]). Observations in humans and animals indicated that myeloperoxidase (MPO) holds a central role in microbial killing. Up-regulation of MPO gene expression in activated phagocytes would seem consistent with the presumed antimicrobial function of the enzyme in these cells. Recent investigations revealed a crucial role of MPO in chronic, nonmicrobial inflammatory processes such as neurodegenerative disease and atherosclerosis (for review see [2]). In addition, epidemiologic data supported a relationship between MPO polymorphism in humans and the risk for lung, larynx, and esophageal cancer and cardiovascular disease in end-stage renal disease (ESRD) patients. Since phagocytes (neutrophils, monocytes, and macrophages, respectively) are considered essential effector cells in (auto)-immunologic and degenerative kidney diseases, it is reasonable to assume a contribution of MPO during these processes. This review summarizes the biochemical features of MPO and presents recent evidence for MPO as a catalyst for oxidation in the pathophysiology of kidney diseases; furthermore, reactions that connect the MPO-hydrogen peroxide-chloride system with pathways of other reactive oxygen and nitrogen species will be described in the context of renal diseases.

**Key words:** glomerulonephritis, MPO-hydrogen peroxide-chloride system, renal disease, hypochlorous acid/hypochlorite, chloramines, advanced glycation end products, LOX-1.

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MYELOPEROXIDASE

The glycosylated arginine-rich, extremely basic MPO protein [isoelectric point (IP) > 10] [1, 3] is comprised of two subunits, encoded within a single mRNA. The approximately 83 kD precursor polypeptide is posttranslationally processed to produce a 59 to 64 kD heavy subunit and a 14 kD light subunit. Two of each subunits are
Fig. 1. Three major pathways leading to formation of reactive oxidants via the myeloperoxidase (MPO)-hydrogen peroxide-system. Adapted and modified from references [2, 10]. Abbreviations are: Tyr, tyrosine; Tyr°, tyrosyl radical; NO₂°, nitrogen dioxide radical; NO₂⁻, nitrite.

assembled with heme molecules to produce the functional enzyme (donor: hydrogen peroxide, oxidoreductase, EC 1.11.1.7). MPO is stored in primary azurophilic granules of leukocytes and the enzyme accounts for up to 5% and 1% of total cell protein content, in neutrophilic polymorphonuclear leukocytes (neutrophils) and monocytes, respectively. Although earlier reports suggested that neither MPO mRNA nor protein is present in mature macrophages, MPO is present in various macrophage subpopulations (e.g., Kupffer cells [4], alveolar macrophages and microglia [5]), and macrophages present in advanced atherosclerotic lesions [6–8] hinting at a specific function of MPO during host defense, tumor surveillance, and inflammation. Alternatively, macrophages can ingest (senescent) neutrophils or neutrophil granules that provide the macrophages with this neutrophil peroxidase. Macrophages exposed to enzymatically active MPO exhibited enhanced secretion of cytokines including tumor necrosis factor α (TNF-α), interleukin-1 (IL-1), and interferons, and increased capacity to phagocytose and to kill microorganisms.

Following phagocyte activation by a variety of stimuli, leading to generation of oxygen, superoxide anion radical and its dismutation product, hydrogen peroxide, MPO is secreted into both the phagolysosomal compartment and the extracellular milieu. A kinetic model for MPO suggested that at ground state, MPO exists in the ferri [Fe(III)] form; however, different stages of activation depending on the respective ligand have been proposed [2, 3]. At physiologic plasma concentrations (approximately 100 mmol/L), chloride is a preferred substrate for MPO and hypochlorous acid/hypochlorite (HOCl/OCl⁻), a potent chlorinating oxidant, is formed. Both, MPO and HOCl appear critical for oxidative killing. Neutrophils isolated from the blood of MPO-deficient individuals kill poorly a variety of microorganisms and inhibitors of MPO impair killing by normal cells. Excessive production of HOCl causes tissue damage and scavenging of HOCl by taurine significantly inhibits or reverses the deleterious effect of this highly reactive oxidant. Indeed, under in vivo and in vitro conditions, HOCl reacts with a wide range of oxidizable biomolecules containing thiols, nitrogen compounds, or unsaturated double carbon bonds. The main biologic chlorination reactions are with pyridine nucleotides, cholesterol, and unsaturated lipids to give chlorohydrins and with amine groups to give chloramines, which are in turn powerful oxidants (Fig. 1). However, under acidic conditions and in the presence of chloride ions the formation of chlorine gas is favoured. Hazen et al [9] could show that human neutrophils employ chlorine gas as an oxidant during phagocytosis and that chlorine gas derived from HOCl is the chlorinating intermediate in the oxidation of cholesterol and cholesterol-enriched low density lipoprotein, respectively.
Human phagocytes use the MPO-hydrogen peroxide-chloride system to generate a family of tyrosyl radical addition products [10] (Fig. 1). Both chlorinated tyrosines (e.g., 3-chlorotyrosine and 3,5-dichlorotyrosine) and chlorohydrins may be used as biomarkers for the estimation of chlorinated molecules [11]. Independent immunohistologic confirmation of a role for chlorinating oxidants, primarily chloramines, may be achieved with specific monoclonal antibodies [7, 12] in those tissues susceptible to MPO-mediated chloramine formation [7, 13–15]. In addition to generation of reactive oxygen species, MPO-generated nitrogen species may be considered to represent alternative biomarkers for the effect of MPO [16] (Fig. 1). MPO may use nitrite, the major end product of nitric oxide radical metabolism, as a substrate to nitrate protein tyrosine residues and to initiate lipid peroxidation; nitrogen dioxide radical, the one electron oxidation product of nitrite, has been implicated in this process [17]. MPO may also generate a nitrating intermediate through secondary reaction of HOCl with nitrite, presumably forming nitryl chloride as reactive intermediate. Thus, MPO-dependent direct and indirect generation of reactive nitrogen species could provide an alternative route leading to formation of proatherogenic and proinflammatory (lipo)protein nitration adducts commonly accomplished by peroxynitrite. Peroxynitrite, formed from nitric oxide and superoxide anion radical, may lead to (lipo)protein nitration and peroxidation in the presence of carbon dioxide, presumably via intermediate formation of nitrosoperoxocarbonate and subsequent formation of nitrogen dioxide radical [16, 17]. From these observations, it is intriguing to speculate that the MPO pathway is coupled to the peroxynitrite pathway and that compensatory mechanisms between both pathways may be functional in the kidney probably under diabetic conditions. Reports on peroxynitrite-induced injury in diabetic nephropathy have been published [18] and treatment of nonobese diabetic mice with peroxynitrite scavengers prevented the development of diabetes mellitus in these animals [19]. A recent in vitro study performed in proximal tubular epithelial cells revealed that high levels of glucose cause generation of peroxynitrite leading to caspase-mediated apoptosis in these cells [20].

Indeed, the role of glucose in oxidative stress-induced kidney injury has been extensively documented. Chronic hyperglycemia contributes to diabetic complications through the formation of advanced glycation end products (AGEs), which are irreversibly formed biochemical end products of nonenzymatic glycation. In vitro experiments have shown that synthesis of acrolein, 2-hydroxypropanal, and glycolaldehyde required chloride, hydrogen peroxide, and MPO, respectively. Also activated neutrophils employ MPO to generate these aldehydes. In particular, α-hydroxylaldehyde, formed from L-serine, mediates protein cross-linking and formation of Nε-(carboxymethyl)lysine, a characteristic AGE [21]. These observations were the first to indicate that the MPO-hydrogen peroxide-chloride system of human phagocytes contributes to formation of AGEs which could act as mediators of inflammation and monocyte activation in chronic renal failure. The candidate receptor for AGEs (RAGE) is expressed in rat, mouse, and human mesangial cells. The functional in vivo importance of the AGE-RAGE system in the development of diabetic nephropathy is underlined in a transgenic mouse model overexpressing RAGE. When these mice were made diabetic by crossbreeding with another transgenic mouse model deficient in the islet production of insulin, the resultant double transgenic animals developed renal insufficiency and advanced glomerulosclerosis having some resemblance to human diabetic nephropathy [22].

**EXPRESSION OF MPO IN KIDNEY DISEASE**

Many authors demonstrated the presence of MPO-containing cells as well as MPO protein and activity in many renal diseases [14, 23–25]. The adherence of neutrophils to the glomerular basement membrane and the degradation of the basement membrane by oxidants at sites of attachment [26] pointed toward a direct involvement of MPO. In vivo experiments (i.e., perfusion with MPO followed by nontoxic concentrations of hydrogen peroxide and chloride ions) revealed MPO-mediated glomerular disease resulting in glomerular morphologic changes, endothelial and mesangial cell injury, activation of platelets, and subsequent proliferative responses mimicking inflammatory and proliferative glomerulonephritis in humans [27].

Originally, MPO activity from inflamed kidneys could not be demonstrated in a model of pyelonephritis even though the presence of large numbers of neutrophils was confirmed histologically [28]. However, the use of specific techniques allowed the assessment of MPO activity in whole kidney tissues by measuring hydrogen peroxide-dependent oxidation of 3,3′,5,5′-tetramethylbenzidine [29] or o-dianisidine [23]. Hillegass et al [23] finally demonstrated that MPO activity from inflamed kidney was significantly greater than that from control tissue. Western blotting experiments revealed immunoreactive MPO heavy subunit in nephrectomy specimens from controls and patients with nephrosclerosis but to a higher extent in nephrectomies from patients with interstitial nephritis, pyelonephritis, and reflux nephropathy [14]. Immunohistochemistry confirmed the presence of MPO in interstitial cells and atrophic epithelial cells. Colocalization of MPO and HOCl-modified epitopes at the glomerular basement membrane in membranous glomerulonephritis [14, 15] supported the participation of the MPO-hydrogen peroxide-chloride system in glomerular dysfunction [27, 30]. Measurement
of urinary MPO was discussed as a possible noninvasive diagnostic marker for renal graft monitoring [31], although the specificity of this test vis a vis bacterial infection can be doubted. Nevertheless, the presence of HOCl-modified epitopes in urine is indicative for neutrophil-/monocyte-mediated respiratory oxidative burst and tubulointerstitial damage in the kidney [32].

**MPO deficiency in mice and humans**

While the detection and quantification of MPO-dependent oxidants may give an insight into a disease process, studies on MPO knockout (MPO−/−) animals might elucidate mechanisms of tissue damage mediated by the MPO-hydrogen peroxide-chloride system; in addition, adaptive responses to this damage might be recognized. MPO−/− mice with different genetic backgrounds have been generated [33, 34] and extensively evaluated in various pathologic processes. Yet, recent findings with MPO−/− mice in a model of atherosclerosis show that this mouse model does not necessarily mirror the proatherogenic action of MPO. Evidence for protein chlorination, a marker for MPO activity, was neither found in atherosclerotic plaques of wild-type nor MPO−/− mice. Surprisingly, the lack of MPO in MPO−/− mice was accompanied by an increased plaque formation [34]. This unexpected proatherogenic effect might reflect species differences in pathways involved in the generation of reactive oxygen species and consecutive tissue reaction. Generally, mice have lower MPO levels in neutrophils than humans (about 10% to 20% of human levels). Furthermore, neutrophils of rodents produce more nitric oxide radical than human neutrophils. Thus, MPO-mediated nitrating reactions that exert important effector functions could be compensated for in the absence of MPO by nitric oxide–dependent pathways via formation of peroxynitrite. The fact that MPO also uses nitric oxide as a substrate and might hence prevent nitric oxide–dependent oxidative reactions could then represent an alternative hypothetical explanation for the protective effect of MPO in mice during development of atherogenesis. Determination of protein (tyrosine) nitration in mouse models with MPO deficiency could indicate these compensating oxidant pathways. This was shown in MPO−/− mice undergoing brain reperfusion injury in which an increased infarct volume and elevated nitrotyrosine levels were found 2 hours after cerebral ischemia as compared to controls [35]. However, analysis of the effect of MPO on the myocardial responses to acute myocardial infarction 72 hours after ligation of the left anterior descending coronary artery revealed that MPO−/− mice exhibited significantly decreased leukocyte infiltration of the infarct area and delayed earlier death to myocardial rupture [36].

Another possibility to gain insight into functional aspects of the MPO-hydrogen peroxide-chloride system is to study differences between MPO-deficient patients and controls. Decreased or deficient MPO activity might be acquired, as shown in case reports in some hematologic diseases (e.g., in acute myelocytic leukemia or in myelodysplastic syndromes), or more commonly inherited. Primary MPO deficiency is a relatively frequent event with a prevalence of 2.5 to 7.5 per 10000 people [37]. It can be attributed to DNA missense mutations/deletions, as well as to impairment of transcriptional activity or attributed to mutations leading to defective proteolytic processing [38–42]. Generally, hereditary MPO deficiency has been considered an autosomal-recessive trait; however, some pedigrees did not show a simple Mendelian mode of inheritance [38, 43]. Most individuals with inherited MPO deficiency have a normal phenotype and do not suffer from a markedly increased risk of infections [44, 45]. Nevertheless, a significant higher occurrence of severe infections and chronic inflammatory processes in states with an additional impairment of immune function (e.g., in diabetes mellitus) was noted. Clinical and laboratory data on acquired MPO deficiency showed a high incidence of diabetes mellitus and thrombotic diseases, as well as a strikingly constant hyperfibrinogenemia in these patients; findings that may indicate the importance of the relationship between neutrophilic granulocytes/monocytes and blood coagulation [46].

Obviously under physiologic conditions, the lack of MPO activity is compensated for by other mechanisms (e.g., increased phagocytic activity and production of superoxide anion radical) [47, 48]. Investigating the course of immunologic or degenerative renal diseases in MPO-deficient patients should give valuable insights into the role of MPO in these diseases. In humans, elevated levels of leukocyte- and blood-MPO are clearly associated with coronary artery disease [49] and data for a protective effect of MPO deficiency against cardiovascular damage were reported [45]. MPO levels are increasing with age and the A-allele of the G-463-A MPO polymorphism was significantly associated with increased levels of apolipoprotein B, total cholesterol, low-density lipoprotein cholesterol, and triglycerides suggesting a possible implication of MPO in influencing the risk of cardiovascular disease [50]; this mutation has a much higher transcription than the alternative allele [41]. In humans, MPO may act as a catalyst for (lipo)protein oxidation [6] and the detrimental impact of MPO-dependent oxidative stress on vascular disease in humans is well established [2].

**MPO in ischemia/reperfusion injury**

Ischemia/reperfusion damage is an important process in kidney transplantation and can cause delayed graft function [51]. In the kidney, neutrophils seem
to be effector cells, although protection against ischemia/reperfusion has not been obtained in all published studies in animals depleted of neutrophils [52]. Other nonneutrophil-dependent mechanisms evidently contribute to renal damage and monocytes/macrophages seem also to be involved in this process. Nevertheless neutrophil influx into the kidney is a dominant feature of experimental ischemia/reperfusion [53]. The fact that these infiltrating cells are actively engaged in tissue damage is supported by observations that knockout mice lacking adhesion molecules (i.e., platelet P-selectin or intercellular adhesion molecule-1) show reduced neutrophil influx into the kidney and are partially protected against reperfusion injury. Similar results were obtained with antibodies known to block neutrophil adhesion [53]. Migration of neutrophils into the injured kidney following reperfusion leads to increased renal activity of MPO [54] suggesting a contribution of MPO-dependent chlorinated or nitrated species to kidney damage. The important role of the MPO-hydrogen peroxide-chloride system in ischemia/reperfusion injury of solid organs is underlined by the fact that tauroine, an important in vivo scavenger of HOCl, prevented neutrophil-mediated damage in an ex vivo model investigating ischemia/reperfusion injury in isolated guinea pig hearts. Furthermore, addition of tauroine ameliorated ischemic preservation of isolated rat kidneys [55]. On the other hand, systemic application of tauroine did not prevent renal microvascular injury following lower limb reperfusion in rats, although being effective in ameliorating pulmonary microvascular injury [56].

Moreover, hydrogen peroxide, the prerequisite for the generation of MPO-derived oxidants, is present in high levels following kidney reperfusion. The main source for the generation of hydrogen peroxide in the kidney following ischemia/reperfusion is, however, not known. Superoxide anion radical and its dismutation product, hydrogen peroxide, may be generated via membrane NADPH oxidases or produced by the mitochondrial NAD(P)H dehydrogenase complex in phagocytic (neutrophils) or nonphagocytic cells (epithelium, endothelium, or fibroblasts) [57, 58]. Another mechanism of hydrogen peroxide formation by elevated activity of xanthine oxidase has not been proven to be relevant in human kidney reperfusion injury. However, in rodent kidneys, ischemia/reperfusion induces the conversion of xanthine dehydrogenase, which uses oxidized NAD as electron acceptor, into xanthine oxidase, which in contrast uses oxygen as a substrate [59–61]; the time, though, needed for conversion exceeds the time of reperfusion-induced generation of reactive oxygen species. Since adenosine triphosphate (ATP) is consumed during ischemia, the purine catabolites xanthine and hypoxanthine may accumulate and in the presence of oxygen, superoxide anion radical, and hydrogen peroxide could be generated by xanthine oxidase during reperfusion (for review see [62]).

However, in normal human renal tissue, only low levels of xanthine oxidase were measured [63, 64]. In addition minor accumulation of xanthine in human kidneys occurred during ischemia suggesting a low activity of xanthine oxidase and/or dehydrogenase. Nevertheless xanthine oxidase localized in renal endothelial cells [65] could at least in part contribute to microvascular oxidative injury following reperfusion. Finally, monoamine oxidases have been discussed as additional sources of hydrogen peroxide generation in ischemia/reperfusion [66, 67]. Since substantial levels of monoamine oxidase are present in human kidney these enzymes could be likely candidates for hydrogen peroxide supply during ischemia/reperfusion.

To date, the role of the MPO-hydrogen peroxide-chloride system in renal ischemia/reperfusion injury has not been sufficiently documented. The interaction of MPO with other important pro-oxidant systems in this process is not well defined (e.g., the interaction of MPO and inducible nitric oxide synthase in ischemia/reperfusion has not been clarified). The latter enzyme is recognized as an important cofactor in ischemia/reperfusion injury [68–70]. Nitric oxide, a relatively long-lived free radical, is involved in MPO-dependent oxidative capacity. As shown for MPO also, the inducible form of nitric oxide synthase colocalizes in the primary granules of leukocytes, and following phagocytic activation, the enzyme is secreted into the phagolysosome and extracellular compartments, and nitration of bacterial proteins can take place. In contrast to intraphagosomal chlorination aromatic nitration in neutrophils is negligible. The selectivity is different in extracellular media, however, where MPO-catalyzed phenolic nitration could possibly compete with chlorination [71]. Elevated nitrotyrosine levels present after renal ischemia and reperfusion may serve as a marker for nitric oxide–dependent oxidative stress either via formation of peroxynitrite or via generation of nitrating species by direct and/or indirect action of MPO [68, 70]. The lack of the inducible form of nitric oxide synthase, either following specific inhibition or gene knockout, resulted in a marked reduction of MPO activity in mice subjected to renal ischemia/reperfusion injury indicating reduced neutrophil infiltration; furthermore, inhibition of the inducible nitric oxide synthase significantly decreased protein nitration and renal injury caused by ischemia/reperfusion in rats and mice [70].

**MPO in degenerative glomerular damage**

*Lipid-associated glomerulopathy.* Monocytes, important effector cells in lipid-associated glomerulopathy, were shown to infiltrate glomeruli during chronic
hyperlipidemia [72]. These cells are recruited by chemotactic signals (e.g., monocyte chemotactic protein-1 and macrophage colony-stimulating factor), and expression of these signals in mesangial cells is induced by different lipoprotein particles. The expression of CCR2, the receptor for monocyte chemotactic protein-1, is up-regulated by native and modified lipoproteins.

In the kidney, monocytes represent the major source of MPO in lipid-associated glomerulopathy a process very similar to lipid-associated development of atherosclerosis; MPO is associated extracellularly and intracellularly with monocytes/macrophages in different stages of lesion severity [7]. HOCl-modified (lipo)proteins and apolipoproteins have been localized in human atheroma [7, 13, 73] and in material from lesions of homozygous and heterozygous low-density lipoprotein receptor-deficient rabbits and rabbits with high response to atherosclerosis after dietary cholesterol intake [74, 75]. The release of reactive oxygen species by monocytes evidently can lead to oxidative modification of lipoproteins, which consequently perpetuate the process of disease. Oxidized-low density lipoproteins can induce adhesion of monocytes to glomerular endothelium and HOCl-modified low-density lipoprotein stimulates production of IL-8 [76]. IL-8, being also chemotactic for monocytes and T lymphocytes, stimulates human polymorphonuclear neutrophils for enhanced production of reactive oxygen metabolites, enzyme secretion, and adhesion to endothelial cells [77]. Endothelial cells are unable to generate HOCl via the MPO-hydrogen peroxide-chloride system themselves, however, MPO derived from circulating leukocytes may bind to endothelial cells and endothelial transcytosis of MPO confers specificity to subendothelial matrix proteins as targets of tyrosine nitration [78]. Recent findings revealed that low-density lipoprotein, modified by HOCl in vitro, inhibits nitric oxide synthesis in endothelial cells via intracellular dislocalization of the endothelial nitric oxide synthase [79], an important mechanism in the development of endothelial dysfunction.

Although different groups and subclasses of scavenger receptors do exist, to date, only lectin-like oxidized low-density lipoprotein (LOX-1) and scavenger receptor class B type I (SR-BI) have been identified as candidate receptors to mediate holoparticle or selective cholesteryl ester uptake from HOCl-modified lipoproteins on endothelial cells [80]. A recent study in passive Heymann nephritis, a rat model for human membranous glomerulonephritis, reported that cholesteryl ester increments in glomeruli and proximal tubular segments were associated with decreased SR-BI expression [81]. These observations support the notion that this receptor is primarily contributing to cholesteryl/cholesterol ester hemeostasis in the kidney. It has not been studied whether HOCl-modified (lipo)proteins may severely impair SR-BI-mediated bidirectional cholesterol flux in the kidney as discussed for peripheral tissues and the liver, respectively [73]. Expression of LOX-1 is increased in ischemia/reperfusion of rat kidney [82] and diabetes-associated conditions in vitro and in vivo [83]. As LOX-1 also serves as a receptor for AGEs, binding of MPO-generated adducts via LOX-1 may similarly introduce oxidative stress, reduce nitric oxide, promote generation of superoxide anion radical, and activate nuclear factor-kappaB (NF-κB), resulting in changes in gene expression (chemokines and adhesive molecules) and cellular phenotype (activation or apoptosis) in the kidney. LOX-1 expression is also up-regulated in experimental hypertensive glomerulosclerosis and in the remnant kidney, an established rat model for chronic renal failure. Staining for LOX-1 was primarily observed in the interstitial cells [84]. Reduction of blood pressure by specific angiotensin II type I receptor antagonists was associated with suppressed renal LOX-1 expression [84]. Taurine protected against renal damage induced by salt loading in Dalt salt-sensitive rats; this was accompanied by suppression of LOX-1 [85], although the exact pathophysiologic meaning of these associations has not been elucidated.

**Diabetic nephropathy.** Blood neutrophils in diabetic patients show reduced MPO activity [86]; however, the activity is restored after stimulation of neutrophils with formyl-methionyl-leucyl-phenylalanine [87]. Since MPO-derived oxidants are capable of forming AGEs [21, 88], a link between the MPO-hydrogen peroxide-chloride system, the generation of AGEs and subsequent interaction with RAGE may be assumed. RAGE is expressed at low abundance in renal endothelial cells, podocytes or parietal epithelium; however, its expression is up-regulated in these cells under various pathologic conditions, including diabetic nephropathy [89]. Binding of AGEs to RAGE leads to prolonged activation of NF-κB in mononuclear and endothelial cells [90]. Up-regulation of proinflammatory genes ensues on polymorphonuclear neutrophils, monocytes/macrophages, and endothelial cells such as expression of adhesion molecules (e.g., intercellular adhesion molecule type 1, vascular cell adhesion molecule 1, or E-selectin) [91], which enhance the adhesion and transmigration of mononuclear cells.

Interestingly, pronounced MPO activity has been detected in epiretinal membranes of patients suffering from proliferative diabetic retinopathy in contrast to membranes of patients with proliferative vitreoretinopathy and macular pucker, pointing toward a significant role of MPO in this diabetic vascular complication [92]. However, increased MPO activity has not been reported in diabetic nephropathy. It is still speculative to regard MPO as a relevant pathogenetic factor for renal complications in diabetes. Epidemiologic studies in diabetic patients with MPO deficiency would represent a valuable cohort to clarify the role of MPO.
MPO in immunologic glomerular disease

Membranous glomerulonephritis. The pathogenesis of human membranous glomerulonephritis has not been fully elucidated although major aspects were deduced from passive Heymann nephritis where reactive oxygen species seem to play a prominent role in the induction of pathophysiologic sequelae such as proteinuria [93, 94]. In passive Heymann nephritis, glomerular epithelial cells may generate hydrogen peroxide by expressing NAD(P)H-dependent oxidoreductase in analogy to neutrophilic granulocytes during the respiratory burst reaction. Evidence for a role of the MPO-hydrogen peroxide-chloride system in the synthesis of reactive oxygen species in damaged glomerular basement membranes from patients with membranous glomerulonephritis [15] was based on immunohistochemistry of HOCl-modified proteins/epitopes colocalizing with immune deposits in affected glomeruli (Fig. 2). In parallel, staining intensity for HOCl-modified epitopes highly correlated with staining intensity for MPO in glomerular basement membranes and the presence of mononuclear cell infiltrates, respectively. This indicates a substantial role of these cells during development of human membranous glomerulonephritis [15]. This must be seen in the context that monocytes, not generally accumulating in membranous glomerulopathy [95, 96], can apparently contribute to the pathogenesis in later stages of the disease with deteriorating kidney function. There is only scant knowledge about MPO in other forms of immune complex glomerulonephritis (e.g., IgA nephritis). HOCl-modified (lipo)proteins were identified in urine of hyperlipidemic rats with mesangioproliferative glomerulonephritis but not in normolipidemic animals with glomerulonephritis [32].

Antineutrophil cytoplasmic antibody (ANCA)-positive small vessel disease-necrotizing glomerulonephritis. In ANCA-positive necrotizing glomerulonephritis pronounced glomerular infiltration with neutrophils and monocytes is found [24, 97]. MPO- and proteinase 3-ANCAs, which bind to MPO or proteinase 3, respectively, recognize specific epitopes on the surface of polymorphonuclear neutrophils. It is generally assumed that they are a common feature of vasculitis of small vessels. ANCAs are suggested to contribute to the pathogenesis of this disease [98–100]. At least in the case of MPO-ANCAs, recent findings strongly indicated a causative role: Splenocytes from MPO−/− mice immunized against MPO were injected into Rag−/− mice (lacking functional lymphocytes) and induced a crescentic glomerulonephritis with a paucity of immune deposits and a systemic necrotizing vasculitis; an analogous effect occurred following application of purified anti-MPO IgG [101]. Whether ANCAs directed against MPO or against proteinase 3 induce different effects has not been reported.

Neutrophils are activated by ANCAs; this activation leads to enhanced binding of polymorphonuclear neutrophils to the endothelial layer and to production of superoxide anion radical and hydrogen peroxide during the oxidative burst [98, 102, 103]. Hydrogen peroxide may serve for the generation of HOCl and for nitric oxide synthase-independent, nonenzymatic formation of nitric
Chemotaxis, activation

Fig. 3. Myeloperoxidase (MPO)- or proteinase-3 (PR3)-directed antineutrophil cytoplasmic antibodies (ANCAs) bind to neutrophils and lead to stimulation. Furthermore, apoptotic bodies of neutrophils are opsonized by ANCAs and are phagocytosed subsequently by macrophages. Activation of macrophages results in secretion of chemotactic factors that enhance neutrophil influx. Tumor necrosis factor-α (TNF-α) released by macrophages is propagating neutrophil apoptosis while cytokines [interleukin-1 (IL-1) and interleukin-8 (IL-8)] mediate chemotaxis. MPO released by activated neutrophils during the oxidative burst generates HOCl while nitric oxide radical (NO) is generated by nitric oxide synthases [inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS)]. Reactive nitrogen intermediates (NOx), also generated by MPO may lead to endothelial damage. Inactivation of MPO by ceruloplasmin is prevented by MPO-ANCAs.

oxide [104]. Thus, ANCA-stimulated neutrophils are producing a variety of oxidants leading to endothelial damage [105]. Moreover MPO-ANCA prevents inactivation of MPO by ceruloplasmin, a plasmatic binding inhibitor of MPO [106]; this contributes to a higher MPO activity and increased generation of HOCl at sites of inflammation. ANCA-stimulated neutrophils release epitopes into supernatant that are recognized by MPO-ANCAs and that bind to the cell surface of unstimulated neutrophils; the binding of these epitopes on unstimulated neutrophils renders them responsive to activation by MPO-ANCAs [103]. Whether the surface structure on neutrophils recognized by MPO-ANCAs corresponds to intact and enzymatically active MPO is, however, unknown.

Following activation by ANCAs, polymorphonuclear leukocytes can undergo apoptosis. Apoptotic bodies are opsonized by ANCAs and phagocytosed efficiently by macrophages [102] (Fig. 3). Apoptotic neutrophils opsonized with proteinase 3-ANCAs stimulated phagocytic activity and production of TNF-α in macrophages [107]. Vice versa TNF-α induced apoptosis and expression of epitopes, which are recognized by both MPO- and proteinase 3-ANCAs in neutrophils [108]. Furthermore, IL-1 or IL-8, consecutively secreted from activated macrophages, help to sustain the inflammatory process [102]. These cytokines are chemotactic for neutrophils and may lead to faster neutrophil-influx into the inflamed site, thus providing a self-amplifying inflammatory process.

Epidemiologic studies in MPO-deficient patients. Recently, substantial progress has been made in the characterization of connections between the MPO-hydrogen peroxide-chloride system and other oxidative pathways so that a very complex tableau of interacting reactions is emerging. To further elucidate the biologic relevance and consequence of different pathways well-defined in vivo models are required. However, as already mentioned, observations in MPO−/− mice have shown interspecies differences for oxidative pathways, suggesting that observations from animal models can only be transferred very carefully to humans. Thus, a most promising alternative may be provided by epidemiologic studies in MPO-deficient patients.

Modulation of the MPO-hydrogen peroxide-chloride system for treatment of inflammatory/degenerative kidney diseases

Studies with MPO−/− mice indicated possible MPO-mediated beneficial effects in inflammatory/degenerative
The protective effect of this nonprotein sulfur containing amino acid in rat models of human kidney disease [114] and attenuation of age-related renal interstitial fibrosis has been reported [115, 116]. In parallel, taurine ameliorated chronic renal lesions in streptozocin-induced diabetes mellitus in rats [117] by decreasing total proteinuria and albuminuria, preventing glomerular hypertrophy, diminishing glomerulosclerosis and tubulointerstitial fibrosis, and decreasing the formation of AGEs.

More clinical experience is available for a contrasting approach. HOCl donor tetrachlorodecaoxide is currently used for topical application in wound-healing disorders and for systemic application as a modulator of monocyte functions in patients with AIDS [118]. Furthermore, animal studies were performed in which tetrachlorodecaoxide was used as a posttransplantation immunosuppressant [119]. Tetrachlorodecaoxide is activated by a reaction with hemoproteins by a two-electron transfer leading to generation of HOCl [120]. Its immunomodulatory properties might be in part due to production of chloramines (e.g., taurine chloramines). It thus is apparent that inhibition as well as stimulation of the MPO-hydrogen peroxide-chloride system may have beneficial effects on the course of disease process; this dual ambivalent action may well be dependent on the stage of the respective renal disease.

OUTLOOK

It is now evident that the MPO-hydrogen peroxide-chloride system plays a relevant role not only in oxidant-mediated antimicrobial defense by granulocytes but also in the progression of degenerative and immunologic diseases of the kidney. The intricate interaction of MPO and its products with other oxidant systems, specifically the nitric oxide pathway, shows the complexity of the redox system. These interactions of oxidant components and the varying biological actions of HOCl concentrations generated in vivo in different disease settings may explain either beneficial or detrimental effects of the MPO-hydrogen peroxide-chloride system. MPO−/− mice tend to incur more acute damage (e.g., brain infarct) and to suffer more chronic vascular damage (e.g., atherosclerosis) than wild-type animals. Drugs that generate HOCl could be immunomodulatory and even enhance wound healing. Yet it seems undisputed that MPO contributes to atherosclerosis and exerts a major influence in the pathophysiology of microscopic polyangitis and necrotizing glomerulonephritis. The precise pathophysiologic role though of the MPO-hydrogen peroxide-chloride system in the majority of human kidney diseases still has to be investigated in detail. Urinary measurement of MPO-mediated protein, lipid, and carbohydrate modifications (including generation of AGEs), immunohistologic and biochemical evidence for reactive MPO-dependent oxygen and nitrogen species and specific inhibition of MPO activity may help to elucidate the participation of MPO
in human glomerular and interstitial disease. Epidemiologic studies of patients with genetic defects in MPO and the study of MPO polymorphisms will probably give further insight into a postulated role of this unique enzyme and its oxidation products in renal disease.

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