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Oxidative stress in allergic respiratory diseases

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There is ample evidence that allergic disorders, such as asthma, rhinitis, and atopic dermatitis, are mediated by oxidative stress. Excessive exposure to reactive oxygen and nitrogen species is the hallmark of oxidative stress and leads to damage of proteins, lipids, and DNA. Oxidative stress occurs not only as a result of inflammation but also from environmental exposure to air pollution and cigarette smoke. The specific localization of antioxidant enzymes in the lung and the rapid reaction of nitric oxide with reactive oxygen species, such as superoxide, suggest that antioxidant enzymes might also function as cell-signaling agents or regulators of cell signaling. Therapeutic interventions that decrease exposure to environmental reactive oxygen species or augment endogenous antioxidant defenses might be beneficial as adjunctive therapies for allergic respiratory disorders. (J Allergy Clin Immunol 2002;110:349-56.)

Key words: *Antioxidants, asthma, superoxide dismutase, oxidative stress*

Oxygen is essential to aerobic life but, paradoxically, can be toxic, even at atmospheric concentrations. In aerobic cells oxygen serves as an electron acceptor in many enzymatic and nonenzymatic reactions; however, addition of electrons to oxygen can result in the formation of toxic reactive oxygen species. All organisms have evolved elaborate cellular defenses that are collectively termed *antioxidants* to overcome this toxicity. The imbalance between reactive oxygen species and antioxidants is termed *oxidative stress* (Fig 1). Oxidative stress occurs in many allergic and immunologic disorders. Although the majority of research on allergic and immunologic diseases has focused on the toxic effects of reactive oxygen species, there is increasing evidence that reactive oxygen species at physiologic concentrations might play additional roles, such as that of cell-signaling mediators.

Abbreviations used
EC-SOD: Extracellular superoxide dismutase
NOS: Nitric oxide synthase
SOD: Superoxide dismutase

REACTIVE OXYGEN SPECIES DEFINED

The major function of the respiratory and cardiovascular system is the delivery of oxygen for use in aerobic energy production by means of oxidative phosphorylation. The features that make oxygen ideal for aerobic energy production (ie, atmospheric abundance and a high affinity for electrons) are also its Achilles' heel. For instance, one electron addition to oxygen produces superoxide, a second electron yields hydrogen peroxide, and a third electron leads to the formation of the hydroxyl radical (Fig 2). These 3 intermediates are called reactive oxygen species because they readily react with other molecules, such as proteins, lipids, and DNA. The hydroxyl radical is the most reactive of all reactive oxygen species (extremely high rate constants $>10^9$ mol/$L^{-1} \cdot s^{-1}$ for reactions with biomolecules suggest that it reacts at the site of production[1]). When oxygen gains a fourth electron, it has been fully reduced to water and no longer readily reacts with other molecules. Other reactive oxygen species include ozone, singlet oxygen, peroxynitrite, hypochlorous acid, and peroxyl, alkoxyl, and hydroperoxyl free radicals.

The term *free radical* denotes molecules with at least one unpaired electron. Oxygen (2 unpaired electrons), superoxide, hydroxyl radical, and nitric oxide (each with one unpaired electron) are examples of free radicals. Hydrogen peroxide is not a free radical because all of its electrons are paired.

SOURCES OF REACTIVE OXYGEN SPECIES

Normal metabolic processes in all cells are the major source of reactive oxygen species (Fig 3). The electron transport chain of mitochondria is the largest contributor. For instance, it is estimated that 1% to 3% of electron flux in mitochondria might form superoxide.[1] Additional major sources of superoxide include the membrane oxidases, such as the cytochrome P450 and *b5* families of enzymes in the endoplasmic reticulum[2] and the reduced nicotin-

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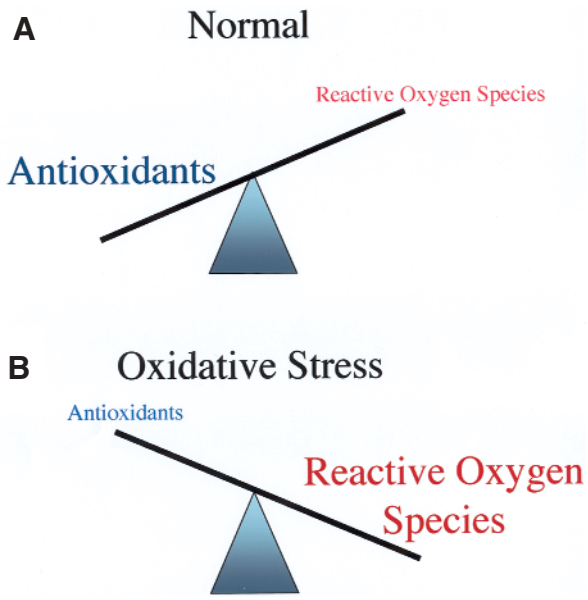

FIG 1. An imbalance between reactive oxygen species and antioxidants can lead to elevated stress. **A,** Normally, there are sufficient antioxidants in the respiratory tract such that the production of a small amount of reactive oxygen species is inconsequential. **B,** If either antioxidants are diminished or production of reactive oxygen species is increased (eg, during an asthma exacerbation), the balance of antioxidants and reactive oxygen species is tipped toward oxidative stress.

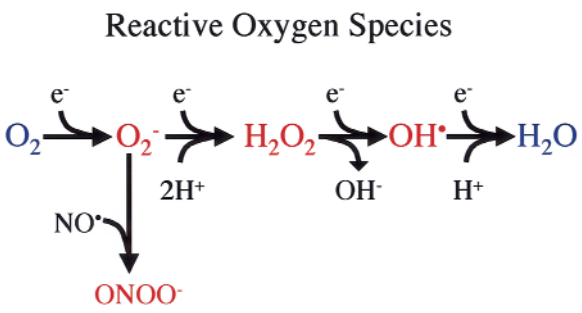

FIG 2. Derivation of reactive oxygen species. Sequential electron *(e^-)* addition to oxygen results in the formation of reactive oxygen species *(red)*: superoxide *(O_2^-)*, hydrogen peroxide *(H_2O_2)*, and hydroxy radical *(OH)*. Superoxide combines with nitric oxide *(NO)* to form peroxynitrite *(ONOO–)*.

amide adenine dinucleotide phosphate oxidases of phagocytic cells. Degenerate cloning of membrane oxidases has recently revealed a new family of membrane oxidases that are found on adventitial smooth muscle cells of coronary arteries[3] and aorta.[4] Hydrogen peroxide is formed during the dismutation of superoxide but also by means of glycolate oxidase in peroxisomes. Peroxisomes contain other hydrogen peroxide–producing enzymes, such as D-amino acid oxidase, urate oxidase, L-α-hyroxyacid oxidase, and fatty acyl-CoA oxidase.[5] Soluble enzymes, such as xanthine dehydrogenase (also known as xanthine oxidase),[6] aldehyde oxidase, dihydrorotate dehydrogenase, flavoprotein dehydrogenase, and tryptophan dioxygenase also can generate reactive oxygen species.[5] Hydroxyl radicals classically form in the presence of metals and hydrogen peroxide (Fenton reaction); however, decomposition from other molecules, such as peroxynitrite, might play a small role in hydroxyl radical formation.[7]

Direct exposure to environmental air is an additional source of reactive oxygen species exposure that is unique to the airways. For instance, cigarette smoke inhalation results in increased exposure to both superoxide and hydrogen peroxide.[8,9] Cigarette smoke–mediated lung damage might also be a result of increased exposure to nitric oxide and nitrites.[10,11] Other sources of environmental oxidants include air pollution, which contains ozone.[12] Ionizing radiation is an efficient method of producing reactive oxygen species in the laboratory but is a minor contributor to reactive oxygen species in vivo. Thus reactive oxygen species are ubiquitous both intracellularly and extracellularly and have a plethora of both endogenous and exogenous sources.

MEASUREMENT AND TOXICITY OF REACTIVE OXYGEN SPECIES

Electron spin resonance is the only method that directly measures free radicals, but the evanescent nature of many reactive oxygen species has made them difficult to measure in vivo, and instead many investigators use spin trapping to trap the free radical in a more stable molecule that can be readily measured in biologic systems. Recently, the ability to collect and analyze exhaled condensates has allowed for the direct assessment of hydrogen peroxide and nitric oxide in allergic respiratory disorders (Table I)[13-31]; however, most investigators rely on indirect measurements to assess reactive oxygen species. Measurement of products damaged by reactive oxygen species is the most common technique to indirectly measure reactive oxygen species. These products are called *footprints* (Table II)[32-46]; however, footprints are not specific for individual reactive oxygen species.

Many oxidative footprints are thought to be the result of nonenzymatic reactions between reactive oxygen species and organic molecules, such as proteins, lipids, or DNA. For instance, reactive oxygen species attack proteins to form carbonyls and can react with nitrogen species and then tyrosine to form nitrotyrosine. Reactive oxygen species react with lipids to liberate ethane and isoprostanes. Reactive oxygen species can react with DNA to form base pair adducts, such as 8-oxo-2-deoxyguanosine. Other examples of footprint reactions include reduction of cytochrome c by superoxide, causing a shift in the absorbance spectrum and oxidation of glutathione.

These footprint reactions might have immediate consequences when the reactants are signaling molecules. For instance, nitric oxide rapidly reacts with superoxide to form peroxynitrite, peroxyl radicals to form alkyl peroxynitrite, and hydroxyl radical to form nitrite. These reactions might decrease the concentration of nitric

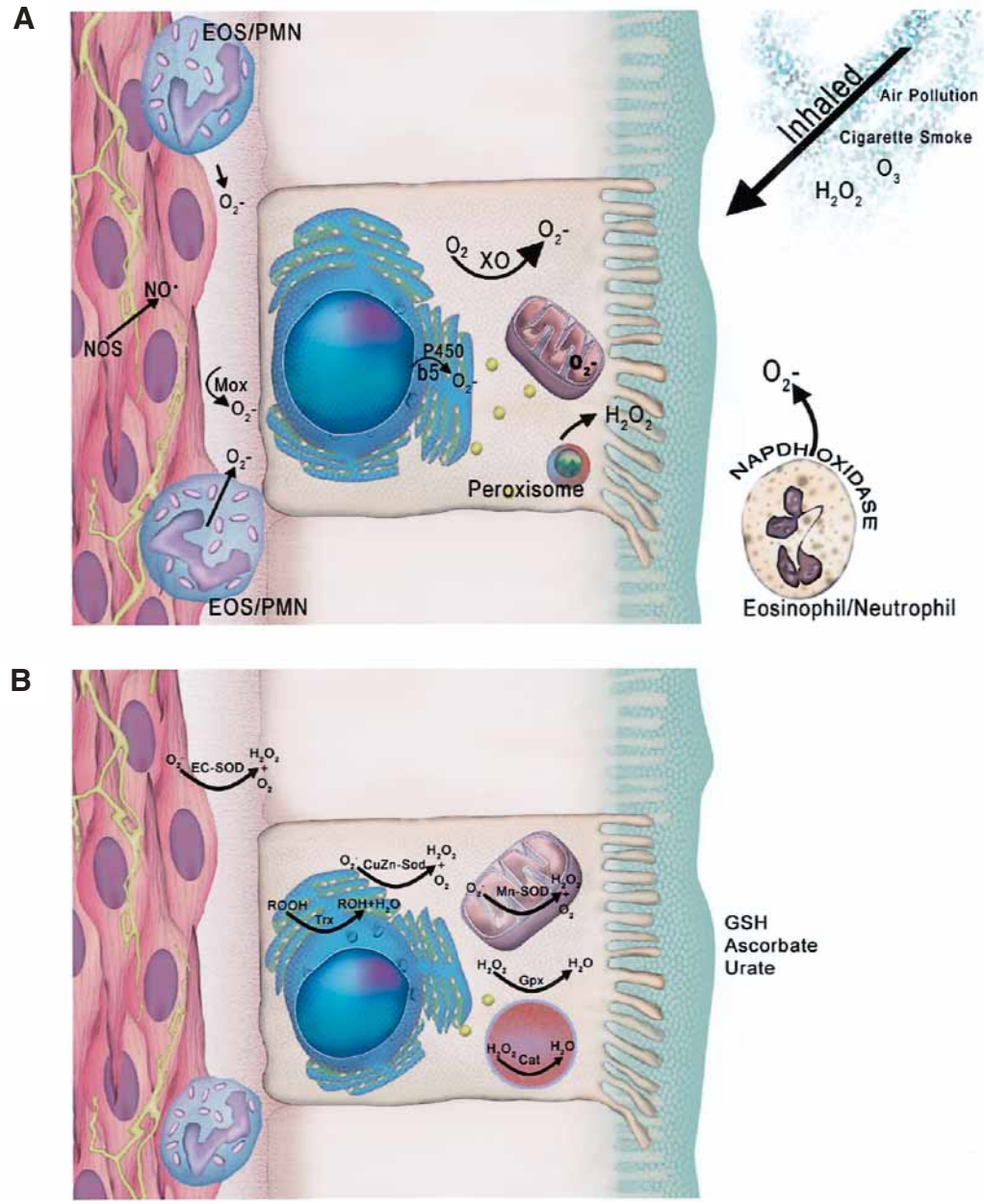

FIG 3. Reactive oxygen species and antioxidant enzymes in airways. **A,** Environmental sources include ozone *(O_3)* and hydrogen peroxide *(H_2O_2)* from air pollution and cigarette smoke. Intracellular sources include mitochondrial respiration, xanthine oxidase *(XO)*, and P450 and cytochrome *b5* enzymes; hydrogen peroxide is a product of superoxide dismutases *(SOD)*, such as Cu,Zn SOD and mitochondrial DOS *(Mn-SOD)*, as well as other oxidases, such as those found in peroxisomes. Extracellular sources of reactive oxygen species include membrane oxidases *(Mox)* and reduced nicotinamide adenine dinucleotide phosphate *(NADPH)* oxidases of eosinophils *(EOS)* and neutrophils *(PMN)*. **B,** Antioxidants found in airways include the SODs, glutathione peroxidase *(Gpx)* and catalase *(Cat)*, thrioredoxin *(Trx)*, and the low-molecular-weight antioxidants glutathione *(GSH)*, vitamin C (ascorbate), and vitamin E (α-tocopherol).

TABLE I. Reactive oxygen species/reactive nitrogen species are increased in allergic diseases

	Asthma	References	Allergic rhinitis	References	Atopic dermatitis	References
Superoxide	↑	14, 15, 17, 18, 21-23	↑	15-17, 24	↑	20
Hydrogen peroxide	↑	13, 25	↑	19	?	
Nitric oxide	↑	26, 27	↑	27-29	↑	30, 31

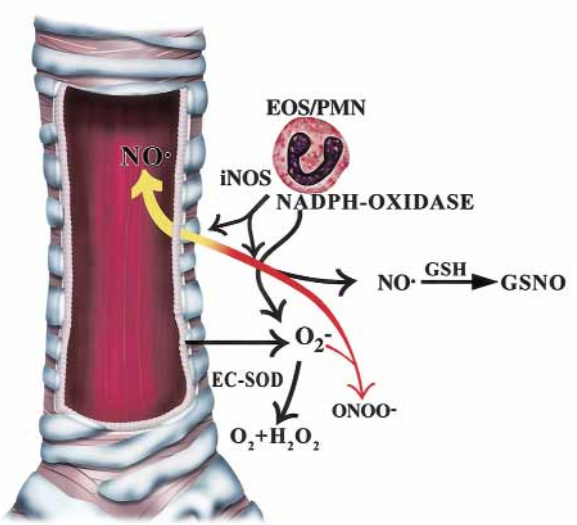

FIG 4. EC-SOD might play a role as both an antioxidant and regulator of signaling. Inflammatory cells, such as eosinophils *(EOS)*, neutrophils *(PMN)*, and macrophages, make reactive oxygen species but also make nitric oxide *(NO)* by means of upregulation of inducible NOS *(iNOS)*. Nitric oxide rapidly reacts with superoxide *(O_2–)* to form peroxynitrite *(ONOO–)*. Nitric oxide can also react with glutathione *(GSH)* to form S-nitrosoglutathione *(GSNO)*. Thus the local bioavailability of nitric oxide might be enhanced by either antioxidants that decrease the concentration of superoxide (eg, EC-SOD) or conversion into a more stable molecular form (eg, S-nitrosoglutathione).

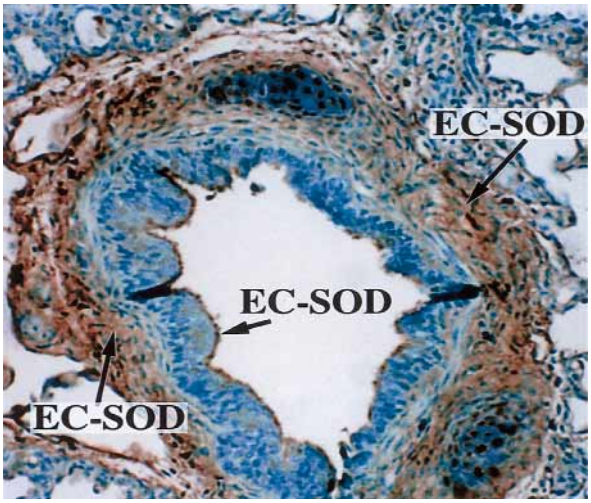

FIG 5. EC-SOD in human airways. In large airways there is strong EC-SOD staining *(brown)* in the bronchial epithelium, beneath the epithelium around smooth muscle cells, and in alveolar septae. There is also strong staining in large and small pulmonary blood vessels (not shown). Used with permission from *J Allergy Clin Immunol.* 1999;104:743-6.

oxide, thereby diminishing its bioavailability (Fig 3). The presence of oxidases and reactive oxygen species scavenging enzymes near areas of nitric oxide signaling (eg, airway smooth muscle) suggests that modulation of reactive oxygen species concentrations might be one method of regulating nitric oxide signaling.

ANTIOXIDANT DEFENSES

The primary defense against reactive oxygen species is endogenous antioxidants, which can be subdivided into enzymatic and nonenzymatic categories. The enzymatic antioxidants include the families of superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione S-transferase, and thioredoxin. Furthermore, each family has isozymes that are distinguished primarily by their distribution. For instance, the 3 mammalian SODs are cytosolic (SOD1), mitochondrial (SOD2), and extracellular (SOD3), and the 2 thioredoxins are cytosolic (Trx1) and mitochondrial (Trx2).[47] Extracellular SOD (EC-SOD) is the primary extracellular SOD enzyme and is highly expressed in lungs. EC-SOD mRNA is abundant in airway epithelium and vascular endothelium and is expressed at levels 4 times that seen in the heart and 15 times that in seen in the liver.[48] The protein is mainly associated with the connective tissue matrix around vessels and airways in the lung but is also found in close proximity to airway and vascular smooth muscle cells (Fig 5).[49,50] Electron microscopic immunocytochemistry reveals that EC-SOD is seen in areas rich in type I collagen, on the surface of smooth muscle cells, and in the extracellular matrix associated with airway and vascular smooth muscle cells. The prominent expression of EC-SOD around airways and airway smooth muscle suggests that it might play a role in allergic airway diseases, such as asthma.

The nonenzymatic category of antioxidant defenses includes low-molecular-weight compounds, such as glutathione, ascorbate, urate, α-tocopherol, bilirubin, and lipoic acid. Concentrations of these antioxidants vary depending of both subcellular and anatomic location. For instance, glutathione is 100-fold more concentrated in the airway epithelial lining fluid compared with plasma.[51] Other high-molecular-weight molecules that might be considered antioxidants include proteins that have oxidizable thiol groups, such as albumin, and proteins that bind free metals, such as transferrin. Albumin and transferrin are found in high concentrations in serum but at much lower concentrations in airway lining fluid.[52] Thus the composition of airways antioxidant defenses and reactive oxygen species depends on both subcellular and anatomic localization.

OXIDATIVE STRESS IN ALLERGIC DISORDERS

Asthma

Many observations suggest that oxidative stress plays an important role in the pathogenesis of asthma. Although it is difficult to get direct measurements of reactive oxygen species in asthmatic patients, recent studies of exhaled gases from asthmatic patients have shown increased hydrogen peroxide[13,53,54] and nitric oxide[55] levels. Furthermore, an increase in reactive oxy-

TABLE II. Footprints of oxidative damage in allergic diseases

Marker	Asthma	References	Allergic rhinitis	References	Atopic dermatitis	References
Protein						
Nitrotyrosine	↑	35, 38	↑	39, 40	?	
Bromotyrosine	↑	36, 41	?		?	
Lipid						
Isoprostanes	↑	32, 34	?		?	
Ethane	↑	33	?		?	
DNA						
8-Hydroxydeoxyguanosine	?		?		↑	42
Miscellaneous						
Oxidized glutathione	↑	37	?		?	
Carbon monoxide	↑	43	↑	44, 45	↑	46

gen species production is inversely correlated with FEV_1.[14] Airway inflammatory cells are the likely source of these increases. For instance, airway macrophages from asthmatic patients produce more superoxide than those from control subjects,[15] and antigen challenge increases spontaneous reactive oxygen species from airway eosinophils in patients with asthma.[16] IFN-γ blunts this response in allergic patients.[56] Circulating inflammatory cells might also be a source. Peripheral blood monocytes are activated to secrete superoxide when IgE binds to membrane receptors,[17] and eosinophils isolated from asthmatic patients 24 hours after antigen challenge produce more hydrogen peroxide when challenged with antigen.[57] Blood eosinophils and monocytes also produce more reactive oxygen species in asthmatic patients compared with control subjects.[15,58] Thus both airway and intravascular inflammatory cells contribute to elevated oxidative stress in asthma.

Multiple investigators have shown that increases in reactive oxygen species that occur during asthma are associated with damage to a wide range of biologic molecules in the lung. Increases in both airway isoprostanes[32] and ethane,[33] as well as urinary isoprostanes,[34] suggest that oxidative stress is occurring both at epithelial cell membranes and endothelial cell membranes. Elevated nitrotyrosine[35] and chlorotyrosine[36,59] levels from airway lavage samples suggest that proteins are also damaged. Although the consequences of oxidative modifications to proteins are not well studied, several investigators have demonstrated diminished activity of proteins, such as α1-protease inhibitor.[60] Steroid therapy attenuates hydrogen peroxide, nitrotyrosine, and ethane formation, suggesting a correlation between inflammation and oxidative stress.

The increase in reactive oxygen species during an asthma exacerbation might overwhelm endogenous antioxidant defenses. Although airway glutathione is increased in asthmatic patients, the ratio of oxidized to reduced glutathione also increases.[37] This increase in reduced glutathione suggests an adaptive response; however, other airway antioxidants, such as ascorbate and α-tocopherol, are decreased,[37] and SOD activity is diminished in cells from lavage and brushing samples of patients with asthma.[61] Several studies reveal that low blood or dietary antioxidants might be a risk factor for asthma[62-64]; however, dietary supplementation with vitamins, such as ascorbate, have not been found to be beneficial.[65,66]

The direct mechanisms by which reactive oxygen species exacerbate asthma could include both effects on airway smooth muscle and mucin secretion. Reactive oxygen species decrease β-adrenergic function in lungs[67] and also sensitize airway muscles to acetylcholine-induced contraction.[68] Hydrogen peroxide activates mitogen-activated kinases in tracheal myocytes[69] and stimulates tracheal smooth muscle to contract.[70,71] Finally, reactive oxygen species have been reported to stimulate mucin secretion.[72]

The association between inflammation and reactive oxygen species could set up a positive-feedback loop that perpetuates lung injury. Numerous cytokines, such as TNF-α, heparin-bound epidermal growth factor, fibroblast growth factor 2, angiotensin II, serotonin, and thrombin, are found in the lung during inflammation and activate oxidases that lead to increases in reactive oxygen species in cell culture.[5] The targets of these reactive oxygen species are unclear but might include receptor kinases, phosphatases, phospholipids, or nonreceptor tyrosine kinases, such as the mitogen-activated protein kinases.[69] Substantial works remains to be done to elucidate these pathways.

Another target of reactive oxygen species might be nitric oxide, and there is increasing evidence that nitric oxide is dysregulated in asthma. For instance, asthmatic patients have increased levels of exhaled nitric oxide that can be suppressed by corticosteroids. The role of nitric oxide in the lung is complicated because there are 3 distinct sources of nitric oxide synthases (NOSs). NOS I (nNOS, or neuronal NOS) is found at nonadrenergic nerve terminals of smooth muscle and might cause nitric oxide–mediated bronchodilation. NOS III (extracellular NOS) is found primarily on endothelium and mediates vasodilation. NOS II (inducible NOS) can be found on a wide variety of inflammatory and epithelial cell types. Both nNOS and extracellular NOS are constitutively active; however, it is primarily inducible NOS that is

induced in asthma and responsible for increased levels of exhaled nitric oxide. The role of nitric oxide in asthma remains to be proved; however, a lack of significant bronchorelaxation associated with nitric oxide in asthma suggests the signaling pathways in bronchial smooth muscle might be impaired. Concomitant increases in the production of reactive oxygen species during inflammation might be the source of this impairment.

The impairment of nitric oxide signaling might be mediated by nitric oxide's reaction with other reactive oxygen species (Fig 4). For instance, nitric oxide rapidly reacts with superoxide to form peroxynitrite.[73] Peroxynitrite formation increases during inflammation and is toxic to microbes; however, peroxynitrite can also cause airway hyperresponsiveness.[74] Presumably, peroxynitrite formation will decrease the amount of nitric oxide available for bronchodilation of smooth muscle. Thus one would expect peroxynitrite formation to be favored at sites of inflammation and infection but discouraged at sites at which nitric oxide–mediated signaling needs to be preserved. Two sites at which nitric oxide signaling is essential are respiratory smooth muscle and the vasculature. Two important pathways to protect nitric oxide signaling are (1) shifting nitric oxide into a more stable species, such as an S-nitrosoglutathione, or (2) reducing the local concentrations of reactive oxygen species by using a high concentration of antioxidant enzymes in the extracellular space. There has been much recent work suggesting that S-nitrosoglutathione might be a major contributor to airway smooth muscle relaxation in asthma.[75,76] The SOD family of enzymes are critical in preserving nitric oxide activity. Indeed, EC-SOD is abundant in both airway smooth muscle and pulmonary vasculature and might be a mechanism of preserving this nitric oxide–mediated smooth muscle bronchodilation, as well as vasoregulation. The specific role of EC-SOD in asthma remains to be proved but should be considered to have a likely role in preserving the bronchorelaxant effects of nitric oxide during oxidative stress (eg, inflammation).

Allergic rhinitis and skin disorders

The role of oxidative stress in allergic rhinitis is not well studied but is likely to be similar to that of asthma. Ozone exposure exacerbates antigen-induced rhinitis, sneezing, nasal secretions, hyperresponsiveness, and eosinophil infiltration in guinea pigs.[77] In allergic rhinitis house dust exposure induces nasal eosinophils to produce hydrogen peroxide.[19] In children initial exposure to environmental ozone increased nasal leukocytes and eosinophils in subjects without allergic rhinitis; however, subsequent exposure did not correlate with nasal inflammation.[78] In adults with pollen allergy, there was also a strong effect of ozone concentrations on rhinitis symptoms.[79]

The role of allergic disorders in the skin has also not been as well studied, but in patients with physical urticarias, there are decreased blood levels of vitamin E, catalase, and glutathione peroxidase; however, increased SOD activity is also found.[80] Furthermore, the peripheral blood monocytes from patients with severe atopic dermatitis are primed to secrete superoxide.[20]

THERAPEUTIC IMPLICATIONS

There are 2 strategies for treating oxidative stress asthma: reducing exposure to reactive oxygen species and augmenting antioxidant defenses. There are already several studies suggesting that reducing exposure to environmental oxidants, such as nitrites and ozone, might decrease asthmatic exacerbations through the attenuation of the activity of pulmonary inflammatory cells.[81,82] For instance, ozone decreases FEV_1 by 12.5% compared with filtered air,[82] and children playing sports (hence more outdoor exposure) have a higher prevalence of asthma in areas with high concentrations of environmental ozone.[83] Augmentation of existing antioxidant defenses with catalytic antioxidants might also be useful in attenuating asthma and other respiratory disorders. For instance, intraperitoneal SOD reduced airways hyperresponsiveness in guinea pigs,[84] and a low-molecular-weight SOD-mimetic attenuates bleomycin-induced fibrosis in mice.[85] The role of these catalytic antioxidants in allergic disorders requires further study. Furthermore, because allergic disorders such as asthma are multifactorial, blocking oxidative stress is unlikely to lead to complete resolution of bronchoconstriction but might be a useful adjunct therapy.

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