The proper study of mankind

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Commentary

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Approximately 30 years ago Klebanoff demonstrated the importance of myeloperoxidase (MPO) in the oxygen-dependent microbicidal activity of human phagocytes (1). The ability of MPO to catalyze the oxidation of chloride and to chlorinate substrates (2, 3), properties unique among members of the protein family of animal peroxidases (4), likely reflects the unusual linkage of its heme to the apoprotein (5). This capacity underlies its potent antimicrobial activity, since HOCl represents the proximal agent generated by the combined efforts of MPO and the products of the NADPH-dependent oxidase released during stimulation of phagocytes (6). However, the significance of peroxidase and H_2O_2 in the modification of biological substrates extends well beyond damaging invading microorganisms and includes processes as seemingly divergent as the synthesis of thyroid hormone (7) and the respiratory burst seen during fertilization of sea urchin eggs (8). The capacity of the MPO-H_2O_2-halide system to modify proteins, lipids, and nucleic acids in a wide variety of biological contexts has been the subject of extensive study (9). Several laboratories have established that the MPO-H_2O_2-halide system, either as reconstituted components in vitro or as the result of phagocyte stimulation, can oxidize lipoproteins and thereby generate atherogenic species. Such a formulation fits well with the concept of atherosclerosis as a chronic inflammatory disease (10), reflecting arterial damage induced by lipoproteins modified by the MPO-H_2O_2-halide system.

The Heinecke lab (11) published, in the *JCI*, the first evidence that linked MPO-dependent oxidation of lipids and lipoproteins to the pathogenesis of atherosclerosis. Detergent extracts of atherosclerotic human vessels contain proteins immunochemically related to MPO and exhibit both peroxidative and chlorinating activities, biochemical properties unique to MPO. Sensitive and quantitative assays for MPO-derived modifications of resident proteins from subintimal plaques (12–14) have since provided compelling evidence that peroxidatively active MPO is present in atherosclerotic lesions [reviewed in ref. 15]. Modifications such as the generation of chlorotyrosine (13) represent specific markers for MPO activity, whereas the detection of other species, such as the products of reactive aldehydes (16) constitute less incriminating evidence for a specific role for MPO. However, direct evidence that MPO-dependent events contribute to the formation or promotion of atherosclerosis was lacking, setting the stage for the report of Brennan et al. in this issue of the *JCI* (17).

As has been previously reported for an independently generated knockout of murine MPO (18), the mutants created by Brennan et al. (17) have increased susceptibility to infection with *Candida albicans*. The murine knockout recapitulates the most dramatic immune defect seen in MPO-deficient humans, as neutrophils from affected individuals exhibit defective candidacidal activity in vitro (19), and several completely MPO-deficient patients have presented clinically with severe or fatal systemic candidiasis [reviewed in ref. 20]. To examine the influence of MPO activity on atherogenesis, Brennan et al. created a double knockout, crossing MPO-deficient mice with those that lack the receptor for LDL. Since the latter is a widely accepted murine model for human hypercholesterolemia (21) and atherosclerosis (22, 23), the authors reasoned that the double knockout would provide a powerful system to examine the contribution of MPO to atherogenesis. Accordingly one would anticipate that the MPO-dependent effect would be manifested as a reduction in the amount or severity of atherosclerosis in the double knockout animals.

Two unexpected results emerge from their studies (17). First, animals that lack both the LDL receptor and MPO have more severe vasculopathy than do the animals with only LDL-receptor deficiency, a result contrary to that predicted if MPO were an important provocateur of atherosclerosis. However, even more unexpected was the second observation, that the atheromata in the positive control group, namely the LDL-receptor knockout animals, lack any trace of MPO. Neither immunochemical evidence of MPO-related proteins nor MPO-specific post-translational modifications of endogenous substrates (e.g., chlorotyrosine) were detected in the lesions of the LDL-receptor knockout mice expressing MPO in their granulocytes. The reader is left with the inescapable conclusion that, at least according to the premise underlying the fundamental question addressed by the authors, the disease produced in the LDL-receptor knockout mice did not mirror human atherosclerosis. In this light, can one draw any conclusions from the data presented? What questions remain unanswered and what alternative approaches to study the role of MPO in atherosclerosis could be pursued?

What questions remain unanswered and what alternative approaches to study the role of MPO in atherosclerosis could be pursued?

First, the authors should be commended for the rigor and integrity with which they assessed how accurately their animal model mirrored human disease, looking beyond simply reproducing its histologic features (17).

Wholesale investment in knockout animal models before rigorously assessing how faithfully they model the human situation can contribute to conclusions unrelated to human disease and propagate unnecessary subsequent studies. This may be especially true when characterizing phenomena in which phagocytic cells play a prominent role. Murine and human phagocytes have significant differences, some of which may undermine the fidelity of the murine system to model human disorders. The granules of murine neutrophils contain less β-glucuronidase, lysozyme, and alkaline phosphatase than do their human counterpart (24) and are devoid of bactericidal permeability–increasing protein or defensins (25), prominent contributors to human granulocyte antimicrobial defense. Furthermore, and especially pertinent to the study under discussion, murine neutrophils possess only 10% (26) to 20% (24) of the MPO present in human cells. Complicating further any interpretation of the functional implications of these interspecies differences in MPO content are the complex interactions of MPO and nitric oxide. Substantial evidence suggests that MPO-generated reactive nitrogen intermediates (RNIs) contribute to the nitration of LDL in human atherosclerotic lesions (27–30), that nitric oxide modulates the activity of MPO (31), and that murine phagocytes generate significantly greater amounts of RNIs than do human phagocytes (32, 33).

Second, the more extensive disease seen in MPO-deficient mice is a reminder that even in chronic inflammation, not all participants are proinflammatory. In fact, various elements may contribute to anti-inflammatory events or participate in termination of the inflammatory reaction. An example pertinent to this study is the compelling, albeit incomplete, suggestion that the MPO-H_2O_2-halide system contributes to termination of activity of the NADPH oxidase, reported two decades ago in the *JCI* (34). Consistent with this potential mechanism is the long-standing observation that monocytes and neutrophils from MPO-deficient individuals exhibit a prolonged respiratory burst (35, 36), suggesting that the normal termination event(s) is delayed or defective in the absence of MPO. Perhaps the MPO detected in human atheromata contributes to the cessation of atherogenic events rather than promotes the disease.

Third, the sources of the two putative causative agents, namely MPO and oxidants, merit revisiting. The obvious candidate for reactive oxygen species, namely the phagocyte NAPDH oxidase (37), may not contribute to oxidant generation at the site of vascular damage. Although subject to all the caveats raised earlier, recent data demonstrate that mice lacking a functional NADPH-dependent phagocyte are not spared atherosclerosis when fed a high-cholesterol diet or bred with a strain deficient in apoE (38). Thus, the source of oxidants in vascular tissue may not be the NADPH oxidase of phagocytes but may be a member of the recently described family of NADPH oxidase homologues, called Nox (NADPH oxidases) in a recent review (39). Nox-1 is expressed in vascular tissue and could be the source of low levels of oxidants sufficient to participate in lipid peroxidation.

The source of MPO is likewise uncertain. Normally, mature MPO resides in the azurophilic granules of polymorphonuclear leukocytes and monocytes (40), its synthesis restricted to the promyelocytic stage of myeloid development (41, 42). The transition from monocyte to macrophage is associated with a decrease in peroxidase activity (43), thought to reflect a loss in cell-associated MPO during differentiation into macrophages. Although the MPO detected within plaques could be the remnants of enzyme released during migration of phagocytes through the lesion early in the genesis of the plaque, neutrophils are not seen in atheromata and monocytes have relatively little MPO. An alternative, and testable, hypothesis is that tissue macrophages, within the context of the unique mix of cytokines present in the atheromatous plaque subintimally, reinitiate transcription of the MPO gene. In contrast to bone marrow promyelocytes, foam cells do not possess azurophilic granules and thus would lack access to the subcellular compartment for storage of MPO. Without an intracellular target and/or targeting mechanism, the newly synthesized MPO precursor in foam cells would enter the secretory pathway and be released into the extracellular space. Were this mechanism operative, immunochemical analysis of such lesions would detect the 90-kDa MPO precursor rather than processed subunits of mature lysosomal MPO.

Lastly, in light of the limitations of the model, the best test of the role of MPO in human atherosclerosis should come from clinical studies. Of course, the least ambiguous study would compare the prevalence or risk of atherosclerosis in individuals with and without MPO. Although complete MPO deficiency is relatively common [reviewed in ref. 20], a large population study would be necessary to provide sufficient numbers to support firm conclusions. Alternatively, one could determine whether the amount of functional MPO within granulocytes correlated with a graded risk of atherosclerosis, analogous to the recently described relationship between factor VII levels and risk for myocardial infarction (44). The promoter region of the MPO gene has an allelic polymorphism wherein the presence of G or A at nucleotide –463 effects 25-fold differences in its transcription (45). This polymorphism is likely reflected in differential levels of MPO protein and activity within circulating granulocytes, affording investigators an accessible means to assess MPO levels and correlate them with the presence of atherosclerotic heart disease.

In summary, the study by Brennan et al. (17), like many excellent studies, raises more questions than it answers, many of which extend well beyond the focus of the initial issue addressed. Most striking is the reminder that the synergy of phagocyte antimicrobial elements, often synonymous with components of inflammation, may complicate the interpretation of murine knockout studies, as elegantly discussed recently by Nathan and Shiloh (33). Despite their utility as powerful analytical tools, perhaps we should temper enthusiasm for this experimental approach with Alexander Pope's admonition from Epistle II of *An Essay on Man*, "The proper study of mankind is man."

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