Commentary

SOD Inactivation in Asthma

Bad News or NO News?

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Changes in the oxidative milieu are well known to accompany inflammatory diseases, including asthma, and the severity of oxidative stress, measured through the accumulation of stable oxidation end products, often directly correlates with the severity of disease. As an example, tyrosine nitration has been reported to positively associate with cardiac disease,¹ and a direct correlation between nitrotyrosine reactivity and functional abnormalities has been reported in patients with asthma.² These observations, coupled with animal studies demonstrating that administration of antioxidant compounds ameliorate various manifestations of inflammatory disorders^{3,4} and that transgenic mice overexpressing antioxidant enzymes display attenuated damage,^{5–7} have provided substantial evidence in support of a causal role of oxidative changes in the inflammatory disease process. Nonetheless, a mechanistic basis underlying such causality is generally lacking, due to an overall failure to pinpoint the critical oxidative targets in relation to functional changes that drive the disease process.

In this regard, the study by Dr. Comhair and colleagues⁸ in this issue of *The American Journal of Pathology* has made significant progress in delineating critical oxidative events in patients with asthma and the role these oxidative alterations may play in the morphological and functional alterations seen in the asthmatic lung. The authors demonstrate in bronchial brush material obtained from patients with mild asthma that SOD is inactivated. Using sophisticated mass spectrometric analyses and immuno-approaches, they also reveal that the mitochondrially localized isoform of superoxide dismutase (SOD), MnSOD, contains multiple oxidations of phenylalanines and tyrosines, including nitrated tyrosine residues. To address the ramifications of such oxidative inactivation of MnSOD, the authors used a chemical inhibitor of SOD in a human bronchial epithelial cell line, or knocked down MnSOD using siRNA, and demonstrated that SOD inac-

tivation or knockdown is sufficient to cause apoptosis. These results are consistent with their observations in epithelial brushings from patients which also revealed evidence of apoptosis, as evidenced by increases in TUNEL reactivity, caspase 3 and 9 cleavage and activation, and PARP cleavage. Lastly, decreases in SOD activity in asthmatics were found to correlate with decreased lung function.⁸ Thus, the scenario emerges that oxidative inactivation of SOD within cells of the conducting airways leads to enhanced apoptosis and a compromised epithelial barrier. These are considered potential contributors to airway hyper responsiveness in patients with asthma and can also fuel airway remodeling.^{9,10} These findings are highly significant in that they not only highlight the strength of translational studies but also provide a much needed mechanistic framework toward elucidating the mechanism of action of oxidants in the pulmonary inflammatory disease process.

Despite the importance of the present study by Comhair et al⁸ in providing evidence for mechanistic links between specific oxidative events, changes in antioxidant function, and decreased epithelial integrity or lung function, a number of unresolved questions remain that will provide a continued challenge for future investigations. First, it remains unclear to what extent the measured tyrosine modifications within MnSOD from samples of asthmatic patients actually contribute to inactivation of the enzyme and consequently drive the apoptotic process. While the efforts to relate changes in SOD activity to specific oxidative modifications are highly commendable, especially considering the challenges associated with analysis of patient specimens, only selected oxida-

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tive modifications in two amino acid residues were analyzed. The total number of oxidations measured ranged from 1.13–1.73 mmol oxidation product/mol precursor tyrosine or phenylalanine, reflecting attack by highly reactive nitrating species or oxidants with hydroxyl radical like activity, which are claimed to have affected up to 6% of total MnSOD. Nevertheless, it is difficult to envision how these quantitatively modest changes account for the extensive enzymatic inactivation of SOD seen in asthmatic patients.^{11–14} Furthermore, the specific location of oxidized tyrosine or phenylalanine residues within the MnSOD protein are unknown, and consequences of modification of these amino acids for alterations in structure and function of MnSOD remain to be determined. In this regard, it is also important to consider oxidative modifications of other amino acids such as cysteine and methionine that have significant impact on the activity of many proteins, as evidenced by the multiple evolutionary conserved redox systems that exist to regulate or reverse such modifications, including thioredoxin, peroxyredoxins, glutaredoxins, and methionine sulfoxide reductase.^{15–20} Indeed, a wealth of evidence suggests that reversible sulfhydryl oxidations could play a prominent regulatory role in cell signaling and apoptosis.^{14,19} However, due to their reversible nature, and the lack of specific reagents, accurate detection of these oxidative modifications is an exceedingly difficult, if not impossible, task that limits our knowledge of the full spectrum of oxidations that could drive the disease process associated with chronic inflammation. Thus, the question that remains unanswered is whether the subset of oxidations measured in the present study are indeed the ones that are relevant to MnSOD inactivation and apoptosis. Clarification of this issue awaits studies with specific mutant proteins, in cell culture or animal models, and the development of much needed additional reagents to adequately probe sulfhydryl and other reversible oxidations.

Another question that remains incompletely addressed is which isoform(s) of SOD is inactivated in asthmatic airways. Three distinct superoxide dismutases exist in humans: MnSOD (SOD2), which contains manganese and is expressed in the mitochondria, CuZn containing superoxide dismutase (SOD1), which is expressed in the cytosol, and extracellular (Ec) SOD (SOD3), which contains Cu and Zn, as well as a heparin binding domain, and is localized on the cell surface or in the extracellular matrix (²¹ for review). While the present study convincingly demonstrates that MnSOD is oxidized in the airways of asthmatic subjects and that its knockdown is sufficient to induce apoptosis, previous studies by the same team of investigators have indicated that lowered SOD activity in asthmatic airways is primarily attributed to decreases in CuZnSOD activity.¹¹ In addition, SOD activity measured in the BAL fluid of asthmatics was also substantially lowered,¹³ which may in fact reflect changes in expression, localization, and/or activity of EcSOD. While decreases in MnSOD can easily be implicated in decreased mitochondrial function and increased apoptosis,^{22,23} such causal relations between apoptosis and changes in other SOD isozymes are much less apparent. Thus, further elucidation of the extent of oxidative modifications in

all SOD isoforms, the ranges at which these occur, and specific assessment of inactivation of each isoform in control subjects and asthmatic patients will be needed to clarify these uncertainties. The importance of this issue may be best illustrated by the several studies that report the effects of genetic deletion of either SOD isoform. While genetic deficiency of MnSOD has significant consequences for mitochondrial integrity and survival,^{24,25} phenotypic changes due to genetic deficiency of either CuZnSOD or EcSOD are more subtle and typically present at more advanced age or in the context of elevated inflammation or oxidative stress.^{6,26,27} Overexpression of CuZnSOD also failed to rescue neonatal lethality associated with the MnSOD knockout genotype,²⁸ clearly confirming that the SOD isoforms have non-redundant roles. Therefore, depending on the isoform of SOD that is targeted in asthma, several scenarios can be drawn to implicate alterations in cell signaling or function.

What is it about SOD oxidation/inactivation that drives apoptosis in epithelial cells, and what are the critical redox changes in this process? These questions are not easily answered because of the complex and multifaceted nature of redox perturbations that will ensue on loss of functional SOD, and the uncertainties regarding the SOD isoform affected in asthmatic airways. The simplest scenario perhaps reflects the increased steady state concentrations of superoxide that will occur following SOD inactivation, which have been shown to inhibit acronitase in association with inhibition of the respiratory chain, destabilization of the mitochondrial membrane, opening of the pore complex, and initiation of the apoptotic cascade.^{29–32}

Another well appreciated scenario through which SOD inactivation may promote redox changes is through the loss of nitric oxide as a consequence of the rapid reaction of superoxide with nitric oxide, causing the formation of the damaging nitrating species peroxynitrite.^{33,34} Whereas the consequences of enhanced superoxide-dependent formation of peroxynitrite are easily implicated in the apoptotic process,^{35–37} it is also important to consider the importance of loss of NO that results from ONOO[–] formation. Such regulation of NO bioactivity would strongly depend on the location of NO production, in association with the isoform of SOD that is inactivated, and could affect NO signaling either extracellularly, in cytoplasmic compartments and/or in mitochondria. NO can be stored in cells in the form of S-nitrosothiol, a chemical form of functional NO associated with protein thiol groups (referred to as S-nitrosation or S-nitrosylation).^{38,39} A role for S-nitrosothiols in the regulation of the apoptotic process has been implicated based on elegant studies demonstrating that the activity of caspases, which are cysteine dependent proteases, is repressed by S-nitrosylation. During the sequelae of apoptosis, caspase denitrosylation occurs and in turn leads to their activation.^{40,41} S-nitrosylation of various caspases has been detected in multiple subcellular compartments,^{41,42} pointing to an important role for NO in the prevention of apoptosis, and suggesting that the loss of functional NO through redox changes may be highly relevant in promoting the apoptotic process. Whether the loss of bioavail-

able NO, which results from SOD inactivation following the formation of ONOO⁻ mentioned earlier or through other redox changes, plays a role in the causation of apoptosis in epithelial cells of asthmatic patients remains to be explored. Nonetheless, it is important to note that S-nitrosothiol levels are markedly suppressed in the asthmatic airways,^{43,44} consistent with this biochemical scenario.

Is oxidatively inactivated SOD functionally silent, or could the inactive protein itself be redox active, thereby contributing to the apoptotic process? This possibility is exemplified by mutant SOD1 isoforms that constitute a fraction of patients with familial amyotrophic lateral sclerosis (ALS),⁴⁵ drawing an interesting parallel between inactivation of SOD1 in asthmatic airways and SOD1 mutations that mediate the clinical manifestations of ALS, which also encompass apoptosis.⁴⁶ These mutant SOD1 proteins are destabilized and are either Zn-deficient or more susceptible to Zn release from their active site, leading to catalysis of aberrant oxidations, tyrosine nitration, enhanced decomposition of S-nitrosothiols, and polymerization.^{47,48} It would be intriguing if oxidatively inactivated SOD1 in asthmatic subjects similarly acquires such a "toxic" gain of function that could thereby promote apoptosis. Formal testing of this possibility will require assessment of the sites of oxidations, and the consequences of these events for enzyme structure and function.

While it is easily envisioned that inactivation of SOD alters subcellular or extracellular oxidative events through processes described above, the source of oxidants and the course of events that lead to the reported oxidative changes in MnSOD remain unclear. It is often implied that oxidants derived from pro-inflammatory cells are responsible for the damage associated with inflammation, and result in certain "signature" oxidative modifications, including bromotyrosines and chlorotyrosines, reflecting the specific involvement of eosinophil peroxidase and myeloperoxidase, respectively.⁴⁹ Such oxidative modifications appear to primarily affect extracellular proteins.⁵⁰ Therefore, it is not entirely clear how such oxidative events specifically affect intracellular or mitochondrial proteins. In this regard, the recent recognition of non-phagocytic NADPH dependent oxidases within the airway epithelium⁵¹ warrants further investigation into their mechanisms of activation, especially within the context of activation inflammatory-immune processes, and their involvement in regulating cellular target enzymes. These endeavors will be facilitated through an amalgamation of both mechanistic and translational studies, exemplified by the study by Comhair et al⁸ in the current issue of *The American Journal of Pathology*. Indeed, this important study paves the way for much needed additional translational studies to unequivocally demonstrate the causal role of specific oxidative processes in cellular dysfunction and the development or propagation of chronic inflammatory diseases.

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