



Review

Molecular mechanisms of reactive oxygen species-related pulmonary inflammation and asthma

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ABSTRACT

Asthma is a highly relevant disorder that can be induced by many environmental factors such as allergens and pollutants. One of the most critical pathological symptoms of asthma is airway inflammation. In order to identify a cause of respiratory inflammation, we thoroughly examine the unique role of reactive oxygen species (ROS). Evidence supports that the inhalation of aggravating compounds such as allergens can promote the increased generation of ROS. Accordingly, ROS have a proven role in the cellular signaling cascades of many respiratory diseases that cause respiratory inflammation, including asthma. Although there is no known cure for asthma, current treatments effectively lessen the inflammation symptom. Based on the investigations of asthma pathogenesis and the mechanism of ROS formation, we have identified several novel anti-inflammatory therapeutic treatments, shedding light on a fundamental understanding for the cure of this disorder. In this review, we will outline the pathogenesis of asthma and its relationship to ROS, oxidative stress, and pulmonary inflammation.

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1. Introduction

Asthma is a chronic inflammatory disorder of respiratory airways induced by cellular mechanisms that produce increased levels of reactive oxygen species (ROS) (Comhair and Erzurum, 2010). According to the National Heart, Lung, and Blood Institute, symptoms of asthma include the inflammation and narrowing of airways which lead to recurring periods of wheezing, chest tightness, shortness of breath, and coughing. Statistics provided by the Asthma and Allergy Foundation of America (AAFA) report that 44,000 people in America have an asthma attack each day while nine people die from asthma each day. In addition, the Centers for Disease Control and Prevention reported a 6% increase in asthma costs from \$53 billion in 2002 to \$56 billion in 2007. While there is currently no cure for asthma, advancements in medicine and new guidelines for asthma treatment have assisted patients in managing its symptoms. For instance, corticosteroids, long acting β -adrenergic agonists (LABA), and leukotriene modifiers are innovative medications developed in the United States during the 1990s which have greatly increased the efficacy of asthma treatment (Szefer, 2011).

In order to develop a full understanding of respiratory diseases such as asthma, it is critical to evaluate the molecular functioning and impact of ROS generation. ROS are influential components of the molecular mechanisms involved in the pathogenesis of asthma and are known to play an important role in other various airway disorders such as adult respiratory distress syndrome, cystic fibrosis, idiopathic pulmonary fibrosis, and chronic obstructive pulmonary disease (COPD) (Gillissen and Nowak, 1998; Rahman, 2005; Rosanna and Salvatore, 2012). Generation of ROS occurs through various enzymatic pathways or chemical processes which are essential in many physiological reactions such as killing invading microorganisms (Henricks and Nijkamp, 2001). ROS can be produced endogenously within locations including mitochondria, microsomes, enzymes, and phagocytes whereas exogenous sources of ROS production have been linked to asbestos, ozone (O_3), coal, diesel fuels, and cigarette smoke (Vallyathan and Shi, 1997; Zuo and Clanton, 2005; Zuo et al., 2011a,b). ROS formation is a normal physiological metabolic process which takes place in every cell (Henricks and Nijkamp, 2001); however, increased levels of ROS can initiate mechanisms that cause harmful pathophysiological disorders such as asthma. Studies have shown that damaged DNA, lipids, proteins, and carbohydrates have been associated with excessive ROS production, ultimately leading to an enhanced inflammatory response in respiratory airways as seen in asthma (Henricks and Nijkamp, 2001; Suski et al., 2012).

In this review, we will discuss updated physiological and pathophysiological events relating to ROS, inflammation, and asthma

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as well as the novel molecular mechanisms and critical therapies which help lessen its deleterious symptoms.

2. Origins of asthma

To characterize the possible sources that can aggravate the detrimental characteristics of asthma, it is critical to primarily identify the role of allergens in the pathogenesis of this disorder. Pollen, animal dander, dust mites and fungi are among numerous airborne allergens that can trigger the airway inflammation associated with asthma when inhaled and mediated by immunoglobulin E (IgE) (Custovic and Simpson, 2012; Jenerowicz et al., 2012). Air pollution micro particles, additional exacerbating agents, have proven to be correlated with heightening asthma exacerbations (McConnell et al., 2002). Arshad et al. investigated allergens as a cause of asthma in a study by exposing mild asthmatic patients to small doses of dust-mite extract in order to mimic natural allergens. They concluded that repeated exposure to doses similar to those found naturally in a home negatively affects bronchial hyperactivity and lung function (Arshad et al., 1998).

Although its relationship to asthma has not been specifically confirmed, extracellular superoxide dismutase (EC-SOD) is proposed to have a significant function in the mechanism of asthma pathology (Qin et al., 2008). In relation to ROS, EC-SOD will break down superoxide anion into hydrogen peroxide, which can then be reduced to water (Leopold and Loscalzo, 2005). EC-SOD is an enzyme most prevalently found in the matrix surrounding pulmonary vessels and airways (Oury et al., 1994) and EC-SOD mimics have proven to diminish airway inflammation in asthma animal models (Chang and Crapo, 2002). Modifications of biologically significant proteins can be initiated by an increase in ROS. This affects the function of the proteins in their initiation and maintenance of inflammation. When superoxide dismutase (SOD) loses its antioxidant capacity, superoxide will catalyze to hydrogen peroxide which will then catalyze to water (Comhair and Erzurum, 2010). Furthermore, oxidation of the enzymes SOD and catalase contribute to the loss of their activity (Comhair and Erzurum, 2010). Thus, increased function of EC-SOD, which is directly associated with decreased oxidative stress, can likely reduce asthma symptoms such as inflammation and airway obstruction (Comhair et al., 2005a).

Furthermore, it is known that the pathogenesis of asthma in childhood and that of adulthood can differ. It is suggested that environmental exposure and various home characteristics such as exposure to dust-mites, mold, and the absence of a dehumidifier can be causes of asthma in children (Reponen et al., 2012). Apart from these allergens, exercise-induced asthma from sports is also commonly developed in children (Del Giacco et al., 2012). In adulthood, one of the sources of asthma development is smoking (Stapleton et al., 2011). Moreover, a study by Pedersen et al. (1996) concluded that asthmatic patients who smoke are resistant to inhaled corticosteroids, a common treatment for asthma. Thus, understanding the pathology of asthma is crucial for the advancement of novel and more effective treatments.

3. The molecular mechanism of ROS and asthma

Throughout normal metabolic processes, the formation of ROS is a constantly occurring phenomenon in all cells; as previously stated, however, overproduction of ROS can lead to compromised cellular functioning and increased inflammation by damaging nucleic acids, lipids, proteins, and mitochondria (Henricks and Nijkamp, 2001; Orrenius et al., 2007; Turrens, 2003). The mitochondria has a significant role in the production of superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), which are the principle types of ROS formation induced *via* activation of an inter-mitochondrial

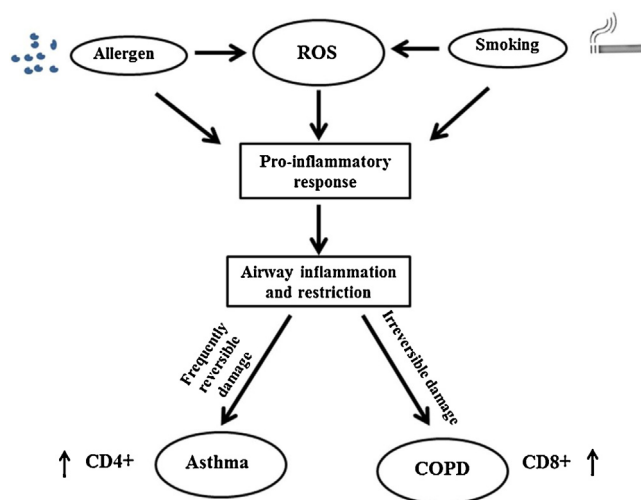


Fig. 1. This schematic diagram outlines the major differences and a proposed redox activation mechanism of asthma and COPD.

signaling network, called ROS-induced ROS release (RIRR) (Aon et al., 2003; Zorov et al., 2006). To confirm this process, several studies have acknowledged the outer mitochondrial membrane, mitochondrial matrix, and the inner mitochondrial membranes as a source for $O_2^{\bullet-}$ production (Turrens, 2003). $O_2^{\bullet-}$ is converted to H_2O_2 spontaneously or by an enzymatic process which greatly increases activity using one of three isoforms of SOD (Leopold and Loscalzo, 2005). Specifically, H_2O_2 can undergo a one electron reduction to the highly reactive hydroxyl radical in the presence of reducing metal ions such as iron, or through a two-electron reduction H_2O_2 can form water by the enzyme catalase (Zuo et al., 2012, 2011b). In addition, oxidants can also be inhaled or released by activated neutrophils, alveolar macrophages, eosinophils, and epithelial cells (Rahman, 2003).

Based on a variety of studies, it is clear that pulmonary ROS formation is a component of the molecular mechanism of asthma. The framework of ROS generation is such that oxidants, which mediate inflammatory responses, activate pro-inflammatory cytokine and chemokine genes that facilitate the up-regulation of adhesion molecules and the increased release of pro-inflammatory mediators (Rahman, 2003). Furthermore, the morphological and functional properties of endothelial cells such as permeability and the expression of adhesion molecules can be altered by ROS, resulting in adhesive interaction between inflammatory and endothelial cells which may contribute to the expression of inflammatory symptoms (Nagata, 2005). More explicitly, ROS may act as signaling modifiers of transcription factors such as nuclear factor- κ B (NF- κ B) and activator protein-1 (AP-1) in epithelial cells (Adcock et al., 1994; Haddad et al., 2000; Krishna et al., 1998; Schreck et al., 1991; Winyard and Blake, 1997). Expression of genes for many pro-inflammatory cytokines, enzymes, and adhesion molecules potentially result from activation by these transcription factors, thus inducing an inflammatory response (Barnes and Karin, 1997; Henricks and Nijkamp, 1998; Kondo et al., 1999; Rahman, 2000; Winyard and Blake, 1997). It can therefore be concluded that there is a dynamic relationship between the molecular mechanism of ROS and expression of asthma symptoms (Fig. 1).

4. The development and effects of oxidative stress in asthma

Oxidative stress, the physiological damage that occurs by ROS attack, has been proven to affect smooth muscle contraction (Zuo et al., 2011a), induce airway hyper-responsiveness (Katsumata

et al., 1990; Zuo and Clanton, 2005), and increase mucus secretion and epithelial shedding within respiratory cells (Doelman et al., 1990; Gillissen and Nowak, 1998; Nabe et al., 2012). It is known that excessive ROS production in asthma can trigger key enzymatic and non-enzymatic alterations, leading to an antioxidant-oxidant imbalance in respiratory airways (Nadeem et al., 2008). This imbalance further promotes a state of oxidative stress in asthma (Fig. 1).

In addition, when factors such as allergens, gaseous pollutants, bacteria, and viruses activate inflammatory cells in asthmatic airways, a respiratory burst releases ROS to surrounding respiratory cells and tissues (Wood et al., 2003). Increased exposure to O₃ alters the distribution of β_1 integrins in bronchial epithelial cells with subsequent damage to cilia and loss of cells, also contributing to oxidative stress (Henricks and Nijkamp, 2001; Jabbour et al., 1998). Exposure to O₃ has the capability to increase the permeability of respiratory epithelial layers in humans and animals, which can potentially induce injury as seen in asthma (Henricks and Nijkamp, 2001; Hyde et al., 1992; Kehrl et al., 1987; Rusznak et al., 1996). Thus, O₃ has been determined to initiate epithelial cell necrosis as a result of proliferation of respiratory cells (Salmon et al., 1998). Interestingly, several studies have demonstrated that cells isolated from bronchoalveolar lavage (BAL) fluid have a tendency to stimulate ROS production once aroused by allergens (Calhoun and Bush, 1990; Calhoun et al., 1992), leading to a significant increase of spontaneous O₂^{•−} production (Jarjour et al., 1992).

Heme oxygenase-1 (HO-1) stress response protein can likewise promote a state of oxidative stress when induced (Choi and Alam, 1996; Wong and Wispe, 1997). However, recent studies have speculated that deleterious effects of the hemeoxygenase (HO) system occur only at high levels while moderate expression of HO-1 may provide protection against the effects of oxidative stress (Suttner et al., 1999). Iron and bilirubin are specific catalytic by-products which draw attention to the HO-1 system by having a significant role in the protection of oxidative stress induced injury within the lung (Choi and Alam, 1996). Bilirubin in particular prevents against oxidative damage due to its effective anti-oxidant properties (Tomaro and Battle, 2002). HO-1 provides a vital role in cellular homeostasis maintenance among different inflammatory diseases such as septic shock and asthma (Chung et al., 2008; Hoshino et al., 2008).

Although it has potential protective qualities, the HO enzyme system is known to produce the majority of endogenously produced carbon monoxide (CO) associated with oxidative stress (Otterbein and Choi, 2000; Ryter et al., 2006). In asthma patients, there is a considerable relationship between increased exhaled CO concentrations and decreased forced expiratory volume (FEV), which is defined as the maximum amount of air that can be expelled in a given number of seconds during a forced vital capacity (Yamaya et al., 2001). This finding verifies that increased CO levels can promote oxidative stress, further worsening asthma symptoms.

5. Asthma, COPD, and airway inflammation

Although asthma and chronic obstructive pulmonary disease (COPD) present many of the same symptoms and are induced by ROS via pro-inflammation cytokine pathways (Rana et al., 2004; Zuo et al., 2012), research has shown that there are differences between the two. Patients with COPD, the fourth leading cause of death in North America, mainly induced by long-term smoking, demonstrate persistent limited expiratory airflow which can progressively worsen with age (Jeffery, 1999; Zuo et al., 2012). Conversely, in asthma, airflow limitation is momentary and frequently reversible; however inflammation from repeated contact with allergens and occupational exposure may lead to irreversibility (Jeffery, 1999). Additionally, research by O'Shaughnessy et al.

reveals a key difference between the two diseases: in COPD, the CD8⁺ lymphocyte subset increases, whereas it is the CD4⁺ lymphocyte subset level that is characteristically increased in asthma (Nabe et al., 2012; O'Shaughnessy et al., 1997). Despite these differences between COPD and asthma, airway inflammation is an occurrence of both diseases and recent work has suggested that there may be similarities in the method of pro-inflammatory cytokine gene expression (Jeffery, 1998; O'Shaughnessy et al., 1997). This airway inflammation experienced in both diseases is related by an increased production of active elastase, which may lead to the promotion of fibroblast migration through the extracellular matrix and the deprivation of elastic fibers (Chetty et al., 1995; Vignola et al., 1998). While the line of distinction between asthma and COPD may initially seem blurred, continued pulmonary research is crucial for understanding the molecular mechanisms of these diseases and thus developing ways to properly diagnose and treat them (Zuo et al., 2012) (Fig. 1).

6. Animal models of asthma

Our objective is to better understand the use of rats and mice in experiments related to asthma studies. These models are economical and hardy organisms with a similar genomic sequence to humans; moreover, genetically modified mice strains are widely available and their usage prevents the exposure of harmful side effects to humans during clinical trials (Corazza and Kaufmann, 2012). Furthermore, numerous significant insights in the understanding of asthmatic conditions have been acquired through animal models of allergic inflammation in the airways (Lieberman and Wang, 2012). Two models for mimicking such airway inflammation responses include the use of C57BL/6 strain derivatives, such as the transgenic mice that are created using this C57BL/6 background, and a Balb/c strain, which is the preferred strain in the research of allergies because it has a propensity to develop immune responses of Th2, a type of helper T cell (Corazza and Kaufmann, 2012; Schroder and Maurer, 2007). The method for establishing non-transgenic mouse models of asthma is often the intraperitoneal injection of a foreign antigen preceded by introducing the antigen directly into the nasal cavity or lung by inhalation, subsequently initiating airway inflammation (Corazza and Kaufmann, 2012). A majority of studies increasingly use antigens such as birch pollen and house dust mite because they are more clinically relevant to asthma patients (Corazza and Kaufmann, 2012).

Although comprehensive efforts have been made to explore allergy-induced asthma in animal models, asthma appears to be a disease generally restricted to humans (Bates et al., 2009). An important characteristic separating human asthma from that of animal models is that mice develop an acute form of airway inflammation; accordingly, increased exposure to antigens fails to lead to chronic inflammation as seen in the human disease (Bates et al., 2009). Despite the fact that there may not be an animal model to perfectly simulate human asthma, the use of such models may help with our understanding and lead to novel medical advancements.

7. Asthma and aging

Asthma in the elderly, defined as age 65 and greater, is frequently under diagnosed and undertreated due to the complexity of the disorder (Enright et al., 1999). When compared to younger asthma patients, elderly asthmatics have an increased rate of severe exacerbations, emergency department visits, and hospitalizations (Moorman et al., 2007). The term "immunosenescence" has been devised to represent changes in the immune system that occur with age which includes deviations in both the numbers and functions of multiple cell types including T lymphocytes (T-cells), B

lymphocytes (B-cells), natural killer cells (NK cells), neutrophils, and eosinophils (Gomez et al., 2008; Sansoni et al., 2008; Weksler and Szabo, 2000). Aging also causes a reduction in the generation of force in respiratory muscles due to degenerative changes in muscle, altered nutritional status, and changes in chest wall configuration on the force generation capacity of the diaphragm (Berend, 2005). Additionally, immunological changes both systemically and within the lung, regardless of the presence of disease, are associated with aging (Berend, 2005; De Martinis et al., 2005). A further complication is neutrophilic inflammation, similar to that noted in COPD and severe non-eosinophilic asthma, which is present in the airways of older patients (Meyer et al., 1998). This inflammation may be attributed to the lifelong antigenic burden, prolonged exposure to environmental stimuli, or to age-related changes to innate immunity (Berend, 2005).

Age can also influence the efficacy of certain asthma treatments. For example, several studies have indicated that Albuterol treatments given in the emergency departments of hospitals are less successful in older patients, resulting in more frequent admission for hospitalization (Banerji et al., 2006). Data from a women's health and aging study showed that increased peripheral blood eosinophils were associated with increased interleukin (IL)-6 levels in older adult women, therefore demonstrating that a marker of allergic inflammation correlates with a marker of age-related systemic inflammation (Leng et al., 2005). Often, this is referred to as "inflamm-aging," and is associated most markedly with increased IL-6 and tumor necrosis factor- α levels in the serum (Franceschi et al., 2007). Moreover, sensitivity to particular indoor allergens such as dust mite and cockroach, are more prominently associated with the presence of asthma in an elderly population (King et al., 2004; Rogers et al., 2002).

8. Antioxidant therapy

The large surface area, blood supply, and high oxygen environment within the lungs make them highly subject to oxidative stress induced injury. Therefore, pulmonary antioxidants are crucial in order to develop protection against oxidant-induced tissue damage (Kirkham and Rahman, 2006). In order to combat the undesirable effects of ROS, various endogenous antioxidant strategies have evolved, incorporating both enzymatic and non-enzymatic mechanisms. For instance, several non-enzymatic antioxidant species exist within the lung lining fluid such as glutathione, uric acid, albumin, ascorbic acid (vitamin C), and α -tocopherol (vitamin E) (Kirkham and Rahman, 2006). In an adaptive process, lung epithelial permeability can be increased by ROS induced lung injury (Li et al., 1994), therefore allowing leakage of plasma constituents containing antioxidants into the lung lining fluid, providing additional antioxidant protection (Kirkham and Rahman, 2006).

Antioxidant intervention to counteract increases in oxidative stress can be achieved through two main approaches: endogenous antioxidant enzyme defenses can be increased or non-enzymatic defenses can be improved through dietary or pharmacological methods (Kirkham and Rahman, 2006). There are two classes of carotenoids which show strong antioxidant activity, attributed to seizing single oxygen atoms and scavenging free radicals: carotenes and xanthophylls (Jackson et al., 2008). Noted studies have reported a link between decreased lung function (Gilliland et al., 2003) and the presence and severity of asthma with reduced dietary antioxidant intake of antioxidants such as vitamins C, α -carotene, and selenium (Harik-Khan et al., 2004; Rubin et al., 2004). However, when compared to current standard therapies for mild to moderate asthma, supplemental intake of vitamins C and E has been shown to have no clinical benefit (Fogarty et al., 2003; Pearson et al., 2004).

Another strategy involves effectively isolating the single electron in free radicals, such as $O_2^{\bullet-}$, and can be performed using a spin

trap, which forms a relatively stable, measurable product. Most spin traps are based around a nitron- or nitroxide-containing molecule. Along with their *in vitro* use, when given therapeutically *in vivo*, spin traps have demonstrated a direct reaction with ROS/RNS (reactive nitrogen species) at sites of inflammation (Chabrier et al., 1999). Nitron spin traps have also been widely used as antioxidants to counter free radical-mediated cellular damage in diseases such as stroke and neurological conditions including Alzheimer's and Parkinson's diseases (Becker, 1999).

A novel, promising asthma therapy uses small compounds which retain the catalytic activity and mimic larger enzyme based molecules, called enzyme mimetics (Kirkham and Rahman, 2006). Antioxidants categorized as enzyme mimetics are the mimics of SOD, catalase, or glutathione peroxidase. Polyphenols are a large group of natural antioxidants principally derived from plants and include benzoic acids, cinnamic acids, coumarins, and flavonoids. Several epidemiological studies have established a beneficial link between polyphenol intake and lower disease risk that is associated with the antioxidant and anti-inflammatory properties of polyphenols (Arts and Hollman, 2005). A significant inverse correlation was observed between polyphenol intake and the incidence of asthma in one study with over 10,000 patients (Knekt et al., 2002). Further investigations into the effectiveness of these antioxidant therapies are necessary in order to improve the management of asthma symptoms.

9. Key studies of asthma

9.1. SARP

When discussing the relationship between asthma and ROS, it is important to acknowledge the recent contributions made by the Severe Asthma Research Program (SARP). In the following we will discuss the updated studies conducted by SARP to further understand the physiological sources of asthma and to develop new potential treatments.

Mast cells play a critical role in the relationship with asthma. There are a lot of persuasive data that associates mast cells with inflammatory diseases and the pathophysiology of asthma (Bradding et al., 2006; Collington et al., 2011). To further clarify this relationship, SARP conducted a study to compare mast cell markers between normal and asthmatic patients using bronchoscopically-obtained mast cell samples from each patient (Balzar et al., 2011). Balzar et al. (2011) concluded submucosal and epithelial mast cells are associated with severe asthma when found in large quantities. In addition, the activation of mast cells within the submucosa and epithelium, result in increased levels of prostaglandin D_2 , a cyclooxygenase metabolite which contributes to asthma pathophysiology upon allergen stimulation (Shichijo et al., 2009). This evidence suggests severe asthma pathology is influenced by mast cells (Balzar et al., 2011).

Another critical question raised by SARP was the relationship between nitric oxide (NO) and patients who suffer from severe asthma. NO has a significant physiological role in pro-inflammatory activities such as asthma, whereby an increased level of NO was detected (Ashutosh, 2000). A recent SARP study hypothesized that levels of forced exhaled nitric oxide (FE_{NO}) may be associated with severe asthma allowing researchers to recognize an at-risk asthma phenotype within the population (Dweik et al., 2010). Due to the close correlation NO has with asthma; the measured FE_{NO} may prove to be a useful marker to treat asthma patients. Inhaled-corticosteroids are a common treatment for asthma and doses are adjusted based on the severity of symptoms of a patient (Smith et al., 2005). This allows FE_{NO} concentrations to be used as an indicator of asthma severity and a good marker to adjust the dosage of corticosteroids based on the symptom of a patient (Smith

et al., 2005). To further examine this relationship between NO and asthma, Dweik et al. (2010) conducted a study with 446 patients with asthma and 49 healthy patients involved in SARP, examining key features of asthma such as airway inflammation and hyper-responsiveness. They found that an increase in airway reactivity, inflammation, and hyperinflation were all common characteristics seen within asthmatic patients who suffered from high FE_{NO} levels (Dweik et al., 2010). This leads to the conclusion that FE_{NO} levels can provide a classification of asthma severity, identifying those patients who suffer from a more reactive asthma phenotype (Dweik et al., 2010).

Interestingly, there may also be a relationship between various biological factors of severe asthma and race. SARP aimed to determine whether specific variables such as physiologic, immunoinflammatory, and sociodemographics contribute to racial disparities between black and white patients suffering from severe asthma (Gamble et al., 2010). Gamble et al. concluded that there are differences in severe asthma seen between black and white patients (Gamble et al., 2010). For instance, the odds of developing severe asthma decreased in white patients who have a family history of asthma whereas the odds for developing severe asthma in black patients nearly doubled (Gamble et al., 2010). In addition, when analyzing IgE levels between black and white patients, Gamble et al. were able to determine there were no significant associations between severe asthma and multivariable analysis IgE levels in white patients but there was a strong association in black patients (Gamble et al., 2010). This information concludes that biological factors vary between black and white asthma patients and can further promote the development of new treatments associated to a specific race.

9.2. Research on eosinophils and neutrophils

In the past ten years, much research has been done regarding the inactivation of antioxidants in allergen challenge, as well as the role of eosinophil and neutrophil-derived oxidant species. We summarize several of their pertinent findings below. Erzurum and Hazen's research has connected oxidative and nitrative stress to the loss of SOD activity and downstream events characteristic of asthma, including apoptosis, shedding of the airway epithelium and hyper-responsiveness (Comhair et al., 2005b). In this regard, it has been directly demonstrated that RNS contribute to oxidative modification of proteins in asthma and that eosinophils play a strong role in generating NO-derived oxidants in severe asthma (MacPherson et al., 2001). Additional investigations have established a potential mechanism of action; *in vivo* activation of eosinophils has been shown to result in oxidative damage of proteins through bromination of tyrosine residues (Wu et al., 2000). Overall, this work suggests that the amplification of inflammation and airflow obstruction is caused by greater oxidant stress which leads to greater inactivation of SOD. Recent findings further suggest a correlation, in that sensitive and quantitative functional measures of global oxidative stress in asthma may be achieved using systemic measures of SOD inactivation (Comhair et al., 2005a).

9.3. The Role of NADPH oxidase in the airway

NADPH oxidase (NOX), a multi-component enzyme complex bound to the membrane of phagocytes and non-phagocytic cells (Lee et al., 2009) has two main roles: the production of superoxide by NOX2 required for phagocytic respiratory bursts and cell signaling regulation (Lee and Yang, 2012; Rahman et al., 2006). NOX is thought to be the key factor in the production of ROS, resulting in a variety of respiratory inflammatory diseases, including asthma mediators (Lee et al., 2009; Lee and Yang, 2012). It is established that this NOX-derived ROS reversibly and specifically reacts with

proteins modifying their placement, activity and half-life (Rahman et al., 2006). Furthermore, activation of the NOX complex consisting of membrane-linked flavocytochromes and a range of cytosolic co-factors, mediates transmembrane electron transfer from NADPH, thus reducing O_2 to $O_2^{\bullet-}$ and H_2O_2 (Bedard and Krause, 2007). Moreover, it has been shown that inhibition of NADPH oxidase and ROS generation reduces mitogen-activated protein kinases activation thus demonstrating the correlation between NOX/ROS and inflammatory gene expression from pro-inflammatory mediators (Lee et al., 2009; Lee and Yang, 2012).

9.4. Outcomes of NCI CARET and ATBC studies

What was once the key contender for the chemoprevention of lung cancer, beta-carotene, has proven to have no benefit as a result of two unfortunate National Cancer Institute (NCI) trials, the beta-carotene and retinol efficacy trial (CARET) studying male and female heavy smokers and those exposed to asbestos, and the alpha-tocopherol beta-carotene trial (ATBC) studying Finnish male smokers (Omenn, 2007; Omenn et al., 1996a,b). Furthermore, the studies not only proved beta-carotene to be non-beneficial, but an increase in the incidence of lung cancer was observed (Omenn, 2007). Specifically, the results of both trials demonstrated an excess in the incidence of lung cancer and mortality in the active treatment group compared with the placebo group. In the ATBC trial, an 18% increase in the incidence of lung cancer and an 8% increase in mortality were observed, while a 28% increase in the occurrence of lung cancer and an 18% mortality increase were discovered in CARET (Omenn et al., 1996a,b). These studies suggested that vitamins are chemicals that may have more actions than are well-known, beta-carotene being a prime example (Omenn, 1998; Omenn, 2007; Salgo et al., 1999).

10. Conclusion

In this review, we have discussed the most recent findings regarding ROS and inflammation which occur in asthma. Based on both clinical and scientific research, we have identified the ongoing investigations of asthma pathogenesis and the molecular mechanisms of ROS formation. Although there is no current cure for asthma, we have noted several beneficial anti-inflammatory therapeutic asthma treatments. This review identifies the complex relationship of ROS and inflammation in asthma, providing a fundamental understanding of the disorder as a basis for further research.

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