Recent developments in the role of reactive oxygen species in allergic asthma

Jingjing Qu[1,2]*, Yuanyuan Li[1]*, Wen Zhong[1], Peisong Gao[2], Chengping Hu[1]

[1]Department of Respiratory Medicine, Xiangya Hospital, Central South University, Changsha 410008, China; [2]Division of Allergy and Clinical Immunology, Johns Hopkins School of Medicine, Baltimore, MD, USA

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*These authors contributed equally to this work.

Correspondence to: Chengping Hu. Department of Respiratory Medicine, Xiangya Hospital, Central South University, Changsha 410008, China. Email: huchengp28@126.com; Peisong Gao, MD, PhD. The Johns Hopkins Asthma & Allergy Center, 5501 Hopkins Bayview Circle, Room 3B.71, Baltimore, MD 21224, USA. Email: pgao1@jhmi.edu.

Abstract: Allergic asthma has a global prevalence, morbidity, and mortality. Many environmental factors, such as pollutants and allergens, are highly relevant to allergic asthma. The most important pathological symptom of allergic asthma is airway inflammation. Accordingly, the unique role of reactive oxygen species (ROS) had been identified as a main reason for this respiratory inflammation. Many studies have shown that inhalation of different allergens can promote ROS generation. Recent studies have demonstrated that several pro-inflammatory mediators are responsible for the development of allergic asthma. Among these mediators, endogenous or exogenous ROS are responsible for the airway inflammation of allergic asthma. Furthermore, several inflammatory cells induce ROS and allergic asthma development. Airway inflammation, airway hyper-responsiveness, tissue injury, and remodeling can be induced by excessive ROS production in animal models. Based on investigations of allergic asthma and ROS formation mechanisms, we have identified several novel anti-inflammatory therapeutic treatments. This review describes the recent data linking ROS to the pathogenesis of allergic asthma.

Keywords: Allergic asthma; reactive oxygen species (ROS); antioxidants

Introduction

Asthma is a characteristic of different phenotypes of chronic airway inflammation, including allergic asthma and non-allergic (intrinsic) asthma. Allergic asthma is a chronic airway inflammatory disease in which many immune regulatory cells within the immune system work together with epithelial cells to cause smooth muscle contraction, enhance airway hyper-reactivity (AHR), increase mucus secretion and stimulate bronchospasm. Non-allergic asthma often develops later in life and, per its definition, has neither immunoglobulin E (IgE) reactivity to allergens nor any obvious involvement of the adaptive immune system, such as type 2 helper T cells (Th2 cells) (1,2). Allergic asthma is more prevalent than non-allergic asthma. In our review, we focus on allergic asthma. In susceptible patients, the main clinical symptoms of allergic asthma are shortness of breath, chest tightness and wheezing (3). Allergic asthma is very common and afflicts 8.5% of the U.S. population and is estimated to cause >3,000 deaths and to cost more than $56 billion annually in lost work and medical expenses (4). Many patients use corticosteroids and a short- or long-acting β-adrenergic agonist to treat asthma; however, in 5–10% patients, therapy fails (5).

It is well known that reactive oxygen species (ROS), endogenous nitric oxide (NO) and NO derived reactive nitrogen species (RNS) have been reported to mediate oxidative stress and airway inflammation and pathogenesis. However, ROS are more common in allergic asthma development (6,7). Hence, in our review, we mainly focus on the relationship between ROS and allergic asthma. ROS, such as hydrogen peroxide, may be directly or indirectly involved in epithelial cell apoptosis and inactivation of antioxidant enzymes and contribute to allergic asthma (8-11). ROS are derived from endogenous or exogenous oxidants. Endogenous ROS are composed of mitochondria, NADPH, and the xanthine/xanthine oxidase (XO) system. Exogenous sources of ROS production have been linked to strong oxidizing gases, such as ozone (O_3), sulfur dioxide (SO_2), nitrogen dioxide (NO_2) and particulate matter (PM), which are a major component of air pollution (12,13). The oxygen stress level in asthma is increased because of inflammatory cells *in vivo*, and cigarette smoke (CS) or PM produces ROS *in vitro*. For example, compared with normal subjects, the level of H_2O_2 and superoxide that are produced by eosinophils and neutrophils are significantly increased in asthma patients (14). Increased levels of ROS can cause harmful pathophysiological disorders of allergic asthma. Studies have reported that excessive ROS can damage DNA, carbohydrates, proteins, and lipids, ultimately leading to an increased inflammatory response in allergic asthma (15,16). In this review, we will discuss the updated pathophysiological and mechanisms of oxidative stress relating to allergic asthma.

Exogenous oxidants and allergic asthma

Patients' exposure to the environmental atmosphere is the main way that exogenous oxidative stress is induced (17). CS is a complex mixture including more than 4,000 different compounds. It has been demonstrated that inhaled CS can increase ROS levels. Most of these compounds have ability to generate ROS during their metabolism. The mechanism of CS induced inflammation is incorporated with oxidative stress leading to cell death and oxidative DNA damage via apoptosis and/or necrosis (7). It was reported that antioxidants have the ability to protect cells from cigarette smoke extract (CSE) induced apoptosis (18). Isolated human airway smooth muscle (hASM) cells were used to determine Ca^{2+} responses and hASM proliferation, as well as ROS level and cytokine generation, by incubating these cells in the presence or absence of CSE. The results revealed that Ca^{2+}

regulatory proteins alter airway remolding and function in smoking-related airway disease, especially allergic asthma (19). CSE can damage bronchial epithelial cells from asthmatic patients through regulation of apoptosis-inducing factors, which is implicated in DNA damage and ROS-mediated cell-death (18). Recently the nanoparticles (NPs) that particle diameter less than 100 nano including engineering NPs and natural formation of the NPs already exist in the air, such as photochemical smog, forest fires, car exhaust. The NPs could exacerbate the inflammatory responses in lungs. PM and is consistently involve in respiratory diseases, such as asthma, through oxidative stress pathways (20). PM is capable of generating free radicals, the metals on their surface takes part in redox cycling or depletion of anti-oxidant glutathione function and $PM_{2.5}$ means the particle diameter less than or equal to 2.5 µm (21,22). Josephine was the first to estimate population-level health effects through ROS activity measurements and to use an epidemiologic approach to link $PM_{2.5}$ ROS to asthma. The results showed that reducing water-soluble $PM_{2.5}$ (WS-$PM_{2.5}$) dithiothreitol (DTT) activity may significantly decreasing the incidence of asthma (23). A meta-analysis showed that particle components play a pro-oxidant role that thereby promote airway inflammation (24). It is well known that the aryl hydrocarbon receptor (AhR) is recognized as a receptor for environmental contaminants. AhR induction of increased mucin production is partially mediated by ROS generation, and antioxidant therapy can mitigate AhR-induced mucus hyper-secretory diseases (25). AhR ligands have been shown to induce mast cells to increase ROS production in asthma (26). Ozone could be an inflammatory mediator by scavenging ROS; for example, electron-rich olefins could be used to treat asthma (27). Additionally, asbestos, coal, and pollens also induce excessive ROS formation levels that are related to immune responses associated with allergic asthma (28,29).

Endogenous oxidants and allergic asthma

Intracellular ROS are mainly composed of mitochondria, NADPH oxidase, and the xanthine/XO system. The generation of superoxide anion is mediated by mitochondria and NADPH oxidase. It was reported that eosinophils and neutrophils could produce more superoxide anion from asthmatic patients compared with healthy subjects due to NADPH oxidase activation during asthma (30). The NADPH oxidase, dual oxidase 1 (DUOX1), critically contributes to airway epithelial secretion of interleukin-33 (IL-33),

which is dependent on Src/EGFR pathway activation in asthma (31). Leukotriene C4 (LTC$_4$) can elicit nuclear translocation of NADPH oxidase 4 (NOX4), ROS accumulation and oxidative DNA damage. LTC$_4$ inhibitors, which are used clinically for asthma, were associated with endoplasmic reticulum (ER) stress activated NOX4 (32). It is interesting that NADPH oxidase 2 (Nox2) deficiency results in induced experimental asthma, which is caused by aggravate Th2 effector function in a T cell intrinsic manner; however, the underlying mechanism must be studied further (32). Superoxide anion can also be generated from mitochondrial respiration chain reactions. Mitochondria are double membrane organelles that possess a proteome and genome (33). Additionally, they are capable of synthesizing and catabolizing metabolites and generating ROS (34). Silencing of mitochondrial transcription factor A (TFAM) not only dramatically reduces bronchial smooth muscle (BSM) cell proliferation but also abolishes the difference between BSM cell proliferation in control subjects and asthmatic patients (35). A study focused on the relationship between mitochondrial ROS and NLRP3 inflammasome in asthma. NecroX-5, a novel mitochondrial ROS inhibitor can decrease protein levels of NLRP3, caspase-1 and IL-1β in tracheal epithelial cells (36). Xanthine oxidoreductase (XOR) is a highly conserved member of the molybdoenzyme family, which exists in two forms, xanthine dehydrogenase (XDH) and XO. The baseline expression of XOR is low; however, transcription can be up-regulated due to several factors, such as hypoxia, lipopolysaccharide (LPS), IFN-γ, IL-1, IL-6, tumor necrosis factor-α (TNF-α), and steroid stimulation. XOR activation is increased in the late allergic response phase (37).

Effect of oxidants on cells during allergic asthma

Epithelial cells

The barrier function of epithelial cells is often injured in allergic asthmatic patients. Epithelial cells are more easily affected by ROS derived from infiltrated inflammatory cells. In fact, IL-13 treatment with primary human tracheal epithelial can stimulate mucus formation and ROS production. However, the muc5ac secretion and ROS levels can be inhibited in epithelial cells that are depleted of the autophagy genes, autophagy related 5 or autophagy related 14, compared with non-depleted control cells. These findings indicate that autophagy is essential for IL-13 to modulate ROS and mucus formation in epithelial cells

during asthma (38). A study suggested that proteinase-activated receptor 2 (PAR-2) is involved in airway inflammation regulation through oxidative stress, leading to increased DUOX-2/ROS pathway activation in airway epithelial cells (39). In human bronchial epithelial cells, lack of glutathione S-transferase Mu 1 (GSTM1) enhances ozone-induced IL-8 production because of ROS generation and subsequent NF-κB activation (40).

Airway smooth muscle and endothelial cells

Asthma can activate mitochondrial ATP-sensitive potassium channels (MitoKATP) in human airway smooth muscle cells (HASMCs) and promote HASMC proliferation and TGF-β1 expression (41). It was reported that even low CSE levels have a substantial impact on ASMC mitochondria and increased Drp1 expression, which subsequently affects mitochondrial morphology and energy production (42). ROS induce increased Ca^{2+}, which results in contraction of airway smooth muscle cells that are treated with ovalbumin (OVA), resulting in airway hyper-responsiveness (43). AMP-activated protein kinase (AMPK) may be a possible therapeutic target for asthma because AMPK can ameliorate airway inflammatory responses by inhibiting ROS production and reducing vascular permeability via the HIF/VEGFA pathway (44). A murine model of asthma used to evaluate the effect of L-2-oxothiazolidine-4-carboxylic acid (OTC), a cysteine pro-drug that acts as an antioxidant, was shown to increase vascular permeability. When OTC was administered in the mouse model, it markedly reduced plasma extravasation and vascular endothelial growth factor (VEGF) levels in allergen-induced asthmatic lungs (45).

Inflammatory cells

Oxidants were reported to have various effects on inflammatory cells. Dendritic cell (DC) function is altered in the absence of a functional NADPH oxidase complex, providing a potential mechanism for the decreased allergic airway inflammation observed in an asthma model (46). Oxidant gasses can directly activate DCs. DCs play an important role in allergic asthma due to their potent ability to activate naive CD4^{+} T cells (47). If mast cells are exposed to oxidants, then they release significantly amounts of histamine and serotonin. Activated mast cells have increased intracellular ROS levels, and ROS enhance histamine release from mast cells (48). Exposure to AhR ligands results in an ROS-dependent increase of reversible oxidation,

leading to enhanced mast cell function in OVA-stimulated mast cells (26). DJ-1 is an antioxidant protein. DJ-1-KO mice exhibited enhanced passive cutaneous anaphylaxis (PCA) and augmented ROS levels in sera and in bone marrow-derived mast cells (BMMCs) (49). During airway inflammation, peripheral blood eosinophils are increased by enhanced spontaneous ROS production and increased cytokines in allergic asthma patients (50,51).

Redox signaling mechanisms in allergic asthma

Hallmark Th2 disorders are the main cause of asthma. Antigen-presenting DCs that recognize and present allergens to T cells in the lymph nodes that activate CD4+ cells that produce IL-4 and IL-5 were found in the bronchoalveolar lavage fluid (BALF) and were correlated with the degree of airway eosinophilia (48). IL-4 induces immunoglobulin synthesis by B cells and release of IgE, which is a critical hallmark of T_H2 immunity (52). IgE binds to its receptor, FcεRI, which is located on mast cells and regulates ROS. IL-5 is responsible for the generation and activation of eosinophils to mediate ROS generation. IL-13 is responsible for AHR and goblet cell metaplasia (53). It was reported that IL-4, IL-5 and IL-13 can regulate DUOX activity that is mediated by H_2O_2, which influences several important features of allergic asthma (54). Moreover, TNF-α and IL-1 can induce ROS formation. These critical mediators may activate mellatoproteinase-9 (MMP-9), intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), which promotes ROS production and inflammation and exacerbates allergic asthma (55). ROS have been shown to increase smooth muscle contraction, enhance AHR and stimulate mucus secretion (*Figure 1*) (1,2).

Current research has also provided more signaling molecules that are related with critical redox reactions during allergic asthma. The expression of copper oxide nanoparticles (CuONPs), which are metal oxide NPs, was increased in an OVA-induced asthma murine model. CuONPs markedly increase AHR, cytokines, and ROS (56,57). PAR-2 mediated DUOX-2 and ROS in airway epithelial cells in a cockroach allergen mice model. However, ENMD-1068, a small molecule antagonist of PAR-2, can inhibit DUOX-2 and ROS in allergic mice (39). NLRP3 inflammasome assembly is activated by mitochondrial ROS, which consequently induces IL-1β production and release (36). In OVA sensitized murine models, a decrease in cellular glutathione, which

is an antioxidant, is involved in the resolution of airway inflammation and respiratory hypersensitivity during the sensitization stage, which has been associated with NOX inhibition and enhanced ROS generation (58). Moreover, decreased glutathione levels have been suggested to induce changes in T_H1 to T_H2 cell differentiation. Under these circumstances, the oxidant and antioxidant balance is lost and AHR is induce by the synthesis of S-nitrosoglutathione and a corresponding shift in NO metabolism (58,59). Another important redox mediator, glutathione S-transferase pi, which was found in lung epithelium, primarily facilitates the conjugation of reduced glutathione to mitigate the electrophilic properties of inhaled antigens and potentially reactive molecules (60). The multifunctional Ca^{2+} and calmodulin-dependent protein kinase II (CaMKII) can be activated by ROS and contribute to inflammation. Oxidative CaMKII was increased in airway epithelium biopsied from mildly asthmatic patients and OVA or *aspergillus fumigatus* challenged mouse models (61). A mouse model that evaluated airway epithelium with a specific deficiency in CaMKII expression showed clinical features that included inhibition of AHR airway epithelial proliferation and mucus secretion (62).

Redox therapeutics

The antioxidant system is well developed in allergic asthma. Generally, antioxidants can be divided into enzymatic [glutathione peroxidase and superoxide dismutase (SOD)] and non-enzymatic (vitamin E, vitamin C) subcategories, which play a critical role in the inhibition and elimination of oxidative damage. Recently, new treatments for ROS in allergic asthma were reported in several studies. *Table 1* shows the antioxidant system.

The toll-like receptor 7 (TLR7) agonist, resiquimod (RSQ)

TLRs play an important role in host defense against bacteria, fungi, viruses and allergies through the innate immune system. Accumulating evidence suggests that TLR-associated inflammation is involved in NOX-deriving ROS generation (39). It was shown that the TLR7 activation is protective in mouse models of asthma. Several mechanisms have been reported that contribute to the ability for TLR-7 to have a critical anti-inflammatory effect in asthma. In a study using OVA intraperitoneally to challenge mice that were intranasally treated with the TLR-7 agonist, RSQ, AHR, inflammation, NOX-4 and nuclear factor E2-related
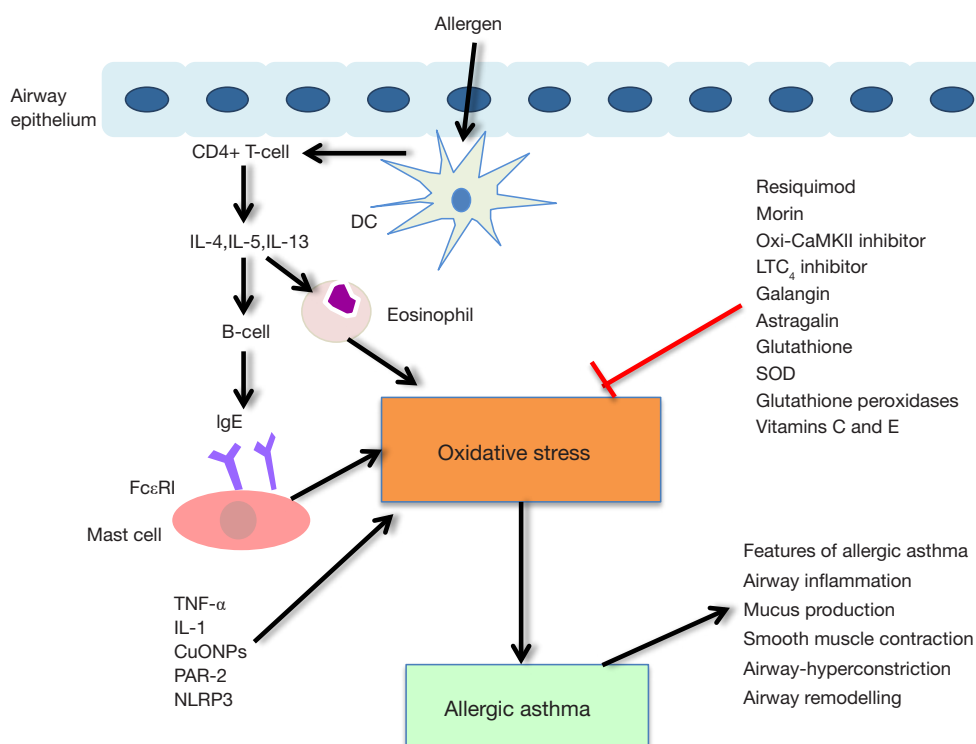
Figure 1 This schematic diagram shows the proposed redox activation mechanism of asthma. Antigen-presenting DCs recognize and present allergens to T cells in the lymph nodes, leading to CD4$^+$ cell activation and IL-4, IL-5 and IL-13 production. IL-4 activation of B cells results in the production of allergic specific IgE, which binds with its receptor, FcεRI, located on mast cells to regulate ROS. IL-5 can generate and activate eosinophils to mediate ROS generation. IL-13 was found to be necessary for AHR and goblet cell metaplasia. Meanwhile, TNF-α, IL-1, CuONPs, PAR-2 and NLRP3 were reported to induce ROS and mediate allergic asthma. However, resiquimod, morin, Oxi-CaMKII inhibitor, LTC$_4$ inhibitor, galangin, astragalin, glutathione, SOD, glutathione peroxidases, and vitamins C and E inhibit ROS generation. DC, dendritic cell; IL-4, interleukin-4; IgE, immunoglobulin E; ROS, reactive oxygen species; AHR, airway hyper-reactivity; TNF-α, tumor necrosis factor-α; CuONPs, copper oxide nanoparticles; PAR-2, proteinase-activated receptor 2; Oxi-CaMKII, oxidative-CaMKII; LTC$_4$, leukotriene C4; SOD, superoxide dismutase.

factor 2 (Nrf2) related signaling levels [NOS, nitrotyrosine, lipid peroxides and copper/zinc superoxide dismutase (Cu/Zn SOD)] were assessed. The results show that treatment with RSQ reduced AHR, inflammation and ROS; however, it improved Nrf2 and Cu/Zn SOD levels in an allergic asthma mouse model. These results from this study suggest that RSQ is beneficial and should be further developed as a therapeutic option in allergic asthma (63).

Morin

Morin (3,5,7,2',4'-pentahydroxyflavone) exists in high concentrations in many herbs, such as Osage orange, *Cudrania tricuspidata*, and other moraceae family members. Morin has been shown to have strong anti-inflammatory

and anti-tumor functions. Accumulating data have indicated that morin suppresses the growth of hepatocellular carcinoma (80) and decreases immune inflammatory responses in chronic colitis (81). However, the role of morin in the treatment of allergic asthma is still unknown. It was reported that in an OVA challenge allergic asthma model, inflammatory cell infiltration around the airways and vessels, goblet cell hyperplasia, collagen deposition/fibrosis and cytokine expression levels were dramatically attenuated following morin treatment. Western blotting and the 2',7'-dichlorofluorescein assay revealed that the intracellular ROS and MAPK phosphorylation levels were abolished with morin treatment (64). A polyphenol flavonol derived morin treatment in an OVA-challenged allergic asthma model also reduced IL-4 and IL-13, lung tissue eosinophil

Table 1 Antioxidants and their functions

Antioxidant	Relevant functions
TLR7 agonist, resiquimod (RSQ)	RSQ reduced allergen-induced AHR, inflammation and oxidative stress; however, it improved Nrf2 and Cu/Zn SOD levels during allergic asthma (63)
Morin	Morin reduced airway inflammation by regulating ROS/MAPK signaling (64)
Oxi-CaMKII inhibitor KN-93	KN-93 significantly decreased goblet cell hyperplasia, bronchial epithelial thickness, airway eosinophilia, the eosinophil chemoattractant molecule, Ccl-11, and NF-κB activity in allergic asthma (61)
Leukotriene C4 (LTC$_4$) inhibitor	A LTC$_4$ inhibitor reduced NOX4 levels and attenuated cell death (32)
Galangin	Galangin demonstrated decreased airway remodeling, angiogenic activity, and ASMC proliferation through the TGF-β1-ROS-MAPK pathway (65-67)
Astragalin	Astragalin suppressed LPS-induced ROS production and eotaxin-1 expression in epithelial cells through the TLR4-PKCγ1-PKCβ2-NADPH signaling pathway (68,69)
Glutathione	GSH balances Th1/Th2 responses, alters nitric oxide metabolism and inhibits ROS (55,59,70)
SOD	SODs protects against the harmful effects of ROS and airway inflammation (71-73)
Glutathione peroxidases	Glutathione peroxidases prevent airway inflammation and alveolar destruction (74-76)
Vitamins C and E	Vitamins C and E reduce AHR, inflammation, and oxidative stress (77-79)

TLR7, toll-like receptor 7; Oxi-CaMKII, oxidative-CaMKII; SOD, superoxide dismutase; AHR, airway hyper-reactivity; Nrf2, nuclear factor E2-related factor 2; Cu/Zn SOD, copper/zinc superoxide dismutase; ROS, reactive oxygen species; NOX4, NADPH oxidase 4; LPS, lipopolysaccharide; Th2, type 2 helper T.

and mastocyte infiltration levels (82). Both of these studies demonstrated novel profiles for morin as a potent asthma management agent.

Oxidative-CaMKII (Oxi-CaMKII) inhibitor

CaMKII is a ubiquitously expressed multimeric serine threonine kinase consisting of 12 subunits. Each subunit is composed of three conserved domains, including an amino terminal catalytic domain that provides the binding sites for ATP and substrates, a central auto-regulatory domain that has an inhibitory pseudosubstrate sequence and several sites for post-translational modifications and a carboxy-terminal associated domain for the oligomerization of subunits to form the holoenzyme and variable regions that are spliced to form various splice variants. CaMKII activity can also be enhanced by ROS, including NADPH oxidase. Mitochondria oxidizes its methionines at 281 and 282, leading to the development of autonomous enzyme activity, which is called Oxi-CaMKII (83). Elimination of ROS generation by blocking either of the pathways results in a significant decline in Oxi-CAMKII levels. Increased Oxi-CaMKII levels were also detected in OVA challenged allergic asthma mice, compared with vehicle treatment,

without any significant increase in total CaMKII levels. Moreover, the Oxi-CaMKII levels were higher in severe asthmatics compared with mild asthmatics and healthy individuals, suggesting that Oxi-CaMKII is not only an indicator of asthma but also a marker of disease severity (61). The p[47] gene encodes for NADPH oxidase, which is required for ROS generation (84). p[47-/-] OVA challenged asthma mice were found to have reduced ROS and Oxi-CaMKII levels compared with wild-type OVA challenged mice. Additionally, inhalation of the Oxi-CaMKII antagonist, KN-93, significantly decreased goblet cell hyperplasia, bronchial epithelial thickness, MUC5AC positive airway cells, Muc5ac mRNA expression, airway eosinophilia, eosinophil chemoattractant molecule Ccl-11, and NF-κB activity in allergic asthma (61).

LTC$_4$ inhibitors

LTC$_4$ production is triggered in ER stress through oxidative stress. The role of LTC$_4$ in allergic asthma has been extensively evaluated (85). LTC$_4$ is produced in mast cells by assembly of a biosynthetic complex at the nuclear envelope, consisting of cytosolic phospholipase A2 (cPLA2), 5-lipoxygenase (5-LO), 5-LO activating protein (FLAP) and

LTC$_4$ synthase (LTC$_4$S). Dvash reported that microsomal glutathione-S-transferase 2 (MGST2)-LTC$_4$ can be elicited by the ER stress. This pathway is the major mediator of ER stress triggered oxidative stress and oxidative DNA damage, which associates with NOX4 and participates in ER stress-triggered cell death. However, the level of NOX4 under extensive ER stress is greatly reduced using LTC$_4$ antagonists. LTC$_4$ receptor antagonists also attenuated cell death and mouse morbidity (32). Now, LTC$_4$ inhibitors are widely used in the clinical treatment of asthma.

Galangin

Galangin (3,5,7-trihydroxyflavone) is a plant that has been used as a herbal medicine for a variety of ailments and exists in high concentrations in honey and alpinia officinarum (65,86). A study showed that galangin exhibited anti-inflammatory and antioxidant activity *in vitro* due to having an affinity towards the COX-2 active site, which was further explored with selective COX-2 inhibitors (66). Galangin also attenuates mast cell function, including decreasing histamine and cytokines release. Furthermore, galangin inhibited IgE-mediated PCA in the inflamed tissue. Galangin inhibited pro-inflammatory cytokine expression, including TNF-α, IL-6, IL-1β, and IL-8, by regulating c-Jun N-terminal kinases and p38 mitogen-activated protein kinase, nuclear factor-κB, and caspase-1 expression (67). Another study revealed that galangin could markedly attenuate the extent of chronic inflammation and airway remodeling in OVA challenged asthma mice, including attenuating inflammatory cell infiltration into the BALF and decreasing the level of OVA-specific IgE in the serum. Furthermore, TGF-β1 and VEGF levels were also reduced following galangin treatment. Additionally, galangin inhibited TGF-β1-induced ASMC proliferation *in vitro*, which involved the ROS level attenuation and ERK, JNK and Akt phosphorylation inhibition. This was the first article to report the potential role of galangin on airway remodeling through TGF-β1-ROS-MAPK signaling, which may provide a promising therapeutic treatment for asthma patients (65).

Astragalin

Astragalin, which is a kaempferol-3-O-glucoside found in persimmon leaves and green tea seeds, possesses anti-inflammatory activity (87,88). It was reported that astragalin inhibited eosinophil infiltration in an OVA-induced asthma model. IL-4, IL-5 and IL-13 were decreased after astragalin treatment. Histological studies demonstrated that astragalin substantially inhibited OVA-induced eosinophilia in lung tissue. All of these anti-inflammatory roles may occur through suppression of cytokine signaling (SOCS)-3 and enhancement of SOCS-5 expression in an asthma model (68). Another study investigated the potential of astragalin and found that it can antagonize oxidative stress-associated airway eosinophilia and epithelial apoptosis. Astragalin suppresses LPS-induced ROS production and eotaxin-1 expression in epithelial cells. The LPS induction of eotaxin-1 was linked to ROS through the TLR4-signaling pathway and PKCγ1-PKCβ2-NADPH oxidases were disturbed by astragalin. Additionally, astragalin endotoxin-instigated epithelial apoptosis was attenuated through manipulating oxidative stress-elicited MAPK signaling in airway epithelial cells. Therefore, astragalin may serve as a modulator against asthma (69).

Glutathione

Glutathione has an SH residue and reacts with oxygen radicals. Glutathione plays an important role in several respiratory diseases and can act against oxidative inflammation along with other enzymatic/non-enzymatic antioxidants. Glutathione can also affect cellular signaling through regulation of redox sensitivity, transcription factors and phosphatases (89,90). Furthermore, glutathione levels can be decreased due to several environment pollutants that have been linked to increased asthma prevalence worldwide (70,91). Glutathione attenuated AHR and inflammation could occur through several mechanisms: (I) the Th1/Th2 balance (70); (II) alteration of NO metabolism through the formation of S-nitrosoglutathione, which was reported to be associated with regulation of airway responses (59); and (III) altering the balance between ROS inhibition and antioxidant reaction (55). Buthionine sulfoximine (BSO) was used for depletion or repletion of glutathione levels during sensitization and challenge phases, respectively, followed by assessment of AHR, inflammation and oxidant-antioxidant balance in an allergy mouse model. A study found that glutathione depletion with BSO induced AHR and airway inflammation and caused a greater oxidant-antioxidant imbalance, as reflected by increased NADPH oxidase expression/ROS generation and decreased total antioxidant capacity. This study indicates that ROS generation in allergic asthma mice was aggravated due to oxidized glutathione and decreased airway responses (58).

SODs

SODs are known as protective antioxidants against the harmful effects of ROS. All forms of SODs act through a common mechanism: dismutation of the superoxide anion to the less potent hydrogen peroxide. Several forms of SODs exist, including Cu/Zn SOD, MnSOD, and extracellular SOD (EC-SOD) (92). Cu/Zn SOD can suppress AHR indicating that the generation of superoxide anion is associated with AHR formation (71). SOD3 is an important isoform of SOD. Several studies have focused on the antioxidant role of SOD3. A study reported for the first time that SOD3 specifically inhibits DC maturation. Subsequently, SOD3 controls T cell activation and proliferation, Th2 and Th17 cell differentiation and inhibits OVA-induced airway remodeling. Interestingly, SOD3 administration in mice alleviated OVA-induced allergic asthma (72,73). Despite the advantages as an anti-inflammatory and antioxidant reagent, it is difficult to obtain large quantities of active recombinant human SOD3 (rhSOD3). A new study found that incorporating metal ions into rhSOD3 after purification maximized the enzymatic activity of rhSOD3. Additionally, albumin or polyethylene glycol prevented rapid inactivation or degradation of rhSOD3 during preparative procedures and long-term storage (72).

Glutathione peroxidases

Glutathione peroxidases are a family of selenium-dependent and selenium-independent antioxidant enzymes. Glutathione peroxidase may decrease airway inflammation and AHR in asthmatic patients because selenium-related antioxidants are powerful scavengers of peroxynitrite (93). An interesting study found that in a susceptible subgroup of mother-child pairs in a population, namely, pregnant women with suboptimal selenium status and offspring with the homozygous minor variant of glutathione peroxidases 4, the incidence of childhood asthma was decreased (74). A study showed the glutathione peroxidase levels were lower in an allergic asthma group compared with a healthy group (75). The expression of human glutathione peroxidase-1 (GPx-1) in transgenic mice significantly increased protein phosphatase-2A (PP2A) and protein tyrosine phosphatase-1B (PTP1B) activities and prevented chronic CS-induced airway inflammation. However, the reverse was observed in GPx-1 knockout mice, including an exaggerated emphysema phenotype and airway inflammation (76).

Vitamins C and E

Dietary intake of vitamin C can reduce the risk of asthma and was associated with increased glutathione peroxidase activity (94). Excessive ROS production decreases enzymatic and non-enzymatic antioxidant levels, such as glutathione, vitamins C and E, β-carotene and glutathione peroxidases, leading to an imbalance of oxidants-antioxidants in airways. It was reported the combination of vitamin C, choline chloride, and selenium reduces AHR, airway inflammation, and ROS levels, probably by inducing IL-10 production by FOXP3$^+$ cells, implicating that vitamin C might be a potential therapeutic against allergic airway disease (77). Vitamin E has antioxidant properties and is comprised of α, β, γ, and δ isoforms for both tocopherols and tocotrienols (95). It is well known that tocopherol is regarded as the isoform that includes the anti-oxidative and anti-inflammatory effects of vitamin E, and some evidence has pointed to γ-tocotrienol as the superior bioactive isoform of vitamin E (95,96). A study demonstrated that oral γ-tocotrienol markedly abated house dust mite (HDM)-induced increases in airway inflammatory, serum IgE levels, cytokine and chemokine levels, mucus production, and oxidative damage biomarkers. The protective mechanisms of γ-tocotrienol are likely mediated by blocking nuclear NF-κB translocation and augmenting Nrf2 nuclear levels, as shown in an allergic asthma model (78). Harada showed that γ-tocotrienol could contribute to the therapeutic airway remodeling benefits in asthma by inhibiting HASMC proliferation and migration (79).

Significance and future directions

In this review, we have discussed the relationships between ROS and inflammation in asthma. The effect of oxidants on cells in asthma was also discussed. Allergic asthma is associated with acute increases in ROS, AHR and mucus secretion levels in response to allergen exposure, leading to oxidant and antioxidant imbalances, initiation of inflammatory signaling pathways, and oxidative stress. Through the use of animal studies and clinical trials, ROS have been found to play a central role in the pathogenesis of allergic asthma. Several new antioxidant methods have been tested in animal studies; however, they have not been evaluated in asthma patients. In the future, human and animal studies should bridge the transition from the bench to the clinic. Moreover, a standard scale should be created to assess the degree of disease progression and severity with regard to the identity and quantity of oxidative stress

byproducts. Further research should be focused on the identification of new therapy methods with regard to ROS in allergic asthma.

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Footnote

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