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Molecular characterization of redox mechanisms in allergic asthma

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ABSTRACT

Objective: To investigate the molecular redox mechanisms in allergic asthma and to examine current studies of the disease to provide a basis for further investigation of oxidative stress in allergic asthma and the signaling cascades involved in its pathogenesis.

Data Sources: Through the use of PubMed, a broad biomedical literature review was conducted in the following areas related to the physiology and pathobiology of asthma: redox therapy, reactive oxygen species (ROS), oxidative stress, allergic asthma, and antioxidants.

Study Selection: Studies pertaining to oxidative stress and redox signaling in the molecular pathways of inflammation and hypersensitivity in the pathogenesis of allergic asthma were reviewed.

Results: Allergic asthma is associated with an increase in endogenous ROS formation, leading to oxidative stress—induced damage to the respiratory system and mitigated antioxidant defenses. Exposure to environmental antigens has been shown to stimulate overproduction of ROS, resulting in abnormal physiologic function of DNA, proteins, and lipids that clinically can augment bronchial hyperresponsiveness and inflammation. Through the use of animal and human studies, oxidative stress has been determined to be important in the pathogenesis of allergic asthma. Thus, recent research suggests that the assessment of oxidative stress byproducts represents a novel method by which disease severity can be monitored. In addition, the use of redox-based therapy to attenuate levels of ROS presents a potential strategy to alleviate oxidative stress—induced airway inflammation in patients with asthma.

Conclusion: Redox mechanisms of oxidative stress in allergic asthma appear to play a key role in the pathogenesis of the disease and represent a promising therapeutic target.

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Introduction

Asthma is a common pulmonary disorder defined by chronic inflammation of the airways, increased contraction of respiratory smooth muscle, and recurrent episodes of bronchoconstriction.¹ The most prominent clinical manifestations of asthma include airway hyperresponsiveness, breathlessness, wheezing, persistent coughing, tightness of the chest, and dyspnea.² Worldwide, the American Academy of Allergy Asthma and Immunology has

estimated that more than 300 million people are affected by this disorder. In the United States alone, the Centers for Disease Control and Prevention has reported that 1 in 11 children and 1 in 12 adults have asthma and has estimated that 9 people die each day of this disorder. Moreover, with an economic burden of \$56 billion a year, asthma is considered a major health concern.

Asthma is a relatively ambiguous term that describes different phenotypes of chronic airway inflammation, including allergic asthma, which is the focus of this review. Allergic asthma is characterized by mucus hypersecretion and the accumulation of eosinophils in the airways in response to inhaled allergens.³ The presence of elevated levels of reactive oxygen species (ROS), or chemically reactive free radicals and peroxides, contributes to the pathogenesis of allergic asthma.⁴ Interestingly, endogenous ROS are important mediators of natural physiological processes—they are generated within mitochondria during cellular respiration and are

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produced by phagocytes in the destruction of foreign pathogens, healing of wounds, and clearance of apoptotic and necrotic cells.⁵ However, when in excess, ROS can have highly deleterious effects on the cell, including protein carbonylation and lipid peroxidation.^{6,7} In response to an antigen, enhanced formation of ROS activates cellular signaling mechanisms, inducing the inflammatory response observed in asthma and many other pulmonary conditions, such as chronic obstructive pulmonary disease, cystic fibrosis, idiopathic pulmonary fibrosis, and respiratory distress syndrome.^{5,8,9} In addition, high levels of ROS can damage DNA and, owing to their roles as signaling molecules and inflammatory mediators, can impede apoptosis and activate proto-oncogenes. Together these can ultimately predispose an individual to an array of pulmonary disorders.⁹ Oxidative stress induced by high levels of ROS has been suggested to result from and contribute to asthma-related inflammation. Although there is no consensus on a strategy that is effective in preventing the onset of asthma or mitigating oxidative stress–induced damage, existing pharmacologic therapies used to manage this disorder include inhaled corticosteroids, β -agonists, and, for severe cases, muscarinic antagonists, systemic corticosteroids, and anti-immunoglobulin E (IgE) antibodies.¹ Further developments in the study of asthma pathology and the importance of redox signaling in disease pathogenesis may shed light on future therapeutics.

Through the use of the PubMed online database, a broad literature review was conducted in the areas of redox therapy, ROS, oxidative stress, allergic asthma, and airway hypersensitivity to provide a basis for further research in the area of redox signaling in allergic asthma. In this review, the authors provide novel insights into recent developments in the pathogenesis of allergic asthma and the corresponding molecular mechanism of oxidative stress, examine allergen-induced ROS production, and highlight potential redox strategies to lessen the morbidity of this disease.

Allergen-Prompted ROS Production

The lungs are highly vulnerable to ROS-related injury owing to their large surface area, ample blood supply, and constant exposure to atmospheric oxygen.¹⁰ Although normal levels of ROS are critical for cellular signaling, growth, and proliferation, heightened levels of ROS initiate oxidative stress, a propagator of tissue injury and a key component in the development of various chronic inflammatory and hypersensitive conditions.¹¹ This being said, in people with asthma, the production of endogenous ROS is accelerated initially in the sensitization stage and during later manifestations of the disease, thus overwhelming the defensive capacity of the antioxidant system and increasing oxidative stress on the respiratory system.¹² In addition, environmental and occupational exposures to antigens and oxidants derived from asbestos, cigarette smoke, coal, diesel fuel, and ozone often induce excessive levels of ROS formation in conjunction with the immune response associated with allergic asthma in hypersensitive individuals. Likewise, pollens consisting of antigenic proteins and enzymes, such as pectate lyase, ole e 1–like proteins, and nonspecific lipid-transfer proteins, have similar effects.^{13,14} New evidence also suggests that phthalates, a common plasticizer, and other high-molecular-weight compounds abundant in many consumer products, may be the cause of oxidative stress and enhanced cytokine activity in allergic asthma.¹⁵ Specifically, these allergens, antigens, and oxidants initiate endogenous ROS production through the activity of inflammatory cells, such as neutrophils, eosinophils, and macrophages, and resident cells, including smooth muscle and epithelial cells.^{4,16} Antigens also can activate mast cells through transmembrane and pattern recognition receptors and, hence, can signal the release of bronchoconstrictors, leukotrienes, and histamines that in turn can stimulate bronchospasm and the early allergic reaction.^{17,18} These

activated inflammatory cells are the main propagators of endogenous ROS overproduction, particularly of superoxide and hydrogen peroxide, observed in people with asthma.^{4,8}

Moreover, Owayed et al¹⁹ reported increased activity of the membrane-bound enzyme complex, nicotinamide adenine dinucleotide phosphate oxidase (NOX), and increased formation of malondialdehyde, an indicator for oxidative stress–induced lipid peroxidation, in the peripheral blood lymphocytes of patients with asthma. NOX is a marked source of endogenous ROS in asthma; hence, inhibition of the NOX pathway has been proved to effectively protect lung tissues against ROS-mediated injuries.^{19,20} NOX also can be assembled by activated neutrophils,²¹ indirectly suggesting that the accumulation of activated neutrophils in asthma causes heightened NOX activity, resulting in oxygen radical–induced airway injury. Interestingly, in a previous study, it was confirmed that NOX derived from certain pollens, including ragweed pollen, can directly generate ROS when introduced to the airway epithelium.¹⁴ In response to pollen NOX, oxidized glutathione and 4-hydroxynonenal, byproducts of oxidative stress, were observed at heightened levels in the airway-epithelial fluid.¹⁴ The same study also found that pollen NOX-derived ROS dynamically amplify the production of IgE, the single most important inflammatory mediator implicated in the pathogenesis of allergic asthma.¹⁴ Specifically, IgE is associated with hypersensitive conditions owing to its initial response to antigens and allergens and initiation of a complex signaling cascade, resulting in inflammation.

Redox Signaling Mechanisms in Allergic Asthma

It is thought that at exposure, dendritic cells present allergens to T cells as foreign antigens, through MHC class II molecules located on B cells.¹⁴ This action initiates the signaling cascade of inflammatory mediators, including mitogen-activated protein kinase and interleukin (IL)-8, which may signal additional mediators through ROS production.¹⁴ Specifically, activated CD4⁺ T cells initiate the production of inflammatory cytokines, such as IL-4, IL-5, and IL-13.²² These proinflammatory mediators activate B cells and stimulate the generation of additional IgE molecules.²³ IgE and cytokines can mediate the activation of immune cells, such as mast cells and eosinophils, which then, through additional signaling molecules, elicit the immune response characteristic of asthma exacerbations—contraction of the smooth muscle,

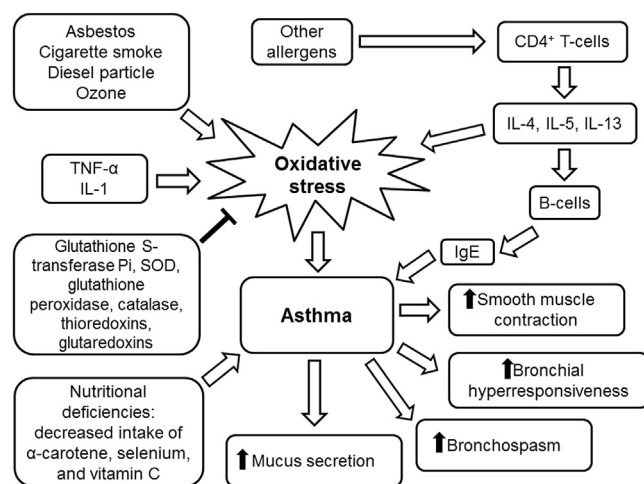


Figure 1. This schematic illustration depicts several putative signaling pathways involving various mediators of oxidative stress associated with asthma. IgE, immunoglobulin E (IgE); IL-1, interleukin-1; IL-4, interleukin-4; IL-5, interleukin-5; IL-13, interleukin-13; SOD, superoxide dismutase; TNF- α , tumor necrosis factor- α .

hypersecretion, inflammation, and airway remodeling (Fig 1).²³ Other research has shown that IL-4 specifically upregulates NOX activity and the transcription of monocyte chemoattractant protein-1, further augmenting oxidative stress.²⁴

With regard to redox signaling implicated in allergic asthma, ROS, produced by the mitochondria as a byproduct of cellular metabolism, may act as second messengers in cellular pathways that stimulate agonists of growth factors and hormones.²⁵ As such, ROS maintain the ability to activate transcription factors and, when in excess, can initiate the activation and modification of nuclear factor- κ B in epithelial cells.²⁶ The resulting cell signaling prompts the upregulation of genes coding for proinflammatory cytokines, enzymes, and adhesion molecules.⁸ Furthermore, amplified ROS formation induces production of tumor necrosis factor- α and IL-1.²⁷ These critical mediators may activate other proinflammatory genes, such as those for metalloproteinase-9, intracellular adhesion molecule-1, vascular cell adhesion molecule-1, and cyclooxygenase-2, that further promote ROS production and inflammatory signaling cascades that result in inflammation and asthma exacerbation.²⁷

Current research also has provided more insight into the relation among critical redox signaling molecules, glutathione homeostasis, and allergic asthma. In ovalbumin-sensitized murine models, a decrease in cellular glutathione, an antioxidant involved in the resolution of airway inflammation and respiratory hypersensitivity during the sensitization stage, has been associated with NOX inhibition and enhanced ROS generation.²⁸ Further, a reduction in glutathione has been suggested to induce changes in T-helper cell type 1 to type 2 cell differentiation, cause an imbalance in oxidant and antioxidant levels, and induce airway hypersensitivity by the synthesis of S-nitrosoglutathione and a corresponding shift in nitric oxide metabolism.^{28,29} In addition, asthmatic oxidative stress–induced S-glutathionylation, or the process by which glutathione binds to thiol groups to protect against further oxidation, is known to activate sarcoplasmic and endoplasmic reticulum calcium adenosine triphosphatase, alleviate airway smooth muscle tension, and regulate inflammatory mediators, nuclear factor- κ B, and activator protein-1.³⁰ Moreover, during a decrease in glutathione levels, consistent with allergic asthma, a corresponding decrease in S-glutathionylation is observed, which may in turn predispose an individual to oxidative stress–induced injury. Accordingly, an influx in glutaredoxins also can decrease S-glutathionylation. A recent study by Kuipers et al³⁰ determined that the rate of S-glutathionylation was markedly decreased in people with asthma and was positively correlated with some clinical manifestations of asthma, including the forced expiratory volume in 1 second, in neutrophilic patients. The same study also observed that glutaredoxin levels were increased in the sputum of patients with asthma, likely as a means of protection against high oxidative stress in times of airway hyperresponsiveness. Another important redox mediator found within the lung epithelium, glutathione S-transferase pi, primarily facilitates the conjugation of reduced glutathione to mitigate the electrophilic property of inhaled antigens and potentially reactive molecules.³¹ Interestingly, glutathione S-transferase pi is downregulated at antigen exposure and may even further contribute to the oxidative stress response.³¹ Furthermore, peroxiredoxin, an important cellular mediator in the resolution of inflammation and the bodily response to oxidative stress, has been found to be hyperoxidized in large proportions of patients with asthma.³² The positive correlation found between degree of peroxiredoxin hyperoxidation and asthma severity may convey an individual's predisposition to oxidative stress and allergic asthma and suggest a potential method of quantitative assessment of oxidative stress–induced damage. However, the exact mechanism behind the pathogenesis of asthma and its many phenotypes remains unclear. Thus, further research is needed to elucidate specific inflammatory and redox signaling cascades

involved in specific phenotypic manifestations of asthma, which may provide insight into future, directed therapeutics.

Oxidative Stress-Induced Damage in Allergic Asthma

Oxidative stress alters the physiologic function of DNA, proteins, and lipids³³ and may cause apoptosis and necrosis.³⁴ The most common cellular effects of ROS overproduction and excessive oxidative stress stem from deleterious oxidant-induced lipid peroxidation and protein carbonylation. ROS initiate the peroxidation of lipids to yield relatively long-lived and highly reactive aldehydes, such as malondialdehyde, acrolein, and 4-hydroxynoneal, all of which cause disruption of the cell membrane lipid bilayer, modification of DNA, alteration of protein structure, and protein carbonylation.⁷ A recent clinical trial has reported that the volatile organic products of lipid peroxidation contained in exhaled breath may be effectively used as predictors of the onset of asthma in children.³⁵ Therefore, development of new methods for the quantitative assessment of oxidative stress–induced damage in allergic asthma may allow for more progressive treatment in the earlier stages of the disease. Clinically, with regard to allergic asthma, oxidative stress has been shown to hinder smooth muscle contraction, enhance bronchial hyperresponsiveness, stimulate bronchospasm, and increase mucus secretion and epithelial shedding within respiratory cells (Fig 1).^{36,37} Oxidative stress also may inhibit superoxide dismutase (SOD), a vital enzyme responsible for catalyzing the conversion of superoxide radicals to less toxic molecules, such as oxygen and hydrogen peroxide, which may be further reduced to water. This inactivation of SOD results in more severe inflammation and airway obstruction.³⁸ However, despite its damaging effects, mild oxidative stress may cause beneficial antioxidant gene expression in asthmatic airways.³⁹ Accordingly, the upregulation of enzymatic antioxidants, such as SOD, glutathione peroxidase, catalase, thioredoxin, and glutaredoxin, acts as a natural defense against asthmatic lung injuries (Fig 1).^{4,16,40,41}

Particular chromosomal loci responsible for the regulation of cytokine generation, atopy, airway inflammation, and hyperactivity in asthma are strongly susceptible to oxidative stress–induced somatic mutation.⁴² It has been postulated that these loci, in conjunction with others, may accumulate mutations throughout the progression of asthma and contribute to molecular mechanisms underlying pathogenesis. A previous clinical study reported significant genetic alteration at the microsatellite DNA level in children and adults with bronchial asthma—at a much higher frequency in adults than in children, suggesting that such genetic instabilities are accumulated throughout the progression of asthma.⁴² The heightened oxidative stress implicated in allergic asthma may be a fundamental cause of the downregulation of DNA mismatch repair systems, rendering the afflicted cells unable to fix microsatellite DNA instabilities. Further, the prevalence of variations in microsatellite DNA has been correlated with augmented eosinophil and IgE levels in patients with asthma.^{23,42}

In addition, recent research has proposed that epigenetic alterations in genomic integrity, such as oxidative stress–induced changes in T-cell phenotypes prompted by maternal cigarette smoking, may predispose an individual to respiratory sensitivity and evoke a certain phenotypic response to a particular antigen.⁴³ Therefore, augmented maternal ROS may increase a child's susceptibility to allergic asthma later in life. Accordingly, it is well known that repeated exposure to an allergen or inorganic pollutant is associated with the onset of asthma.^{44,45} Interestingly, Gruziova et al⁴⁵ found that prolonged exposure to environmental air pollution in infancy was positively associated with asthma onset later in childhood. Furthermore, in a recent study of children with asthma, it was suggested that mitochondrial dysfunction is highly correlated with onset of asthma.⁴⁶ The study examined mutations in mitochondrial DNA and found that certain mitochondrial genetic polymorphisms evoke changes in

the rate of ROS production and the severity of asthma.⁴⁶ The study also found factors that attribute sex differences in the etiology of asthma to variants in sex-specific loci involved in mitochondrial ROS production.⁴⁶ Thus, oxidative stress has a prominent role in clinical manifestations of allergic asthma through its modification of vital cellular components, epigenetic and genetic alterations of DNA, and induction of polymorphisms of cellular subunits.

Redox Therapeutics

It has been shown that intra- and extracellular pulmonary antioxidants are effective in regulating levels of ROS and lessening oxidative stress–induced tissue and organ damage.⁸ In general, antioxidants, divided into enzymatic (glutathione peroxidase and SOD) and nonenzymatic (vitamin E, vitamin C, and albumin) subcategories,⁸ have a critical role in the inhibition, delay, and elimination of oxidative damage and in the mediation of redox signaling pathways to sustain cellular redox environments.²⁵ Various approaches have been developed to treat and prevent oxidative stress–related injuries using several redox therapies, such as antioxidant intervention,⁴⁷ to lower heightened levels of oxidative stress. This therapy can be carried out through increasing the existing endogenous antioxidant defense system or introducing nonenzymatic antioxidants exogenously through dietary supplementation.⁸

Previously, a thorough examination of antioxidant levels in children with asthma determined a direct association between decreased lung function and low levels of enzymatic and nonenzymatic antioxidants.⁴⁸ The presence of high levels of oxidative stress, especially those introduced exogenously through cigarette smoke,⁴⁹ cause an increase in the permeability of the lung epithelial layer, resulting in the leakage of plasma components, including intracellular antioxidants,⁵⁰ and the initiation of natural antioxidant defense mechanisms. Current research suggests that one of these defenses stems from the antioxidant properties of clusterin—a glycoprotein and biomarker of oxidative stress, mediated by activator protein-1.⁵¹ In a recent clinical trial, it was determined that clusterin may be upregulated in people with asthma and has important antiapoptotic and cytoprotective effects.⁵¹

Another strategy to combat oxidative stress involves the sequestration of the single electron in free radicals through the use of spin traps. This technique uses nitroso-containing compounds to effectively isolate lone electrons and form spin adducts, or nitroxide-based persistent radicals, which can be visualized by electron paramagnetic resonance spectroscopy.⁵² Spin traps also can be applied in the same fashion as antioxidants⁵³ and can be used therapeutically to counter reactive species at sites of inflammation *in vivo*.⁸ This defense mechanism against oxidative stress–induced damage has been successfully used for neurologic conditions, such as Parkinson and Alzheimer diseases, and as a strategy for neuroprotection in stroke and cerebral ischemia.⁸

By virtue of their ability to retain the catalytic function of their larger analogous enzymatic antioxidants, such as glutathione peroxidase and SOD, enzyme mimetics have promising applications to decrease asthmatic injuries.^{8,50} Glutathione peroxidase and SOD catalyze the reduction of peroxides and convert superoxide into hydrogen peroxide, respectively.^{8,25} Decreased levels of active SOD have been observed in patients with asthma, suggesting that the introduction of a SOD mimetic may have a protective effect.⁵⁴ Research also has shown that the removal of superoxide by a mimetic decreases respiratory and histopathologic abnormalities.⁵⁴ In addition, an inverse correlation between polyphenol intake and the occurrence of asthma has been reported,⁵⁵ suggesting that polyphenols, a class of natural antioxidants derived from plants, might contribute to the prevention of oxidative damage through an anti-inflammatory process.⁵⁶

Importantly, it has been proposed that nutritional deficiencies, especially in the intake of α -carotene, selenium, and vitamin C, and an increased demand for antioxidant counterbalances directly correlate with poor pulmonary performance (Fig 1).⁵⁷ Carotenoids, one class of nonenzymatic antioxidants, are abundant in many fruits and vegetables and often form chemical adducts with various types of ROS, prompting the release of harmless degradation products.⁵⁸ Furthermore, it has been observed that vitamin C intake, through the consumption of fresh fruit or dietary supplementation, relates directly to improved airway and pulmonary function.⁵⁹ Although vitamin C might decrease the morbidity of asthma, it is still unclear whether supplemental intake of vitamin C, and of vitamin E, would have a significant clinical benefit.⁸ In a cohort study by Knekt et al,⁵⁵ it was determined that elevated dietary intake of the flavonoids, quercetin, hesperetin, and naringenin markedly decreased the incidence of asthma. For example, intake of apples and oranges provided the strongest correlation with the decreased occurrence of this disease, which is not surprising given the high flavonoid content in fruit.⁵⁵ Thus, future studies should be focused on elucidating the exact relation between antioxidant intake and incidence of asthma, which may be useful in the development of potential redox treatments for asthma and related lung disorders.

Perspectives, Significance, and Future Directions

Allergic asthma is a common phenotype of asthma, associated with an acute increase in levels of ROS in response to an allergen, leading to oxidant and antioxidant imbalances, initiation of inflammatory signaling cascades, and oxidative stress–induced damage to the airways. Through the use of animal studies and clinical trials, ROS have been found to play a central role in the pathogenesis of allergic asthma. Transgenic murine models with the deletion, knockout, or overexpression of specific genes implicated in asthma, such as those corresponding to IgE, IL-4, and IL-5 production, and detailed, longitudinal studies examining the pathogenesis of asthma in children and infants have been especially useful in gaining further insights into the disease. In addition, airway remodeling is a major factor in the morbidity of asthma, resulting from inflammation and fibrogenesis elicited by ROS and redox signaling.⁵¹ Thus, the development of new techniques to assess and monitor oxidative stress and related damages is imperative and may hasten the creation of new therapeutics. To date, there are several valuable methods by which researchers can obtain quantitative measurements of byproducts of oxidative stress imposed by allergic asthma—namely the assessment of lipid peroxidation products, malondialdehyde and 4-hydroxynoneal,⁷ clusterin,⁵¹ spin adducts,⁵² and the hyper-oxidized peroxiredoxin ratios in peripheral blood mononuclear cells.³² This allows for the approximation of asthma severity and the degree to which the system is impaired by allergen-imposed oxidative stress. Current research also supports the use of exhaled breath analysis in conjunction with emerging analytical and computing technologies to evaluate biomarkers of oxidative stress in patients to better understand the role of oxidative stress in the pathogenesis of allergic asthma.⁶⁰ Although translational, this research must be solidified in further human and animal studies to bridge the transition from the bench to the clinic. Perhaps a standard scale could be created to objectively assess the degree of disease progression and stage of severity with regard to identity and quantity of oxidative stress byproducts. The specific cellular oxidative stress pathways involved in allergic asthma also must be delineated further. Accordingly, byproducts of oxidative stress may be traced to their source, and possible mediators of interest involved in the regulation of oxidant and antioxidant balance, such as glutathione S-transferase pi,³¹ might be

accurately targeted in potential interventions. Further research should be focused on the identification of the major source of ROS generation in allergic asthma and efficient methods to evaluate the therapeutic index of interventions.

Conclusion

In this review, the authors have described the critical role of redox signaling cascades, the pathway of chronic inflammation, and the molecular mechanisms implicated in allergic asthma. In examining oxidative stress associated with allergic asthma, exploring potential redox strategies to mitigate lung damage, and characterizing current models of the disorder, they have provided a basis for further investigation into molecular processes responsible for disease pathogenesis and novel therapeutics.

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