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COMPREHENSIVE INVITED REVIEW

Glutathione Redox Control of Asthma: From Molecular Mechanisms to Therapeutic Opportunities

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Abstract

Asthma is a chronic inflammatory disorder of the airways associated with airway hyper-responsiveness and airflow limitation in response to specific triggers. Whereas inflammation is important for tissue regeneration and wound healing, the profound and sustained inflammatory response associated with asthma may result in airway remodeling that involves smooth muscle hypertrophy, epithelial goblet-cell hyperplasia, and permanent deposition of airway extracellular matrix proteins. Although the specific mechanisms responsible for asthma are still being unraveled, free radicals such as reactive oxygen species and reactive nitrogen species are important mediators of airway tissue damage that are increased in subjects with asthma. There is also a growing body of literature implicating disturbances in oxidation/reduction (redox) reactions and impaired antioxidant defenses as a risk factor for asthma development and asthma severity. Ultimately, these redox-related perturbations result in a vicious cycle of airway inflammation and injury that is not always amenable to current asthma therapy, particularly in cases of severe asthma. This review will discuss disruptions of redox signaling and control in asthma with a focus on the thiol, glutathione, and reduced (thiol) form (GSH). First, GSH synthesis, GSH distribution, and GSH function and homeostasis are discussed. We then review the literature related to GSH redox balance in health and asthma, with an emphasis on human studies. Finally, therapeutic opportunities to restore the GSH redox balance in subjects with asthma are discussed. *Antioxid. Redox Signal.* 17, 375–408.

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I. Introduction and Conceptual Framework

Asthma is a complicated disorder characterized by variable and recurrent episodes of airway inflammation and airflow limitation in response to specific triggers. In both adults and children, asthma is the most common chronic lung disease and affects between 8% and 10% of the entire U.S. population and ~300 million people worldwide (3, 310). While asthma symptoms are intermittent in some individuals, a significant number of subjects with asthma have persistent symptoms that necessitate daily treatment with anti-inflammatory inhaled corticosteroids (3). Among these subjects requiring daily anti-inflammatory therapy, a small subset (~10%) has severe asthma that is refractory to treatment with very high doses of inhaled corticosteroids and in some cases, oral corticosteroids as well (1). These individuals with severe asthma suffer from an excessive burden of respiratory symptoms and have extreme morbidity, including an increased risk of exacerbations and hospitalization (85, 229). However, severe exacerbations can occur in all asthmatics regardless of baseline symptoms and underlying disease severity. Thus, despite the heterogeneity of asthma within the larger population (89, 230), all affected individuals with asthma, regardless of their baseline severity, share the risk for adverse outcomes, including asthma-related death (310).

Despite considerable advances in the understanding and treatment of asthma over the past decade, the etiology of asthma and the specific mechanisms underlying the variable severity of the disorder are not understood. Inflammation is the hallmark of asthma and is associated with airway hyperresponsiveness and airflow limitation in response to specific triggers, including allergens and respiratory viruses. Whereas inflammation is important for tissue regeneration and wound healing, the profound and sustained inflammatory response associated with asthma may result in airway injury through complex interactions between cells and inflammatory mediators (42). This process is commonly referred to as airway remodeling and involves smooth muscle hypertrophy, epithelial goblet-cell hyperplasia, and permanent deposition of airway extracellular matrix proteins that may increase airflow obstruction and the respiratory symptom burden of the disorder (61).

While the specific mechanisms responsible for asthma have yet to be unraveled, oxidative stress from free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) is likely to play a key role in the development and pathogenesis of the disorder. ROS and RNS contribute to airway inflammation and increased concentrations of both ROS and RNS have been observed in the airways of asthmatic individuals (54). Importantly, these ROS and RNS can lead to oxidation and nitration of proteins important for the resolution of inflammation. This frequently occurs through modification of critical cysteine residues present on the surface of proteins, resulting in S-sulfhydration, S-nitrosylation, S-cysteinylation, S-glutathionylation, and other covalent post-translational modifications, which may or may not be reversible (158). Ultimately, these modifications can alter the function and vital role of the proteins in the inflammatory response. One example involves catalase, the enzyme that catalyzes the breakdown of hydrogen peroxide (H_2O_2), which undergoes oxidative modification (nitration and chlorination) after airway allergen challenge and loses its enzymatic activity as a result (108). The activities of manganese superoxide dismutase and glutathione-S-transferase, which detoxify superoxide and other electrophilic substances, respectively, are also inhibited by nitration (203, 346). Another example involves S-glutathionylation of the p50 subunit of the transcription factor, nuclear factor kappa B (NF-κB), which has been associated with repressed DNA-binding activity (256).

Although oxidative stress has been increasingly implicated in aging (187) and a number of chronic diseases such as cancer (247), diabetes (297), and cardiovascular disease (114), the concept of oxidative stress has evolved considerably since its inception >25 years ago. In the traditional view, oxidative stress was thought to result from a global disruption in the pro-oxidant and antioxidant balance (296). In this view, disease was due to an excessive burden of ROS and RNS with an accompanying deficiency of free radical-scavenging antioxidants (Fig. 1) (296). This view of oxidative stress ultimately led to several large antioxidant interventional trials that were undertaken in a variety of chronic disease states, but many of these failed to demonstrate clear benefits with regard to clinically relevant health outcomes (33–35, 75, 193, 292). These disappointing findings are likely related to a number of

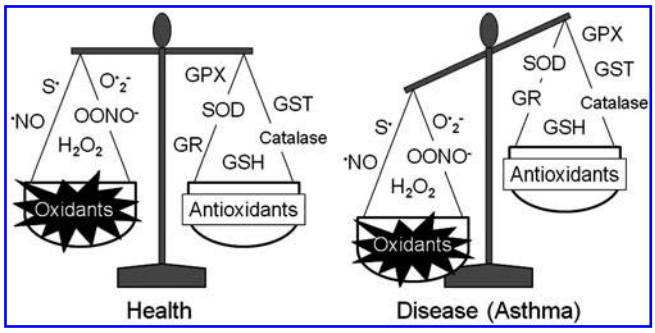

FIG. 1. The traditional concept of oxidative stress in health and disease. Originally, oxidative stress was thought to result from a global disruption in the pro-oxidant and antioxidant balance. In this view, disease was attributed to an increased burden of free radicals (thiyl radical [$S^{\bullet}$], nitric oxide radical [$NO^{\bullet}$], superoxide [$O_2^{\bullet-}$], peroxynitrite [OONO–], and hydrogen peroxide) and a deficiency of endogenous antioxidants (glutathione, reduced [thiol] form [GSH], catalase, glutathione-S-transferase [GST], glutathione reductase [GR], superoxide dismutase [SOD], and glutathione peroxidase [GPX]).

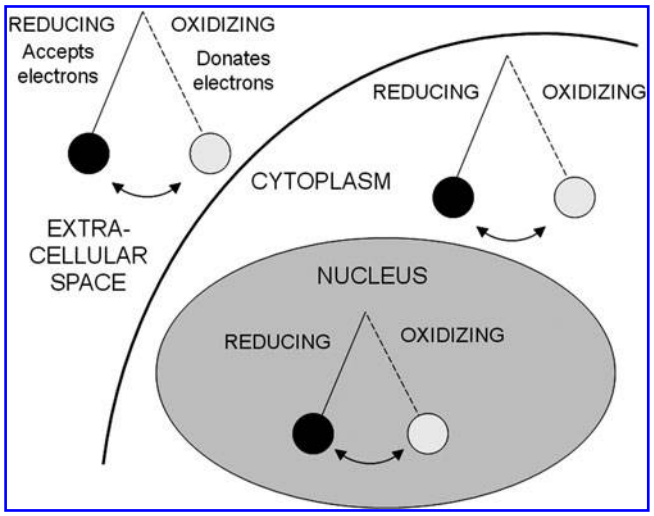

FIG. 2. A modified concept of oxidative stress. As opposed to a simple disruption in the pro-oxidant and antioxidant balance, oxidative stress is better conceptualized as a disruption of redox signaling and control. In this view, oxidative stress is a dynamic process in constant flux, similar to a pendulum. These oxidative shifts are highly compartmentalized and are not necessarily in equilibrium between the extracellular space, the cytoplasm, and the organelles.

factors, including (i) insufficient knowledge of the clinical pharmacology of antioxidants, (ii) insufficient dosing or duration of the antioxidant therapy due to limited dose–response studies, (iii) the method of antioxidant delivery (*i.e.*, oral *vs.* inhaled), (iv) the composition of the antioxidant agent itself (*i.e.*, solubility, adjuvants, and additives), (v) selection of un-standardized, un-validated, or otherwise soft primary outcome indicators, including outcomes of limited relevance to the mechanism of antioxidant action, and (vi) lack of surrogate markers of oxidative stress to accompany functional outcomes. However, as knowledge of oxidation/reduction (redox)-dependent signaling and molecular mechanisms of antioxidant therapies has increased, many are now questioning the traditional view of oxidative stress as a pro-oxidant/antioxidant imbalance and instead are focusing on oxidative stress as "a disruption of redox signaling and control" (156). This alternative view of oxidative stress assumes that (i) oxidative stress occurs through discrete redox pathways within cells, and (ii) oxidative stress involving a disruption of redox circuitry can occur without a global pro-oxidant/anti-oxidant imbalance (156, 157) (Fig. 2). Thus, oxidative stress can lead to both organ-specific and pathway-specific toxicity without macromolecular damage (157). This re-conceptualization of oxidative stress as a disruption of redox signaling and control may explain why some of the previously conducted antioxidant supplementation trials focused solely on the mitigation of global oxidant/antioxidant imbalance have failed to yield significant results. Furthermore, re-conceptualization of asthma as a disorder characterized by disruptions of redox signaling and control may also help to unravel unanswered questions regarding the pathogenesis and severity of the disorder. Because single genes and proteins responsible for asthma susceptibility and severity have yet to be identified (17), asthma remains a complex disorder (or perhaps even a clinical syndrome) associated with significant clinical heterogeneity of symptoms, inflammatory patterns, and airway physiology. Indeed, there are a number of phenotypes or endotypes of asthma associated with unique clinical and biological features that likely require individualized therapeutic interventions (89, 196, 230). While this broad clinical spectrum of asthma may preclude the widespread use of antioxidant therapies in all affected patients, greater understanding of oxidative stress in terms of redox signaling and control may enhance the development of therapeutic strategies to prevent or alleviate asthma morbidity within selected, highly defined asthma subpopulations.

This review will focus on disruptions of redox signaling and control in asthma with a focus on glutathione. Although there are a number of redox-related proteins relevant to asthma, glutathione, in its thiol-reduced form (GSH), is the most abundant antioxidant in the airway epithelial lining fluid, with concentrations 100-fold higher than what is observed in the plasma (46). GSH also serves a number of important functions relevant to asthma, including (i) detoxification of electrophilic compounds such as xenobiotics, (ii) scavenging of free radicals, and (iii) modulation of cellular processes such as DNA synthesis and repair, differentiation, apoptosis, and immune function (198, 212, 334, 337). This review will discuss regulation and homeostasis of GSH in asthma as well as the potential therapeutic uses of GSH and other thiol-derived compounds in the clinical setting.

II. Glutathione Synthesis

GSH is an organosulfur tri-peptide (γ-glutamyl-cysteinyl-glycine) produced from metabolism of the essential sulfur amino acid, methionine, which is not synthesized *de novo* in mammals (Fig. 3). After ingestion, dietary methionine is absorbed across the intestinal epithelium and enters the plasma as a free amino acid. Although the majority of methionine is removed by the liver from the portal circulation, some metabolism of methionine also occurs directly in the intestine (19, 295). Once inside the cell, methionine forms *S*-adenosylmethionine through an ATP-dependent reaction catalyzed by methionine

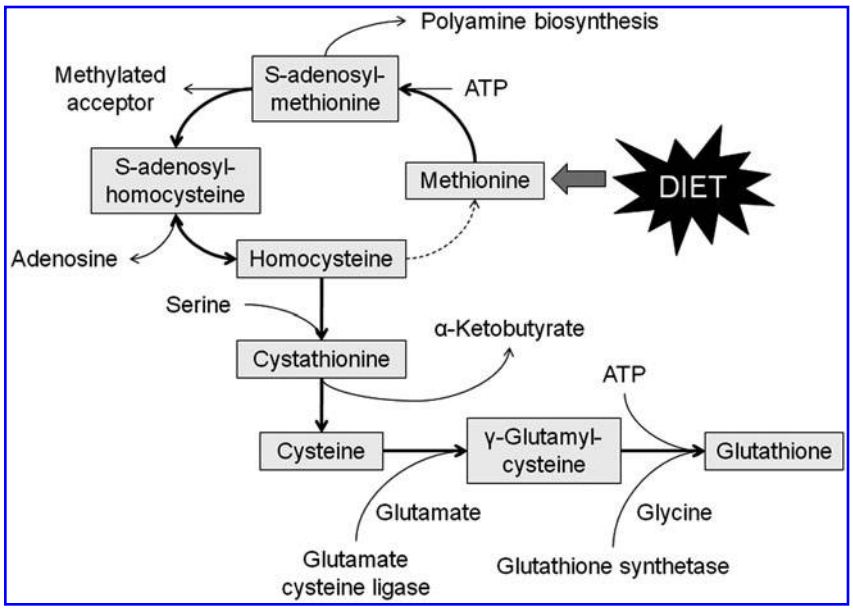

FIG. 3. Methionine metabolism. Methionine is metabolized to homocysteine, which is either (i) methylated back to methionine through the remethylation pathway or (ii) converted to cysteine through the trans-sulfuration pathway. The cysteine that is formed may be used for protein synthesis or formation of glutathione.

adenosyltransferases. *S*-adenosylmethionine functions as the primary methyl donor in mammalian cells (215). The co-product of *S*-adenosylmethionine transmethylation, *S*-adenosylhomocysteine, is then hydrolyzed by *S*-adenosylhomocysteine hydrolase to form adenosine and homocysteine. Homocysteine is either methylated back to methionine through the remethylation pathway or is converted to cysteine through the trans-sulfuration pathway.

For trans-sulfuration, homocysteine condenses with serine to form cystathionine through a reaction catalyzed by cystathionine β-synthetase. Cystathionine is then hydrolyzed by cystathionine γ-lyase to form α-ketobutyrate and free cysteine, which may be used for GSH formation (56, 119, 306). The synthesis of GSH from glutamate, cysteine, and glycine involves two steps, catalyzed by glutamate cysteine ligase (also referred to as γ-glutamylcysteine synthetase) and GSH synthetase, respectively, as shown below:

1. Glutamate + cysteine + ATP → γ-glutamylcysteine + ADP + Pi, and
2. γ-glutamylcysteine + glycine + ATP → GSH + ADP + Pi.

The first step of GSH synthesis is primarily controlled by negative feedback from GSH. However, when GSH is consumed and feedback inhibition is impaired, the availability of cysteine becomes the major limiting factor for GSH synthesis (198, 199, 334).

III. Distribution of Glutathione in the Body

GSH is present in all tissues and fluids throughout the body. Although the GSH content of bodily fluids is significantly lower (up to 1000-fold) than that found in tissues, all cells release GSH, suggesting that it is essential for cellular function and defense (231). The total amount of GSH in the body is therefore ~15 g, of which 5 g is cysteine. This bodily GSH is distributed in all the major organ systems, including the lungs. However, the liver has the highest GSH content (about 4 g or 20% of all bodily GSH), although liver GSH concentrations fluctuate somewhat according to the time of day, the composition of the diet, and bodily demands (36). The cysteine content of these liver GSH stores is nearly equal to the daily recommended dietary intake of total sulfur amino acids (methionine plus cysteine), which is ~1.1 g/day for a 60-kg woman (equal to 2.7 g/day of GSH) and ~1.4 g/day for a 75-kg adult male (equal to 3.3 g/day of GSH) (93). Thus, the GSH content of the liver can be quickly depleted in ~1 day with fasting and starvation. In otherwise healthy individuals, homeostatic mechanisms prevent hepatic GSH stores from becoming too low during these conditions and GSH is instead derived from muscle and other tissues (210). However, the entire body has only a 4-day GSH reserve. Although the majority of adult males and females in the United States consume more than the daily recommended intake of sulfur amino acids (90), these GSH losses can be of importance during prolonged states of protein/energy insufficiency (including catabolic illness) or in individuals with chronic inflammatory illnesses such as alcoholism where GSH stores are otherwise depleted (94, 352).

IV. Glutathione Function and Homeostasis

The biological activity of GSH is derived from cysteine. Cysteine contains a carbon-bonded sulfhydryl (–SH) group that is highly nucleophilic and easily oxidized, particularly in the extracellular space (161) (Fig. 4). With oxidation, two cysteine residues covalently link and form a disulfide bond (–SS), yielding the oxidized moiety cystine (CySS). Within a protein, these disulfide bonds contribute to a protein's tertiary structure during the course of protein folding. Disulfide bonding between cysteine residues further contributes to multi-unit protein quarternary structure through the formation of covalent bonds between peptide chains. In the case of GSH, which is not a protein, the disulfide bonding of the –SH group contributes to thiol-disulfide equilibrium and many other vital GSH functions.

A. Glutathione as a cysteine reservoir

Because cysteine is relatively unstable and rapidly oxidizes to CySS, GSH serves as the primary cysteine reservoir (Fig. 5).

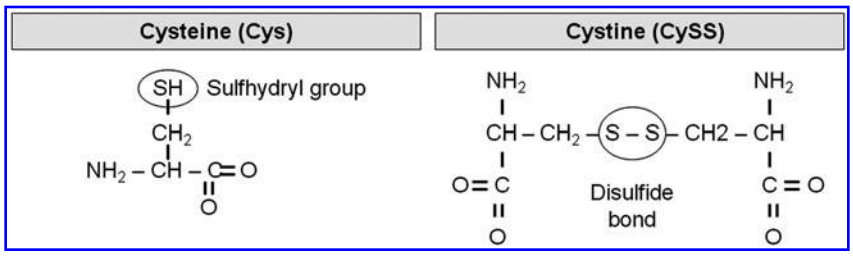

FIG. 4. Chemical structures of cysteine (Cys) and cystine (CySS).

The γ-glutamyl cycle allows GSH to serve as a continuous cysteine source for the body when cysteine reserves are not depleted (199, 245) (Fig. 6). Through this cycle, γ-glutamyl transferase, which resides in the cell membrane, transfers the γ-glutamyl moiety of extracellular GSH to cysteine, forming γ-glutamyl-cysteine and cysteinyl-glycine. The cysteinyl-glycine is broken down by dipeptidase in the extracellular space to generate glycine and cysteine, which are readily taken up by the cell. The γ-glutamyl-cysteine that is formed in the extracellular space may also be transferred back into the cell, where it undergoes further metabolism to cysteine, which can be used for additional GSH synthesis or protein incorporation.

B. Xenobiotic conjugation and detoxification

GSH is also crucial for detoxification of xenobiotics (95, 199, 334). Xenobiotics are electron-loving substances that are otherwise highly reactive. Xenobiotics form conjugates with GSH either spontaneously or enzymatically through reactions catalyzed by glutathione-S-transferases (Fig. 7). These conjugates are then excreted from the cell, where they undergo cleavage by γ-glutamyl transferase and further breakdown by dipeptidase, resulting in a cysteinyl conjugate. The cysteinyl conjugate then undergoes N-acetylation, forming a mercapturic acid, which is then excreted by the kidney. While this process is important for detoxification of the parent compound, which is often highly reactive, GSH conjugation also irreversibly consumes intracellular GSH (199).

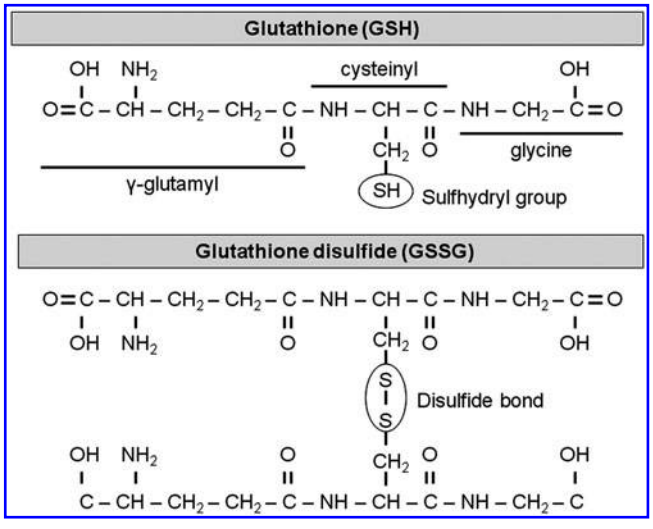

FIG. 5. Chemical structures of GSH and glutathione disulfide (GSSG).

C. Free radical scavenging

Free radicals are produced through a number of physiologic processes, such as mitochondrial respiration, and can also be encountered exogenously though environmental exposures. While some free radicals are important for physiologic function, intermediates such as superoxide anion ($O^{\bullet}{}_2^-$) and organic hydroperoxides such as H_2O_2 can ultimately result in lipid peroxidation. GSH provides the primary defense against lipid peroxidation through donation of a reducing equivalent ($H^+ + e^-$) to organic hydroperoxides by means of a glutathione peroxidase or a glutathione-S-transferase-dependent reaction. However, in doing so, it becomes reactive (SG–) and reacts readily with another reactive glutathione (SG–) to form glutathione disulfide (GSSG). GSSG is then reduced back to GSH by glutathione reductase through an NADPH-dependent reaction, forming a redox cycle (Fig. 8). GSSG can also be actively exported from the cell or react with a protein –SH group, forming a protein mixed disulfide.

D. Maintenance of thiol equilibrium

Because GSH is the predominant intracellular thiol, GSH also plays a key role in maintaining the essential thiol status of proteins. GSH participates in thiol-disulfide exchange through a reversible reaction catalyzed by thiol-transferases as shown below, where Protein-SSG is a mixed disulfide and Protein-SH is reduced protein:

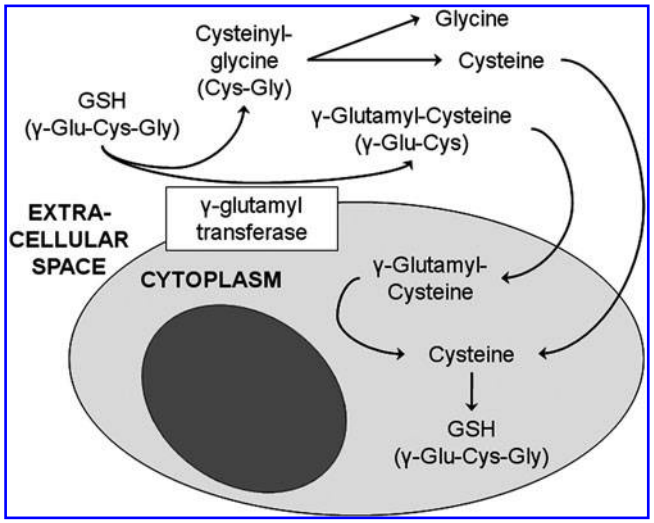

FIG. 6. The γ-glutamyl transferase cycle. The γ-glutamyl cycle allows glutathione to serve as a continuous source of cysteine. The γ-glutamyl-cysteine and cysteinyl-glycine are broken down to form cysteine, which can be used for glutathione synthesis or protein incorporation.

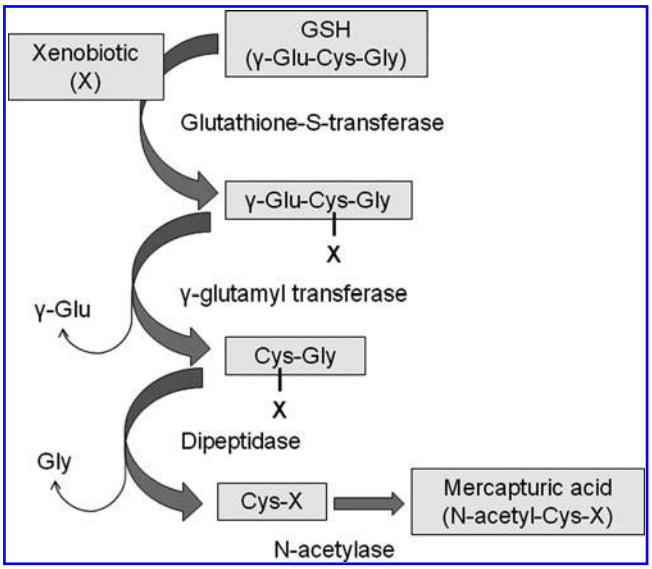

FIG. 7. The role of glutathione in xenobiotic conjugation and detoxification. Glutathione conjugates xenobiotics to a less reactive form, yielding a mercapturic acid that is excreted by the kidney.

$$\text{Protein-SSG} + \text{GSH} \rightarrow \text{Protein-SH} + \text{GSSG}.$$

The thiol-disulfide equilibrium is determined by the redox state of the cell, which is based on GSH and GSSG concentrations. Typically, GSSG concentrations are low and proportional to the log of $[GSH]^2/[GSSG]$ such that protein-mixed disulfide formation is limited. However, with increased formation of GSSG, thiol-disulfide equilibrium is disrupted and protein mixed disulfide formation is increased. While these shifts in the GSH/GSSG balance are essential for redox signaling and the maintenance of cellular metabolism and homeostatic function, sustained disequilibrium from excess GSSG formation ultimately increases protein mixed disulfide formation.

E. Protein S-glutathionylation

Proteins contain a number of cysteine-sulfhydryl (–SH) residues that undergo post-translational modifications as part of their normal function. Protein S-glutathionylation (S-SG) is one example that occurs when a protein –SH moiety forms a disulfide bond with GSH (59, 219). This can occur through a number of mechanisms (Fig. 9). While protein S-glutathionylation occurs more frequently in response to ROS or RNS, it is also important for redox signaling and regulation of protein activity and is therefore present to some extent during basal physiologic conditions (333). For example, the cellular functions of actin are mediated by reversible S-glutathionylation (58) such that impairment of actin S-glutathionylation alters stress fiber formation and cytoskeleton organization (83).

Although protein-GSH bonding is usually transient and reversible under reducing conditions, during chronic oxidizing conditions the protein-GSH bond can persist indefinitely (22, 109). Under these oxidizing conditions, S-glutathionylation may serve as a reservoir for GSH inside the cell and may also protect from irreversible oxidation to sulfinic and sulfonic acid, which leads to proteasomal degradation of the protein (209). However, if the cysteine residue is essential, then S-glutathionylation may disrupt critical protein functions and lead to compromised cellular activities (59), such as is the case with the transcription factor, NF-κB. S-glutathionylation of the p65 and p50 subunits of NF-κB inhibits NF-κB binding to the DNA (256, 260), while S-glutathionylation of the inhibitory kappa B kinase beta subunit represses NF-κB transactivation (219, 275).

F. Regulation of cell surface proteins

Thiols such as GSH are also present in the extracellular space, where they play an important role in cell surface protein regulation and signal transduction. The effects of the extracellular thiol redox balance on cellular functions have been studied previously. Whereas reducing states promote proliferation of epithelial cells (153, 241), oxidation of the extracellular thiol pools promotes epithelial cell apoptosis (151). Similarly, oxidation of the extracellular thiol redox potential stimulates proliferation and pro-fibrotic signaling of

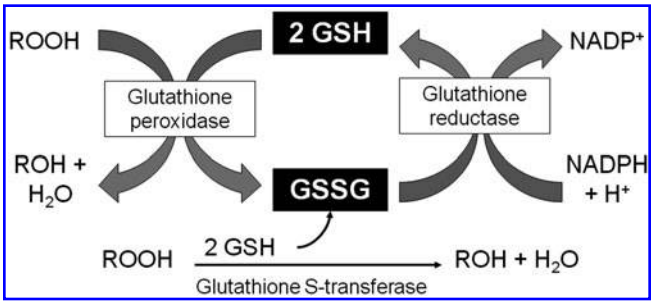

FIG. 8. The glutathione redox cycle. In the presence of organic hydroperoxides (ROOH), GSH is converted to GSSG, yielding a less reactive product (ROH). GSSG is converted back to GSH by glutathione reductase through an NADPH-dependent reaction.

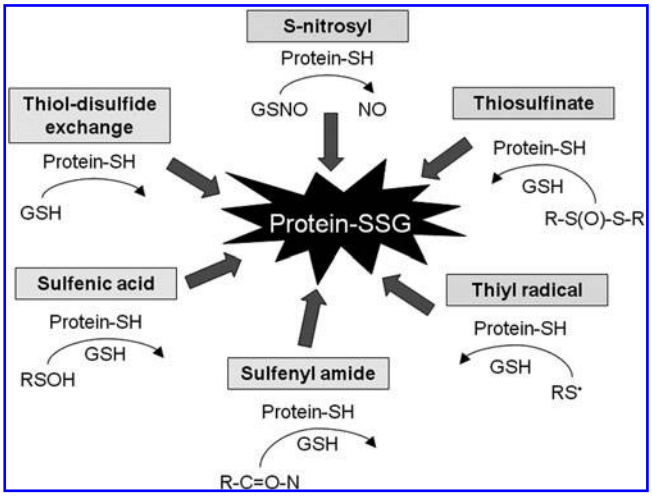

FIG. 9. Mechanisms of protein S-glutathionylation. Proteins contain a number of cysteine-sulfhydryl (-SH) residues that may form a disulfide bond with GSH, resulting in protein S-glutathionylation (protein-SSG). "R" represents an organic substituent.

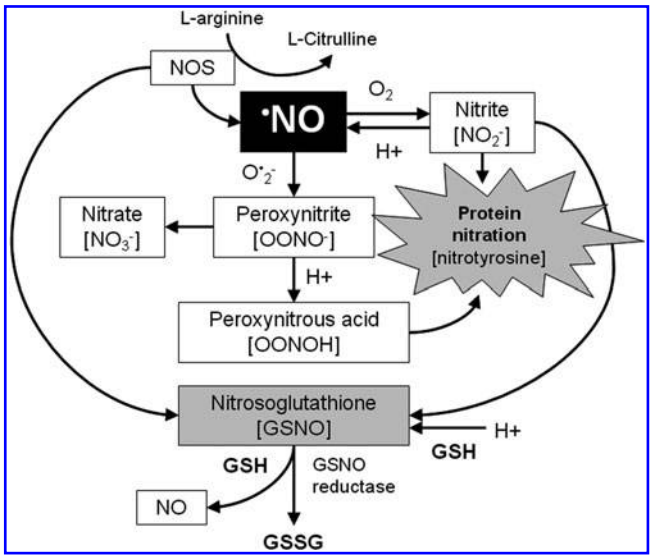

FIG. 10. Reactive nitrogen species biochemistry. •NO is produced from nitric oxide synthases and is then metabolized to the nitric oxide oxidation products nitrite (NO_2^-) and nitrate (NO_3^-) through a series of reactions involving superoxide anion ($O^{\bullet}{}_2^-$) and oxygen. The OONO– that is generated is then protonated to peroxynitrous acid (ONOOH). ONOOH may also react with GSH and other thiol residues to form S-nitrosothiols such as nitrosoglutathione (GSNO). •NO, nitric oxide radical

transforming growth factor beta-1 (TGFβ1) within fibroblasts (269) and also activates NF-κB and stimulates monocyte adhesion to vascular endothelial cells (111). These findings suggest that direct uptake of GSH and related thiols by cells is not essential for cellular function. Rather, beneficial cellular functions can be derived from preservation of cell signaling functions through maintenance of the extracellular GSH and thiol redox balance.

G. Protection against nitrosative stress from RNS

Nitric oxide (•NO) is the primary RNS in the lung. •NO is produced from nitric oxide synthases, which convert L-arginine to •NO and L-citrulline. •NO is then metabolized to the nitric oxide oxidation products nitrite and nitrate through a series of reactions involving $O^{\bullet}{}_2^-$ and oxygen (Fig. 10). The peroxynitrite (OONO–) that is generated is then protonated to peroxynitrous acid (ONOOH), which can nitrate tyrosine residues, leading to either a gain or loss of protein function (14, 214). ONOOH may also react with GSH and other thiol residues to form S-nitrosothiols such as nitrosoglutathione (GSNO), a beneficial endogenous bronchodilator (261) that may function as an important signaling mechanism in the presence of nitrosative stress (97, 105, 134).

V. Glutathione Redox Balance in Health

The redox state of GSH and GSSG in healthy individuals has been well described (154, 156, 157, 231, 288). Because GSSG and GSH concentrations are not directly proportional, the redox state of the GSH/GSSG pool is best expressed with the Nernst equation, rather than a simple ratio of GSH to GSSG. The Nernst equation incorporates the correct stoichiometry of 2 GSH units oxidized per GSSG unit formed and is expressed as follows:

$$E_h = E_o + (RT/nF) * \ln([GSH]^2/[GSSG]).$$

In this equation, E_h represents the redox potential at a given pH and is defined as the electromotive force in millivolts relative to a standard hydrogen electrode (1 atm H_2, 1 M H^+). The E_o term represents the standard potential for the redox couple at a given pH, R is the gas constant, T is the absolute temperature, F is Faraday's constant, and n is the number of electrons transferred. The Nernst equation is not limited to the GSH/GSSG pool but can also be applied to other redox-active biomolecules and therefore provides a means to assess the tendency of the redox couple to donate or accept electrons. Whereas couples with more positive E_h values are more oxidized, couples with more negative E_h values are more reduced (154, 156, 157, 231, 288). Given the compartmentalization of the redox state within the body (156, 157), intracellular and extracellular GSH/GSSG redox states are discussed separately below.

A. Intracellular glutathione redox status

GSH is the predominant intracellular thiol. Within cells, the majority of GSH is found in the cytosol, the primary site of GSH synthesis, with concentrations ranging from 1 to 11 mM (298). While GSSG concentrations are typically very low, GSH and GSSG concentrations vary somewhat as the cell undergoes proliferation, differentiation, and apoptosis, leading to shifts in the GSH/GSSG redox state (154, 170, 231). During cellular proliferation, the GSH/GSSG redox state is most reduced and ranges from −230 to −260 mV. With the onset of differentiation and growth arrest, the redox state becomes more oxidized and ranges from −220 to −190 mV, possibly due to contact inhibition (142, 170). Further oxidation of the GSH/GSSG couple by 40 mV to the range of −170 to −150 mV results in cellular apoptosis (154, 231). These changes are relatively small and suggest that the GSH/GSSG pool within the cells is tightly regulated.

Although total cellular GSH concentrations typically reflect the cytosolic pool, organelles also contain GSH. For instance, cells such as hepatocytes have a higher volume of mitochondria that can comprise up to 15% of the total GSH pool (217). Within the mitochondria, GSH concentrations are similar to those in the cytoplasm and range from 5 to 11 mM (103, 120, 330). However, the GSH/GSSG redox state of the mitochondria (−330 mV) is significantly more reduced (112, 113, 126). Similarly, the GSH content in nucleus comprises 5%–10% of the total cellular GSH pool and 10%–20% of this GSH content is independent from that found in the cytosol (30, 298, 301). Like the mitochondria, the GSH/GSSG redox state of the nucleus (−280 mV) is also more reduced than the cytoplasm (113, 127, 155, 338). By contrast, the GSH/GSSG redox state of the endoplasmic reticulum is more oxidized compared to the cytosol, with a redox potential of approximately −150 mV (112, 113, 143). Within the endoplasmic reticulum, the ratio of GSH to GSSG ranges from 1:1 to 3:1, compared to >30:1 for the overall GSH to GSSG ratio of the cell (288). Although the specific roles of GSH and the GSH/GSSG redox balance in these organelles are still being unraveled, the oxidizing environment of the endoplasmic reticulum may be essential for

the production of proteins with disulfide bonds (143, 288). Likewise, the reducing environment of the mitochondria and nuclear compartments may promote redox-sensitive transcriptional regulation and inhibit oxidative DNA damage (113).

B. Plasma glutathione redox status

Although GSH and GSSG are present outside of the cell, their concentrations are usually 100 to 1000 times less than what is observed inside the cell (288). In the plasma of healthy adults, GSH concentrations range from 2 to 4 μM, with a GSH/GSSG redox potential of approximately -137 ± 9 mV (159, 160). Thus, the redox state of the plasma is considerably more oxidized than that of cells and tissues.

Whereas GSH is the predominant intracellular thiol, cysteine is the most abundant thiol in the plasma. However, given the more oxidizing environment, the majority of cysteine in the plasma exists in its disulfide form, as CySS. In healthy adults, plasma cysteine concentrations range from 8 to 10 μM, whereas plasma CySS concentrations are at least 40 μM (159). Thus, the cysteine/CySS redox potential is significantly more oxidized than the GSH/GSSG redox potential, with normal values approximating -80 ± 9 mV (159). The difference of 57 mV between the redox potentials of cysteine/CySS and GSH/GSSG suggests that they are not in redox equilibrium and likely play distinct roles in redox signaling and control (161). For that reason it has been suggested that plasma GSH/GSSG and plasma cysteine/CySS redox states be used together as a measure of oxidative stress *in vivo* in humans, since the plasma GSH/GSSG redox potential may less sensitive to acute oxidative disturbances (156).

Although the cysteine/CySS pool is more abundant than the GSH/GSSG pool in the plasma, plasma GSH and GSSG concentrations do yield useful information about the oxidative burden of an otherwise healthy individual. Plasma GSH/GSSG redox may also be highly informative in cases of disease. In a previous study of age-related macular degeneration (285), individuals >60 years of age had oxidation of the plasma GSH/GSSG redox couple by about 25 mV compared to individuals <43 years of age. In a related study, the plasma GSH/GSSG redox potential grew steadily more oxidized in otherwise healthy individuals after the age of 45 years, with an average rate of oxidation of ~0.7 mV/year (162). Similarly, in the case of cigarette smoking, plasma GSH concentrations were significantly lower in smokers (1.8 ± 1.3 μM) than in nonsmokers (2.4 ± 1.0) and the resulting GSH/GSSG redox potential was significantly more oxidized (-128 ± 18 mV *vs.* -137 ± 17 mV), with a difference of ~9 mV between groups (232). This difference has important implications for the function of thiol-containing proteins, since a 9 mV change is sufficient to induce a twofold change in the ratio of reduced to oxidized proteins (154).

C. Epithelial lining fluid glutathione redox status

In contrast to the plasma where cysteine (as CySS) is the predominant thiol pool, in the epithelial lining fluid, GSH is the most abundant thiol (Fig. 11). In healthy nonsmoking adults, total glutathione concentrations in the epithelial lining fluid, measured as the sum total of the GSH and GSSG pools, are ~400–500 μM and therefore approximate the values found in some cell types (46, 233). Furthermore, in healthy adults, <5% of the total GSH/GSSG pool exists in the oxidized (GSSG) form (46, 233), resulting in a relatively reduced GSH/GSSG redox potential of approximately -200 mV (352). Why GSH (as opposed to cysteine) predominates in the epithelial lining fluid is unclear but may be related to barrier defense against inhaled oxidants and microorganisms. In isolated type II alveolar epithelial cells, extracellular GSH inhibits hyperoxia-induced injury and pro-inflammatory cytokine release and promotes cell growth (273, 282, 345). GSH is also essential for airway innate immune defense and regulates alveolar macrophage apoptosis and phagocytosis of infectious particles (43, 140). GSH depletion further leads to activation of NF-κB and increased pro-inflammatory gene transcription and cytokine release from histone deacetylase suppression in epithelial cells (268, 351).

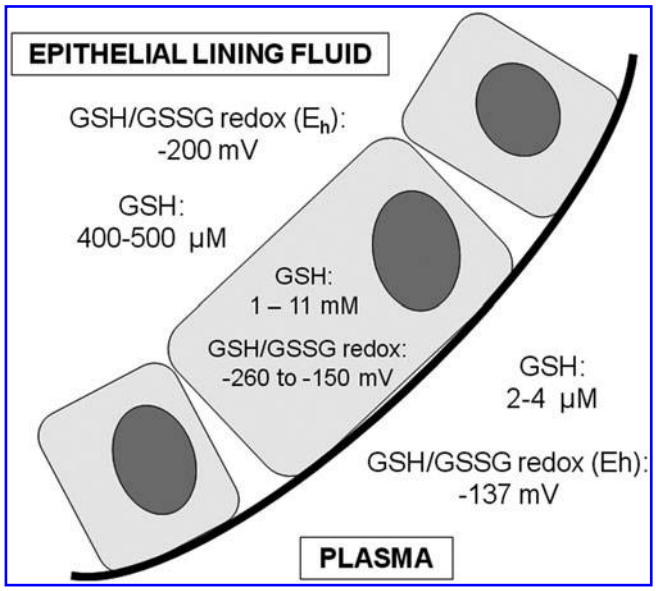

FIG. 11. Glutathione distribution in the plasma, cell, and epithelial lining fluid of healthy adults. The GSH/GSSG redox potential within the cell varies as a function of differentiation, proliferation/growth arrest, and apoptosis.

Human studies support these observations and confirm the important role of GSH in maintaining lung health. Although acute insults such as smoking and diesel exhaust exposure initially increase GSH concentrations in the epithelial lining fluid (23, 46, 352), chronic insults such as long-term smoking over many years lead to increased airway GSSG formation and protein carbonylation (238). Likewise, in cases of severe lung injury such as acute respiratory distress syndrome (40, 44, 246), pulmonary fibrosis (21, 25), and hypersensitivity pneumonitis (24), total and reduced GSH concentrations are significantly lower than values observed in healthy adults, with differences up to 10-fold. GSSG further accounts for up to 30% of the total GSH pool in these patients (44), suggesting that the GSH/GSSG redox balance in the epithelial lining fluid may be responsible for some of the respiratory morbidity associated with pulmonary disease.

VI. Glutathione Redox Balance in Asthma

Glutathione was first characterized in subjects with asthma in the late 1970s. In the earliest available publication, 22 adults

with asthma across a wide range of severities were exposed to ozone or filtered air (sham) for 2 h in a cross-over design (191). With ozone exposure, blood GSH levels decreased and asthma symptoms worsened, although no changes in pulmonary function were noted (191). Whether the reduction in blood GSH content was related to increased airway oxidative stress is not clear. However, in a similar study where 400 ppb of ozone was administered to healthy subjects for 2 h, increased 8-isoprostane concentrations were noted and were associated with decreased pulmonary function (225). Oxidative stress reflected by 8-isoprostane levels is also apparent after allergen challenge (224).

While the specific mechanisms linking GSH disturbances to asthma are still being unraveled, several studies characterizing GSH and related factors in the airways and systemic circulation of subjects with asthma have been undertaken. These findings, as well as their potential physiological significance, are discussed below. A summary table of the major findings related to GSH/GSSG redox balance in healthy controls and subjects with mild-to-moderate and severe asthma is provided in Table 1.

A. Airway glutathione concentrations in asthma, measured invasively

Sampling of the airway epithelial lining fluid is difficult and therefore only seven studies to date have directly examined GSH and GSSG content in the airways of asthmatic subjects. Given ethical and practical considerations, the majority of these studies were limited to inhaled corticosteroid-naive asthmatic adults with mild asthma and good symptom control (52, 166, 234, 299, 300). These subjects underwent invasive bronchoscopy with bronchoalveolar lavage for sampling of the epithelial lining fluid constituents.

In the first studies conducted on the subject, total glutathione concentrations in the bronchoalveolar lavage cells (measured as the sum total of the GSH and GSSG pools) did not differentiate between asthmatics and healthy controls (300). However, the subjects with asthma had nearly twofold higher baseline concentrations of total glutathione in the airway lavage fluid (299). Total glutathione concentrations were highest in the bronchial *versus* the alveolar space (299), consistent with the bronchial features of asthma (such as airway hyperresponsiveness) and the nature of the inflammation in these subjects, which is primarily localized to the airways. While GSH and GSSG were not measured, the increased total glutathione concentrations may reflect increased airway oxidative stress from increased GSSG formation, as opposed to increased airway reducing capacity from increased GSH synthesis. In keeping with this notion, in similar studies, increased total glutathione concentrations were associated with decreased basal expression of the antioxidants superoxide dismutase, ascorbate, and alpha-tocopherol in asthmatics *versus* controls (234, 300). Other studies in which both GSH and GSSG were assessed further revealed significant increases in GSSG between asthmatics and controls, particularly in the bronchial *versus* the alveolar airspaces (166, 234), with no differences in GSH concentrations between groups (52, 166). Furthermore, while acute antigen challenge did not lead to further increases in bronchoalveolar GSSG content in the asthmatic subjects, it did significantly decrease GSH concentrations (52). These findings are of relevance and suggest that the baseline perturbations in airway GSH and GSSG homeostasis in subjects with asthma may increase the risk of airway injury from secondary insults such as antigen exposure.

More recently, we characterized the airway GSH/GSSG redox state in controls and children with mild-to-moderate and severe asthma treated with inhaled corticosteroids who underwent bronchoscopy with bronchoalveolar lavage for clinical indications (86–88). Whereas the total glutathione (GSH + GSSG) concentrations in the epithelial lining fluid of healthy adult controls were similar to other published reference values (436 μM) (46, 233), these concentrations were reduced by more than threefold in children with severe asthma (129–134 μM) (88). Whether age influences the airway GSH/GSSG pool is not clear since children have not been previously studied due to the invasive nature of bronchoscopy and a number of ethical and practical concerns. However, total airway glutathione concentrations remained more than twofold lower in children with severe asthma than in nonasthmatic children with current respiratory symptoms (260 μM) (88). Total airway glutathione concentrations in children with severe asthma were also nearly half the levels observed in age-matched children with mild-to-moderate asthma (86). Within the severe asthmatics, these lower total airway glutathione concentrations were further characterized by near-total depletion of GSH and a two- to fourfold increase in GSSG compared to mild-to-moderate asthmatics (86, 87) and controls (88). These findings were also accompanied by up to a 75-mV shift in the epithelial lining fluid GSH/GSSG redox potential (E_h) toward the more oxidized state in children with severe asthma (adult control, −172 mV; pediatric control, −146 mV; mild-to-moderate asthma, −124 mV; severe asthma, −94 to −120 mV [Note: the values for mild-to-moderate asthma are expressed here per mL of epithelial lining fluid and do not correspond directly to reference (86)]) (86–88).

These observations suggest that profound disequilibrium of the airway GSH/GSSG redox balance accompanies severe *versus* milder forms of asthma. This GSH/GSSG disequilibrium may account for some of the morbidity associated with the disorder. In otherwise stable subjects with severe asthma refractory to inhaled corticosteroid treatment, the perturbations in the epithelial lining fluid GSH/GSSG redox potential (*i.e.*, −75 mV difference between severe asthmatics *vs.* controls) were most striking in subjects with airflow limitation and were directly associated with lipid peroxidation and DNA oxidation byproducts, potentially indicative of airway structural damage (87, 88). The GSH/GSSG perturbations in severe asthma were further associated with cysteine depletion and increased oxidation of the cysteine/CySS redox potential in the epithelial lining fluid, suggestive of global airway thiol imbalance and possibly decreased cysteine reserves for additional GSH synthesis (86). These observations may account for the refractory nature of severe asthma, in which airway inflammation persists despite intensive corticosteroid therapy (1).

B. Airway glutathione concentrations in asthma, measured noninvasively

Given the invasive nature of bronchoscopy and the practical and ethical considerations prohibiting its use in most research studies, a few investigators have attempted to characterize airway GSH concentrations in induced sputum from

Table 1. Summary Table of Glutathione, Reduced (Thiol) Form, and Glutathione, Reduced (Thiol) Form–Redox Balance in Asthma

Reference	*Assay method*	*Subjects*	*Total GSH+GSSG*	*GSH*	*GSSG*
Whole blood/erythrocytes					
Hasbal *et al.* (129)	DNTB reduction assay	19 pediatric controls	—	2.52 μmol/g Hb	—
		30 children with MMA, before treatment		2.72 μmol/g Hb	
		30 children with MMA, after treatment[a]		2.40 μmol/g Hb	
Mak *et al.* (206)	DNTB reduction assay	135 controls	—	—	158 μM
		26 mild asthmatics[a]			162 μM
		50 moderate asthmatics[a]			172 μM
		28 moderate-to-severe asthmatics[a]			
Nadeem *et al.* (236)	DNTB reduction assay	23 controls	830 μM	—	—
		38 symptomatic mild-to-severe asthmatics[a]	610 μM		
Nadeem *et al.* (237)	DNTB reduction assay	97 stable MMAs[a]	710 μM	—	—
		32 MMAs with acute exacerbation[a]	700 μM		
Pennings *et al.* (253)	DNTB reduction assay	40 stable MMAs, before treatment	7.0 μmol/g Hb	—	—
		40 stable MMAs, after treatment[a]	6.6 μmol/g Hb		
Vural and Uzun (329)	DNTB reduction assay	43 controls	0.49 μmol/g Hb	—	—
		40 mild asthmatics	0.59 μmol/g Hb		
Wood *et al.* (348)	DNTB reduction assay	31 controls	908 μM	836 μM	24.8 μM
		44 stable mild-to-severe asthmatics[a]	834 μM	832 μM	5.7 μM
Plasma					
Ercan *et al.* (74)	DTNB reduction assay	68 controls	—	~6 μM	—
		187 controls (separate sample)		~6 μM	
		196 children with mild asthma		~4.8 μM	
		116 children with moderate-to-severe asthma[a]		~4.2 μM	
Fitzpatrick *et al.* (86)	HPLC	38 children with MMA[a]	1.3 μM	~0.8 μM	0.3 μM
		51 children with SA[a]	0.6 μM	~0.4 μM	0.5 μM
Sackesen *et al.* (284)	DTNB reduction assay	173 pediatric controls	—	~5.9 μM	—
		164 children with mild asthma		~4.7 μM	
Shanmugasundaram *et al.* (293)	DTNB reduction assay	94 preadolescent controls	—	58 μM	—
		86 adolescent controls		56 μM	
		118 preadolescent MMAs		41 μM	
		92 adolescent MMAs		40 μM	
		39 preadolescent MMAs with exacerbation		38 μM	
		26 adolescent MMAs with exacerbation		35 μM	
Bronchoalveolar lavage and epithelial lining fluid					
Comhair *et al.* (52)	HPLC	5 controls, at baseline	—	268 μM	9 μM
		5 controls, after allergen challenge (10 min)		192 μM	3 μM
		5 controls, after allergen challenge (48 h)		231 μM	11 μM
		7 mild asthmatics, at baseline		282 μM	8 μM
		7 mild asthmatics, after allergen (10 min)		165 μM	15 μM
		7 mild asthmatics, after allergen (48 h)		284 μM	9 μM

(*continued*)

Table 1. Continued

Reference	*Assay method*	*Subjects*	*Total GSH + GSSG*	*GSH*	*GSSG*
Fitzpatrick *et al.* (86)	HPLC	38 children with MMA[a]	1.2 μM	~0.6 μM	0.6 μM
		51 children with SA[a]	0.7 μM	~0.35 μM	0.4 μM
Fitzpatrick *et al.* (87)	HPLC	21 children with moderate asthma (BAL)[a]	—	0.33 μM	0.13 nM
		21 children with moderate asthma (ELF)[a]		37 μM	13 μM
		43 children with SA (BAL)[a]		0.26 nM	0.45 μM
		43 children with SA (ELF)[a]		24 μM	32 μM
Fitzpatrick *et al.* (88)	HPLC	35 adult controls (ELF)	436 μM	~390 μM	~10 μM
		6 symptomatic pediatric controls (ELF)[a]	260 μM	~225 μM	~30 μM
		35 children with SA without airflow limitation (ELF)[a]	134 μM	~100 μM	~40 μM
		30 children with SA with airflow limitation (ELF)[a]	129 μM	~50 μM	~75 μM
Kelly *et al.* (166)	DTNB reduction assay	20 controls (bronchial wash)	—	0.30 μM	0.09 μM
		20 controls (BAL fluid)		0.41 μM	0.08 μM
		20 mild asthmatics (bronchial wash)		0.41 μM	0.52 μM
		20 mild asthmatics (BAL fluid)		0.30 μM	0.28 μM
Mudway *et al.* (234)	DTNB reduction assay	15 controls, baseline (bronchial wash)	0.46 μM	—	0.11 μM
		15 controls, baseline (BAL fluid)	0.88 μM		0.07 μM
		15 controls, after ozone (bronchial)	0.98 μM		0.31 μM
		15 controls, after ozone (BAL fluid)	0.94 μM		0.30 μM
		15 mild asthmatics, baseline (bronchial)	1.08 μM		0.27 μM
		15 mild asthmatics, baseline (BAL fluid)	0.98 μM		0.24 μM
		15 mild asthmatics, after ozone (bronchial)	1.20 μM		0.35 μM
		15 mild asthmatics, after ozone (BAL fluid)	1.40 μM		0.43 μM
Smith *et al.* (299)	DTNB reduction assay	17 controls (bronchial)	13 nM/mg protein	—	—
		17 controls (alveolar)	23 nM/mg protein		
		10 mild asthmatics (bronchial)	24 nM/mg protein		
		10 mild asthmatics (alveolar)	37 nM/mg protein		
Induced sputum					
Beier *et al.* (27)	DTNB reduction assay	31 controls, baseline	12.1 μM	—	—
		31 controls, 72 h (no intervention)	9.8 μM		
		12 mild asthmatics, baseline	20.2 μM		
		12 mild asthmatics, 72 h (no intervention)	18.1 μM		
Beier *et al.* (28)	DTNB reduction assay	15 controls	9.2 μM	—	~1.08 μM
		20 mild asthmatics	8.7 μM		~1.29 μM
		19 moderate asthmatics[a]	4 μM		~1.20 μM

(*continued*)

Table 1. Continued

Reference	*Assay method*	*Subjects*	*Total GSH + GSSG*	*GSH*	*GSSG*
Beier *et al.* (29)	DTNB reduction assay	12 mild asthmatics, early allergen responders, baseline	—	3.3 μM	4.5 μM
		12 mild asthmatics, early allergen responders, postallergen challenge		5.9 μM	2.9 μM
		12 mild asthmatics, late allergen responders, baseline		3.5 μM	3.7 μM
		12 mild asthmatics, late allergen responders, postallergen challenge		2.8 μM	3.2 μM
Dauletbaev *et al.* (60)	DTNB reduction assay	10 controls (saliva)	1.2 μM	—	0 μM
		10 controls (sputum)	3.9 μM		50.9 μM
		10 MMAs (saliva)[a]	0.9 μM		0 μM
		10 MMAs (sputum)[a]	6.4 μM		72.3 μM
Deveci *et al.* (66)	DTNB reduction assay	11 controls	—	0.34 μM	—
		11 stable MMAs[a]		8.53 μM	
		10 MMAs with acute exacerbation[a]		2.85 μM	
Kongerud *et al.* (178)	DTNB reduction assay	18 controls (sputum cells)	628 ng/10^6 cells	—	—
		16 mild asthmatics (sputum cells)	1016 ng/10^6 cells		
Wood *et al.* (348)	DTNB reduction assay	6 controls (whole sputum)	10.4 μM	2.7 μM	3.5 μM
		20 controls (sputum supernatant)	7.0 μM	1.2 μM	2.6 μM
		10 mild-to-severe asthmatics (whole sputum)[a]	13.6 μM	2.2 μM	3.5 μM
		37 mild-to-severe asthmatics (sputum supernatant)[a]	15.3 μM	4.1 μM	5.9 μ
Exhaled breath condensate					
Corradi *et al.* (57)	HPLC	10 pediatric controls	—	14.1 nM	
		12 children with MMA, with exacerbation[a]		5.96 nM	
		12 children with MMA, after exacerbation[a]		8.44 nM	
Dut *et al.* (70)	HPLC	191 pediatric controls	—	~17 nM	—
		110 children with mild asthma		~8 nM	
		30 children with moderate asthma		~8 nM	

Values represent the mean.
[a]Treated with inhaled corticosteroids.
MMA, mild-to-moderate asthma; SA, severe asthma; BAL, bronchoalveolar lavage; ELF, epithelial lining fluid; GSSG, glutathione disulfide; GSH, glutathione, reduced (thiol) form; DNTB, (5,5'-dithiobis-(2-nitrobenzoic acid)); HPLC, high-performance liquid chromatography.

asthmatic subjects. However, the results have been conflicting, most likely due to a number of methodological challenges associated with sputum GSH and GSSG analysis (26, 27). Whereas some studies have failed to demonstrate differences in sputum total glutathione concentrations (GSH + GSSG) between mild asthmatics and controls (27, 60, 178), others have shown increased basal total glutathione concentrations in induced sputum from asthmatics at baseline (28, 66, 348) and after allergen challenge (29). Although sputum GSSG concentrations were not consistently elevated in subjects with mild asthma in these studies (28, 60, 348), others have observed significant associations between increased GSSG concentrations, increased eosinophil counts (29), and decreased pulmonary function reflected by the ratio of the forced expiratory volume in one second to the forced vital capacity (348). While these findings may reflect a greater airway oxidative burden, they may also reflect differences in the sample processing methodology. Dithiothreitol, which is commonly used to minimize the viscosity of sputum samples, is a strong reducing agent and concentrations above 0.001% artificially increase total glutathione concentrations while decreasing GSSG (27). Sputum induction time (27) and sample storage conditions (26) may also alter GSH content. Thus, additional studies using standardized methodology are necessary before conclusions about the sputum GSH/GSSG redox state can be reached. Studies comparing GSH concentrations in sputum *versus* the epithelial lining fluid would also be of benefit.

Exhaled breath condensate, though extremely dilute given the high concentration of exhaled water, is another potential source of airway constituents. Previous studies have shown that GSH/GSSG redox balance can be measured in the exhaled breath with sensitive methods such as high performance liquid chromatography (353). Only two studies to date have assessed GSH in the exhaled breath condensate of subjects with asthma. In these studies, children with mild-to-moderate asthma had significantly lower concentrations of exhaled GSH than healthy controls who were further associated with increased malondialdehyde formation, a marker of lipid peroxidation (57, 70). Within the asthmatics, a 5-day course of systemic corticosteroids increased GSH and decreased malondialdehyde concentrations to levels observed in controls (57). These observations are similar to what we have previously observed in the epithelial lining fluid of asthmatic children (88) and highlight the potential utility of exhaled breath condensate in the assessment of airway GSH/GSSG redox status. Given the noninvasive nature of exhaled breath condensate, which is particularly advantageous for field studies and studies of subject populations (such as children) in whom bronchoscopy is not feasible, additional validation studies are needed. Although there are a number of methodological issues associated with exhaled breath condensate measurement, including the significant dilution of the sample and lack of normalization standards, the exhaled breath does contain leukotrienes, lipid peroxidation byproducts, prostaglandins, H_2O_2, nitric oxide oxidation products, and ionic constituents (116, 223). Therefore, it would be worth studying the effects of pharmacological asthma treatments on exhaled breath condensate concentrations of GSH/GSSG and 8-isoprostanes (226), a specific marker of lipid peroxidation, which has been associated with airway hyperreactivity (18) and increased asthma severity (286) in affected patients.

C. Systemic glutathione concentrations in asthma

Although several studies have examined systemic glutathione in subjects with asthma, these studies have yielded conflicting results given differences in the type of sample analyzed (*i.e.*, whole blood, erythrocytes, and plasma) and the clinical characteristics of the subjects enrolled. For instance, in two studies, whole blood and erythrocyte total glutathione concentrations (measured as the sum total of the GSH and GSSG pools) were not significantly different between asthmatics and healthy controls at baseline (348) or during acute exacerbations (237). However, in both of these studies, the subjects enrolled were mild asthmatics who had achieved good symptom control on low doses of inhaled corticosteroids (237, 348). By contrast, two other studies of treatment-naïve asthmatics with mild airflow limitation (329) and asthmatics with current symptoms on inhaled corticosteroids (236) demonstrated increased whole blood and erythrocyte total glutathione concentrations in asthmatics *versus* controls. While previous findings of increased total glutathione in subjects with asthma may reflect an adaptive response whereby synthesis and mobilization of GSH is increased to combat acute insults, these previous findings are more likely due to increased GSSG formation (206) since protein –SHs are also reduced in these subjects (15, 236). Furthermore, in other studies, asthmatics with higher total glutathione concentrations also had decreased pulmonary function evidenced by reductions in the forced expiratory volume in one second (237, 243) and higher concentrations of systemic protein carbonylation and lipid peroxidation byproducts (236). Given the important role of GSH/GSSG redox in free radical scavenging, these findings likely represent increased GSSG formation or relative GSH depletion. However, GSH and GSSG were not measured in the majority of these studies, so the GSH/GSSG redox state cannot be estimated.

Although few studies have studied systemic GSH and GSSG in asthma, the few studies that have been conducted do reveal important insight on the systemic GSH/GSSG redox balance of these subjects. In a recent study, plasma GSH concentrations were decreased by nearly 1.5-fold in inhaled corticosteroid-naïve asymptomatic asthmatics compared to healthy controls (284). The decreased plasma GSH concentrations in these subjects were further associated with decreased expression of superoxide dismutase and increased lipid peroxidation byproducts (284). Although the asthmatics enrolled in this study did not have an obvious burden of respiratory symptoms, these findings suggest that even well-controlled asthmatics may have a significant oxidative burden, which may be a risk factor for future exacerbations or other respiratory complications. Other studies of poorly controlled asthmatics with obvious airway inflammation have likewise revealed lower systemic GSH concentrations (74, 293) and higher GSSG concentrations (206) in these subjects than in healthy controls. With acute asthma exacerbations, these GSH concentrations decreased further and were associated with an increased burden of free radicals and lipid peroxidation byproducts in the plasma and circulating cells (293). While the specific mechanisms underlying these GSH/GSSG redox disturbances are not clear, these findings highlight the role of GSH/GSSG redox in asthma control and suggest that systemic measures of GSH and GSSG, as opposed to total glutathione alone, may be particular relevance

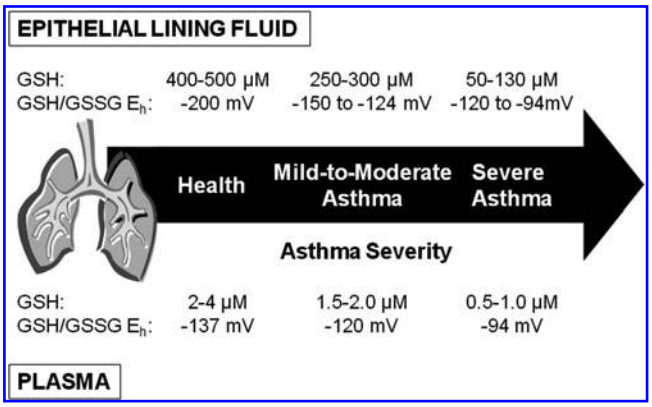

FIG. 12. Hypothesized continuum of glutathione redox disturbances in asthma. Values for GSH and the GSH/GSSG redox potential (E_h) were estimated from existing literature.

in these subjects. Given that GSH and GSSG are also differentially regulated between the plasma and circulating cells (156, 157), these findings also underscore the need for compartmentalized (*i.e.*, plasma or erythrocyte *vs.* whole blood) assessment of both GSH and GSSG in asthma to approximate the underlying systemic GSH/GSSG redox state.

We recently published the first report of the GSH/GSSG redox state in children with mild-to-moderate asthma and severe asthma refractory to inhaled corticosteroids that included assessment of the GSH/GSSG redox potential (86). In this study, children with severe asthma had lower total glutathione (GSH + GSSG) concentrations in the plasma (0.6 μM) than children with mild-to-moderate asthma (1.7 μM) (86). Although nonasthmatic controls were not enrolled, the total glutathione concentrations observed in both groups of asthmatics were significantly less than the range of 2–4 μM previously reported for healthy adults (159, 160). Whereas plasma GSSG concentrations did not differentiate children with severe and mild-to-moderate asthma, children with severe asthma had twofold lower concentrations of GSH and a shift in the GSH/GSSG redox potential (E_h) by 30 mV to the more oxidized state (−94 *vs.* −120 mV for severe *vs.* mild-to-moderate asthma, respectively) (86). The total plasma cysteine pool (cysteine + CySS) was also significantly lower in children with severe (3.1 μM) *versus* mild-to-moderate asthma (4.5 μM) and was likewise associated with cysteine depletion and a shift by nearly 30 mV in the cysteine/CySS redox potential (E_h) (−62 *vs.* −89 mV for severe *vs.* mild-to-moderate asthma, respectively) (86). These findings were mirrored in the airway lavage fluid from these same children (86) (Fig. 12). Given that the extracellular GSH/GSSG and cysteine/CySS redox pools are under tight regulation during physiologic health (156, 157), these observations reflect a global disturbance in both thiol availability and thiol redox signaling in individuals with severe asthma that likely has important clinical and therapeutic implications.

D. Glutathione redox balance in asthma: Effect of corticosteroids

Because inhaled corticosteroids are the cornerstone of treatment for persistent asthma (3), clinical studies of GSH/GSSG redox balance in subjects with asthma are frequently confounded by corticosteroid exposure since both GSH and corticosteroids exert broad anti-inflammatory effects. Therefore, it is not entirely clear how inhaled corticosteroids affect GSH/GSSG redox balance *per se*. In studies of corticosteroid-naïve children (129) and adults (253) with elevated systemic total glutathione levels (irrespective of GSH or GSSG content), treatment with inhaled corticosteroids for 6 to 8 weeks resulted in a significant reduction of total glutathione concentrations. These reductions mirrored improvements in asthma symptom control and were further accompanied by decreased blood eosinophils, decreased systemic markers of DNA oxidation, and increased systemic catalase activity, suggesting an attenuation of systemic oxidative stress with inhaled corticosteroid treatment (129, 253). Similarly, *in vitro* studies involving treatment of human airway epithelial cells with hydrocortisone resulted in a reduction in basal and tumor necrosis alpha-stimulated intracellular total glutathione content, although no detectable changes in GSSG were noted (266, 332). Whether corticosteroids decrease GSH synthesis in asthmatic patients is not entirely clear (265). Given previous observations of GSH depletion in severe asthma (86–88), a disorder necessitating treatment with high doses of corticosteroids, additional investigations of the effect of corticosteroids on GSH/GSSG redox balance are needed.

VII. Other Glutathione-Related Proteins and Redox Systems in Asthma

A number of other GSH-related proteins and redox systems are also present in the body and are important for antioxidant defense. These include glutathione peroxidases, glutathione reductases, glutathione-S-transferases, GSNO, thioredoxins, glutaredoxins, and peroxiredoxins. The relevance of these proteins and redox systems to asthma is discussed below.

A. Glutathione peroxidases

Glutathione peroxidases catalyze the reduction of hydroperoxides by GSH and are therefore essential for maintaining GSH/GSSG redox balance. Glutathione peroxidases have been extensively studied in the peripheral blood of subjects with asthma. Although some studies have found no differences in systemic glutathione peroxidase activity or protein expression between asthmatics and healthy controls (55, 207, 257, 312, 347), a few have noted increased glutathione peroxidase expression and activity in asthma subjects (130, 131, 148, 315). In these subjects, many of whom had milder forms of asthma, increases in glutathione peroxidase may reflect a beneficial physiological response to an increased hydroperoxide burden. However, when this burden is severe or chronically sustained, the functional utility of this enzyme can be exhausted as a function of GSH/GSSG redox disturbances (267). This likely explains the results of many studies that have shown decreased glutathione peroxidase activity in the circulating erythrocytes (165, 206, 254, 258, 284), leukocytes (236, 328), platelets (132, 222, 250), and whole blood (91) and plasma (263) of asthmatics *versus* controls. In keeping with this notion, systemic glutathione peroxidase activities have been shown to decrease as function of asthma severity (255) and during acute asthma exacerbations (31, 123, 293). Decreased glutathione peroxidase activities in subjects with asthma have also been associated with increased systemic lipid peroxidation and malondialdehyde formation (76, 123,

236) and lower pulmonary function (122). While treatment with inhaled corticosteroids alleviates airway inflammation, it has not been shown to increase systemic glutathione peroxidase activity in subjects with asthma (82, 253).

Similar to the systemic circulation, within the lung, investigators have not been able to consistently demonstrate alterations of glutathione peroxidase in asthmatic subjects. In three studies of mild asthmatics and healthy controls, no differences in glutathione peroxidase activity in airway cells or bronchoalveolar lavage fluid were observed at baseline (52, 62, 300) or after allergen challenge (52) despite significant differences in superoxide dismutase activity between groups. By contrast, Comhair *et al.* found increased expression of glutathione peroxidase in the epithelial lining fluid and bronchial epithelial cells of subjects with mild asthma compared to controls (53). In that study, increased expression and activity of glutathione peroxidase was attributed to increased gene expression from ROS exposure and GSH/GSSG redox alterations (53). While more studies are needed, these findings suggest that the airway GSH/GSSG redox balance may lead to induction of glutathione peroxidase transcription, protein expression, and secretion and activation in the airway. However, this response is likely to be overwhelmed by chronic and sustained GSH/GSSG airway redox alterations and GSH depletion. This might explain why we previously failed to observe differences in epithelial lining fluid glutathione peroxide activities between children with severe asthma and controls despite an increased oxidative burden in the severe asthmatic group (88).

B. Glutathione reductases

Glutathione reductase is a flavoprotein that catalyzes the reduction of GSSG to GSH. Few studies have assessed glutathione reductase in asthma. While three studies found no differences in glutathione reductase activity in the airway lavage cells (300), epithelial lining fluid (88), and erythrocytes (254) of asthmatics *versus* healthy controls, others have observed decreased glutathione reductase activity in erythrocytes from asymptomatic asthmatics as compared to controls (124, 208). In a separate study, erythrocyte glutathione reductase activity declined significantly in the asthmatics during acute exacerbations as a function of increased lipid peroxidation (123). Similar reductions in basal glutathione reductase activity have also been observed in subjects with chronic obstructive pulmonary disease (179) and chronic inhalation exposures (194). These findings may explain previous observations of GSH deficiency in asthmatics, particularly in those with severe disease. More studies are needed to understand the relationship of glutathione reductase activity to GSH/GSSG redox signaling in these individuals.

C. Glutathione-S-transferases

The glutathione-S-transferase supergene family, which contains enzymes of the mu (*GSTM1*), theta (*GST1*), and pi (*GSTP1*) classes, catalyzes the conjugation of GSH and is therefore critical for detoxification of endogenous compounds and xenobiotics. Much of the interest in glutathione-S-transferases in asthma stems from genetic polymorphism studies. In the original study, the frequency of the *GSTP1* Val(105)/Val(105) genotype, the form with greater activity, was significantly lower in asthmatics (99). Furthermore, the *GSTP1* Val(105)/Val(105) homozygotes had a reduced asthma risk of six- to ninefold and a decreased severity of airway dysfunction compared to *GSTP1* Ile(105)/Ile(105) homozygotes (99). Other studies have also demonstrated associations between asthma and the *GSTM1* null genotype (which lacks the *GSTM1* gene) and the *GSTT1* null genotype (163, 164, 280, 311).

Because these glutathione-S-transferase polymorphism studies included relatively small populations, a systematic review and meta-analysis were recently undertaken to better understand the associations between *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms and asthma in adults and children (221). These analyses did not identify a substantial role of glutathione-S-transferase genes in asthma risk given significant heterogeneity that existed between these studies (221). However, these analyses were limited to asthma risk and did not focus on the role of these genes in the severity of the disorder. Another important knowledge gap is how glutathione-S-transferase polymorphisms affect protein expression or activity of the glutathione-S-transferase enzymes or related proteins. Whereas one study observed decreased airway neutrophil flux in response to ozone exposure in *GSTM1* null *versus GSTM1* wild-type asthmatics (7), another failed to demonstrate differences in glutathione-S-transferase enzymatic activity in subjects with asthma stratified by genotype (205). That same study also observed higher total glutathione-S-transferase activity in asthmatics *versus* healthy controls (205), while others found no difference in glutathione-S-transferase activity between groups (84, 300). Further studies linking glutathione-S-transferase genotypes to protein expression and functional activity of the enzyme and related proteins are needed.

D. Nitrosoglutathione

Nitrosative stress, evidenced by increased formation of $^{\bullet}$NO and nitric oxide oxidation products, is a characteristic feature of the asthmatic airway and is particularly pronounced in subjects with severe *versus* mild-to-moderate asthma (71, 84, 204). This process may ultimately contribute to protein dysfunction and cellular destruction. In the airways, nitrosative stress has been associated with cellular apoptosis and necrosis as well as eosinophil recruitment, microvascular leakage, and airway hyperresponsiveness (10, 279). The physiological influence of nitric oxide ($^{\bullet}$NO) in the airways is largely due to post-translational modification of cysteine residues on proteins through S-nitrosylation (97). This process is primarily regulated by transfer of NO groups between proteins and GSH, which results in the formation of GSNO. Whereas excessive $^{\bullet}$NO and nitrosative stress may result in airway injury, GSNO is an endogenous bronchodilator associated with airway smooth muscle relaxation (79) that protects against methacholine-induced airway hyperresponsiveness (98, 261) and NF-κB activation during allergic airway inflammation (244). GSNO also suppresses 5-lipoxygenase, the rate limiting enzyme for the formation of the inflammatory and bronchoconstricting cysteinyl leukotrienes, in airway epithelial cells (357).

Although airway GSNO concentrations are relatively stable and range from 200 to 500 n*M* in healthy subjects (106, 107), in subjects with asthma, GSNO concentrations are significantly decreased ($\sim$60 n*M*) (262) and are virtually undetectable during severe acute exacerbations resulting in respiratory failure (107). These GSNO reductions are associated with increased

activity of the GSH-dependent formaldehyde dehydrogenase, class III alcohol dehydrogenase (also referred to as GSNO reductase) (262), which catalyzes the reduction of GSNO (192). Although the mechanisms underlying increased GSNO reductase activity in asthma are not clear, recent studies have demonstrated increased GSNO reductase activity in response to formaldehyde (303, 316), which is increased in the airways of asthmatics *versus* controls (as formate) and is associated with airway hyperreactivity and asthma severity in a concentration-dependent manner (116). Formaldehyde-induced GSNO depletion ultimately leads to the formation of glutathione sulfonamide and glutathione sulfinic acid, which inhibit glutathione-S-transferase activity (303). Alternatively, increased GSNO reductase activity in asthma may be related to underlying genetic polymorphisms in the *GSNOR* gene, which have been associated with an increased risk of asthma in children (349) and decreased bronchodilator responsiveness (48, 228). However, the functional relevance of these polymorphisms is not presently known.

E. Thioredoxins

Thioredoxins, including thioredoxin 1 and thioredoxin 2, are oxidoreductase enzymes that contain a dithiol (–SH HS–) group that forms a disulfide bridge (–S–S–) (139). Whereas thioredoxin 1 is found in the cytoplasm, thioredoxin 2 is located in the mitochondria (11). Both thioredoxins scavenge ROS and inhibit apoptosis (127, 139). Thioredoxins also play a key role in the reduction of methionine residues in proteins. Similar to protein –SH groups, protein methionine residues undergo oxidation to methionine sulfoxide (MetO) in efforts to protect critical residues at the active protein site from oxidative modification and inactivation (80, 201). MetO reductases catalyze the reduction of MetO back to methionine (339) and then undergo reduction by thioredoxins, thus readying the MetO reductase enzyme for another catalytic cycle. Thioredoxin reductases in the cytoplasm and mitochondria also maintain thioredoxins in a reduced state and function similarly to glutathione reductases.

Studies of thioredoxins in asthma are limited and the majority of work that has been conducted in focused on thioredoxin 1. In animal models of allergic airway inflammation, thioredoxin suppresses eosinophil influx and goblet cell hyperplasia and mucin production (144, 319) and further inhibits airway smooth muscle hyperplasia and airway hyperresponsiveness (144). Thioredoxin 1 also inhibits eotaxin and regulated on activation, normal T-cell expressed, and secreted (RANTES)-induced chemotaxis of eosinophils from human subjects (175). While studies in asthma are limited, one study noted increased serum concentrations of thioredoxin 1 in subjects with acute asthma exacerbations compared to asthmatics who were well controlled that were inversely correlated with lung function (350). A separate study of isolated T-lymphocytes from subjects with asthma further observed increased expression of thioredoxin 2 compared to healthy controls (150). However, the role of thioredoxins in the protection against oxidative stress in these subjects is not clear and warrants further study.

F. Glutaredoxins

Glutaredoxins are thiol-disulfide oxidoreductases that reduce protein mixed disulfides (protein-SSG) with glutathione through a process called deglutathionylation (219). Thus, the activity of glutaredoxins is entirely dependent upon GSH availability and the GSH/GSSG redox balance of the cell. Although there are four known glutaredoxins, the dithiols glutaredoxin 1 and glutaredoxin 2 are most commonly studied. Glutaredoxin 1 is a cytosolic protein, whereas glutaredoxin 2 is found in both the mitochondria and the nucleus. Both glutaredoxins exhibit deglutathionylating activity for peptide and protein substrates, but the activity of glutaredoxin 2 is $\sim$10-fold lower than that of glutaredoxin 2 (101, 200).

In mice, glutaredoxin 1 is associated with suppression of airway neutrophil influx and pro-inflammatory cytokine release as well as decreased activation of NF-κB in the lung (6, 49). Suppression of glutaredoxin 1 is further associated with impaired terminal differentiation of airway macrophages resulting in impaired phagocytic function and altered inflammatory mediator release (5). In experimental models of asthma induced by ovalbumin sensitization, glutaredoxin 1 is increased after allergen challenge while glutaredoxin 2 is not altered (276). However, glutaredoxin 1 decreased significantly in response to TGFβ1 (276), which is commonly found in the asthmatic airway (145). While human studies of asthma are not available, these findings suggest that glutaredoxin1 inhibition may play an important role in asthma-related oxidative stress and airway structural changes given its suppression by TGFβ1. Other studies of adults with fibrotic airway disorders (251) and chronic obstructive pulmonary disease (252) have demonstrated reduced glutaredoxin 1 expression in bronchial epithelium and airway macrophages that associated with increased airflow limitation. Additional studies in asthma are needed.

G. Peroxiredoxins

Peroxiredoxins are nonseleno peroxidases with a wide spectrum of activity against organic hydroperoxides and OONO–. There are six mammalian peroxiredoxins, which are located throughout the cytosol, mitochondria, and peroxisomes (278) and protect the lung from oxidative injury and oxidant-mediated cell death (190, 335). Within the human lung, peroxiredoxins are expressed in abundance in critical areas of oxidant protection, including alveolar macrophages, bronchial epithelial cells, and the alveolar epithelium (169). Although expression of peroxiredoxins initially increases with oxidative stress (167, 186), severe and sustained oxidative stress can lead to over-oxidation and inactivation of the enzyme (186, 278). However, the role of peroxiredoxins in asthma and other lung disorders is not understood. In murine models of allergic airway inflammation, peroxiredoxin 1 and peroxiredoxin 2 are increased in airway and lung tissue of sensitized mice (78, 146, 227). Ablation of peroxiredoxin 1 and 2 is further associated with increased T helper type 2 (Th2)-mediated cytokine release, airway hyperresponsiveness to methacholine, B-cell activation, and T-cell proliferation (146, 227), suggesting that peroxiredoxins are central to the airway antigenic response. Further studies in asthma are needed.

VIII. Physiological and Biological Implications of Altered Glutathione Redox Balance in Asthma

Despite accumulating evidence that the GSH/GSSG redox balance is altered in the airways and possibly even the

systemic circulation of subjects with asthma, the functional and ultimately clinical relevance of the altered GSH/GSSG redox balance in asthma is not entirely clear. Several *in vitro* studies have demonstrated that physiologic concentrations of airway GSH ($\sim 500\,\mu M$) are important for maintaining the structure, function, and defenses of airway epithelial cells. Upon exposure to endogenous or environmental toxins or respiratory viruses, GSH content decreases in alveolar epithelial cells in both a dose-dependent manner and a time-dependent manner (152, 189, 235) and is associated with increased apoptosis (344), increased pro-inflammatory cytokine and chemokine release (152), and increased superoxide production (248). In otherwise healthy cells, other defenses help restore airway GSH pools to minimize these adverse effects. These include increased expression of glutamate cysteine ligase (also referred to as γ-glutamylcysteine synthetase) to promote GSH synthesis (272), upregulation of glutaredoxins that catalyze the reduction of S-glutathionylated proteins in the mitochondrial membrane (22), and increased expression and activation of the transcription factor, nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which regulates the expression of several genes essential for GSH synthesis and homeostasis (188). However, these mechanisms may be overwhelmed in asthma, a disorder characterized by chronic airway inflammation. For instance, glutamate cysteine ligase activity in airway epithelial cells may be sensitive to airway thiol deficiency (271), which is particularly pronounced in subjects with severe asthma (86). Nrf2 activity is also altered in cases of severe asthma (86), potentially resulting in increased expression of Th2-related cytokines and other inflammatory features that accompany the disorder (4, 270, 343).

Because human studies are lacking, the functional consequences of the GSH/GSSG redox imbalance in the asthmatic airway have been studied through the use of several animal models. In these studies, guinea pigs and BALB/c mice with allergic airway disease induced by ovalbumin sensitization had significantly lower total lung thiol content, with up to 40% lower GSH concentrations and threefold higher concentrations of GSSG in the lung tissue homogenate (172, 177). The decreased whole lung GSH levels in these animals were further accompanied by a Th2-mediated inflammatory response evidenced by increased airway eosinophil influx; higher concentrations of interleukin (IL)-4, IL-5, IL-10, eotaxin, and RANTES; and decreased concentrations of IL-12 and interferon gamma in the bronchoalveolar lavage (177). In a similar study of BALB/c mice sensitized to ovalbumin, reductions in the GSH/GSSG ratio in lung tissue after ovalbumin challenge were also associated with increased airway mucin production and airway hyperresponsiveness, which persisted for several days after the initial exposure (249). The ability of GSH/GSSG to regulate airway tone was further suggested in a recent study where γ-glutamyl transferase-deficient mice (with high extracellular GSH concentrations) were protected from methacholine-induced airway hyperreactivity (197). In that study, the increased airway GSH content was also associated with decreased airway mucin production, decreased mucous cell hyperplasia, and decreased protein carbonylation after a secondary insult with IL-13 (197). These findings suggest that the airway GSH/GSSG redox balance may play an important role in asthma independent of Th2-mediated inflammation (Fig. 13). Additional functional studies of GSH and airway structure and function are needed.

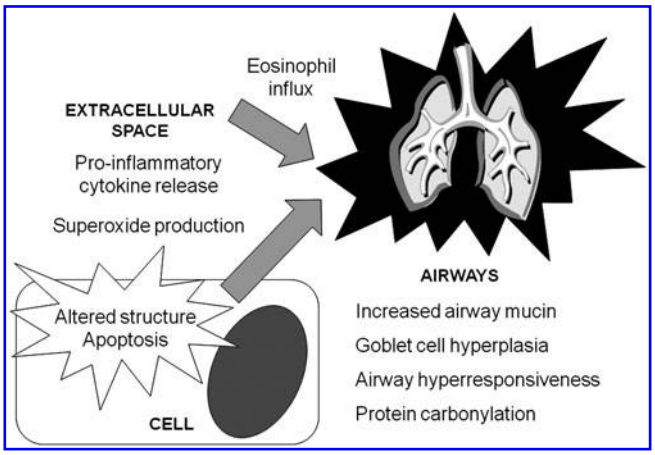

FIG. 13. Consequences of altered GSH/GSSG redox balance in asthma.

IX. Altered Glutathione Redox Balance in Asthma: Therapeutic Opportunities

Current treatment paradigms for asthma are focused on relaxation of airway smooth muscle and suppression of airway inflammation. These effects are achieved primary with bronchodilators and inhaled corticosteroids, which are the cornerstone of asthma therapy given their widespread effectiveness in most asthma populations (3). However, in patients with severe asthma that is refractory to inhaled corticosteroid treatment, or in other subpopulations of asthmatics such as smokers, obese asthmatics, or asthmatics with nutrient deficiencies, other interventions may also be warranted to restore the GSH/GSSG redox balance. To this end, several studies have attempted to increase the GSH pool in subjects with asthma through direct delivery of GSH or GSH-ethyl esters. Because cysteine is the functional unit of GSH, studies have also attempted to increase cysteine pools through delivery of cysteine precursors. Others have focused on dietary interventions for subjects with asthma to increase sulfur amino acid and micronutrient availability to restore GSH/GSSG redox balance. These are discussed below.

A. Glutathione and glutathione-ethyl esters

GSH-ethyl esters are efficiently transported into the cell, where they are hydrolyzed to GSH (288). For that reason several studies have used GSH-ethyl esters as opposed to GSH to restore the airway GSH/GSSG redox balance. In one such study, significant reductions in airway inflammatory cell infiltration (including eosinophil chemotaxis) and airway pro-inflammatory cytokine synthesis were observed in ovalbumin-sensitized mice with intraperitoneal GSH-ethyl ester administration (177). GSH-ethyl esters administered to the airways through aerosolization or direct instillation (after isolation) also decreased ovalbumin-induced airway methacholine hyperresponsiveness, thus blunting the airway obstruction that is typically observed during an acute asthma response (172, 173). In another study, direct GSH treatment of isolated bronchi from ovalbumin-sensitized guinea pigs *in vitro* resulted in similar decreases in smooth muscle contraction and bronchodilation (47). These observations suggest

a possible therapeutic role for GSH and GSH donors in the management of airway inflammation and obstruction.

In light of these observations, two studies have attempted to administer GSH *via* inhalation to subjects with asthma. In the first study, a 600-mg glutathione solution (composition unclear) was administered by nebulization to 12 young adults with mild-to-moderate asthma in a double-blind, placebo-controlled, cross-over study (16). Subjects with asthma were pretreated with the glutathione solution or placebo and then immediately underwent challenge with ultrasonically nebulized distilled water. Compared to placebo, the glutathione solution attenuated the fall in the forced expiratory volume in one second resulting from the challenge (6.04 L decline *vs.* 20.41 L decline for glutathione solution *vs.* placebo) (16). However, in another study involving eight adults with mild asthma, a similar glutathione solution (600 mg, composition unknown) administered by nebulization resulted in increased airway resistance, decreased pulmonary function measured by the forced expiratory volume in one second, and increased asthma symptoms, including severe wheezing and breathlessness ($n=1$), breathlessness ($n=2$), and cough ($n=4$) (213). The reasons underlying the bronchoconstricting effect of the glutathione solution are not clear but may be related to sulfite sensitivity since the hypertonic acid solution that served as the placebo resulted in cough in only one subject (213). These findings are in contrast with those from other studies, which did not reveal toxic or adverse effects with similar inhaled glutathione solutions (300–600 mg) administered to subjects with cystic fibrosis (32, 118, 128, 283), chronic otitis media (314), idiopathic pulmonary fibrosis (39), human immunodeficiency virus (138), and chronic rhinitis (313). In those studies, nebulization of the glutathione solution resulted in lower airway GSH deposition (118) in treated subjects with a significant increase in epithelial lining fluid GSH concentrations (39, 118, 138, 283). Increased airway GSH availability in those studies was further associated with decreased $O^{\bullet}{}_2^-$ release by airway alveolar macrophages (39, 283), decreased airway prostaglandin E_2 synthesis (128), and increased circulating T cells in the bronchoalveolar lavage of treated subjects (128). A small randomized, double-blind, placebo-controlled pilot study of glutathione inhalation in children with cystic fibrosis also demonstrated greater peak expiratory flows and improved respiratory symptoms in the glutathione-treated group (32). While meta-analyses have found no evidence to recommend the routine clinical use of nebulized glutathione or thiol derivatives (240), these analyses were limited by the quality of the trials conducted. Given the profound GSH/GSSG balance that accompanies asthma, further research on the safety and efficacy of inhaled glutathione solutions for asthma may be warranted.

B. Cysteine precursors

N-acetylcysteine and L-2 oxothiazolidine-4-carboxylic acid are examples of cysteine precursors that have been used in animal models of allergic asthma. These studies have shown that *N*-acetylcysteine, when administered before ovalbumin sensitization, maintains lung GSH/GSSG homeostasis and attenuates lung protein carbonylation and lipid peroxidation after a secondary insult from diesel exhaust or antigen exposure (37, 289, 342). In sensitized animals, *N*-acetylcysteine further attenuates many of the characteristic features of asthma, including airway cellular infiltration, airway mucin and pro-inflammatory cytokine expression, and airway hyperresponsiveness (37, 249). These beneficial effects on airway inflammation and tone have also been noted after administration of *N*-acetylcysteine amide and *N*-acetyl cysteine proline cysteine amide, which have increased membrane permeability and greater reducing activity than *N*-acetylcysteine (168, 183). Pretreatment of sensitized animals with L-2-oxothiazolidine-4-carboxylic acid has also yielded similar results (184, 185), suggesting that repletion of cysteine pools may be of benefit in asthma.

Although the clinical efficacy of cysteine precursors has not been explored in asthma, several studies have focused on the role *N*-acetylcysteine in subjects with chronic obstructive pulmonary disease. Although chronic obstructive pulmonary disease is distinct from asthma, it is increasingly recognized that cigarette smoking is not the sole factor underlying the epidemiology and natural history of the disorder (73). Indeed, asthma and chronic obstructive pulmonary disease share a number of clinical features, including airway inflammation, airflow limitation, and respiratory exacerbations (110). In a systematic review of eleven placebo-controlled trials involving subjects with chronic obstructive pulmonary disease, oral *N*-acetylcysteine at a dose of 400–600 mg was more efficacious than placebo in reducing respiratory symptoms, with no significant adverse effects (305). A similar meta-analysis also showed a 23% reduction with *N*-acetylcysteine *versus* placebo in the number of exacerbations over 6 months (115). However, in contrast to these findings, a more recent large-scale randomized trial found no differences in the number of chronic obstructive pulmonary disease exacerbations per year or the yearly rate of decline in the forced expiratory volume in one second when 600 mg of *N*-acetylcysteine was taken orally once daily (63). This finding may reflect confounding by inhaled corticosteroids since in secondary analyses, the number of exacerbations was significantly lower with *N*-acetylcysteine treatment in subjects not taking inhaled corticosteroids (risk ratio 0.79 *vs.* 0.99 overall) (63). Alternatively, this finding may reflect inadequate dosing of *N*-acetylcysteine, since up to 1800 mg daily may be necessary to increase plasma GSH levels by 50% (41) due to its limited systemic bioavailability (64). Indeed, in a smaller study of critically ill subjects with chronic obstructive pulmonary disease exacerbations who were treated with supplemental oxygen, daily oral administration of 1200 to 1800 mg of *N*-acetylcysteine preserved erythrocyte GSH concentrations and attenuated systemic levels of oxidized protein as compared to placebo (96). A separate double-blind, cross-over study also found less air trapping and greater exercise performance in subjects with chronic obstructive pulmonary disease when *N*-acetylcysteine was administered at a daily 1200-mg dose (304).

The mechanisms underlying the benefits of *N*-acetylcysteine in subjects with chronic obstructive pulmonary disease are not completely understood. It is certainly possible that *N*-acetylcysteine may not be functioning as a GSH precursor, but rather as a mucolytic in these subjects. It is also possible that the benefits are only present in selected subpopulations. For example, in critically ill subjects or subjects with protein-energy malnutrition from catabolic disease (with increased energy expenditure), *N*-acetylcysteine supplementation may simply restore sulfur amino acid availability from decreased sulfur amino acid intake. However, 99% of adult

males and females in the United States consume greater than the recommended daily intake for sulfur amino acids, while 50% consume more than twice the recommended intake (90), suggesting that additional approaches may be needed to address a functional need for GSH in most Americans.

C. Dietary interventions

The specific role of the diet in maintaining or restoring GSH/GSSG redox balance has not been studied in asthma. However, given that GSH is synthesized from sulfur amino acid metabolism, increased dietary intake of methionine and cysteine may be of benefit in subjects with asthma, particularly those with severe disease in whom GSH stores are low. Other dietary components relevant to GSH synthesis and function, such as selenium, may also be beneficial. The potential role of the diet as a complementary therapy for modification of the GSH/GSSG redox balance in asthma is discussed below.

1. Selenium. Selenium is an essential trace element that plays a vital role in glutathione peroxidase activity. The mechanism by which the glutathione peroxidases catalyzes the reduction of hydroperoxides by GSH involves it selenocysteine (selenol) residue. The selenol is oxidized to selenenic acid by hydroperoxides, which then undergoes a reaction with GSH to form a selenenyl sulfide adduct. The selenenyl adduct reacts with another GSH molecule to regenerate the selenol, releasing GSSG as a byproduct (Fig. 14).

Given the important role of selenium in maintaining GSH/GSSG redox balance, dietary selenium requirements are based on the minimum intakes thought to be necessary for the maximization of glutathione peroxidase activities (317). Although true selenium deficiency is rare, low selenium intakes have been associated with a number of chronic diseases, including asthma (77). Several studies have demonstrated lower selenium concentrations in the plasma, whole blood, and erythrocytes of asthmatics compared to healthy controls (91, 165, 307). In these studies, subjects with the lowest selenium concentrations had a twofold increase in the relative risk of asthma (91) and lower activities of the enzymes glutathione peroxidase (124, 222), glutathione reductase (124), and catalase (124) associated with a significant reduction in total antioxidant defense (176). Furthermore, in mild-to-moderate asthmatics with airflow limitation, low plasma selenium concentrations were also associated with greater lipid peroxidation byproducts, increased concentrations of high-sensitivity C-reactive protein, and an increased ratio of CD4+/CD8+ lymphocytes, suggesting a shift in Th2 polarization (124). These findings were further accompanied by increased concentrations of the trace element, copper (124). Because copper may complex with GSH, resulting in a complex that reacts with oxygen and generates $O^{\bullet}{}_2^-$ (302), these findings merit further investigation and may account for decreased copper and zinc superoxide dismutase activities in asthmatics *versus* controls (62).

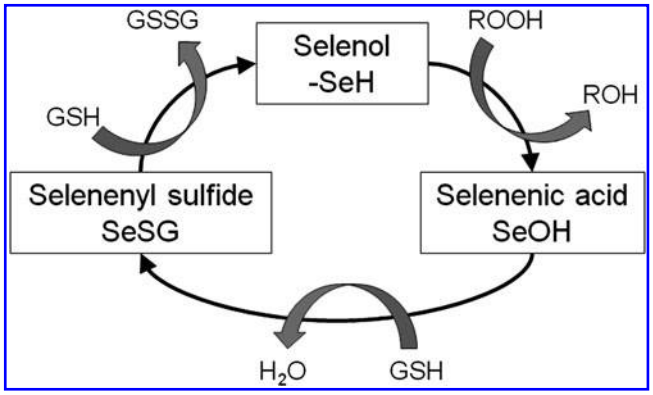

FIG. 14. Role of selenium in glutathione peroxidase activity. The selenol residue (–SeH) of glutathione peroxidase is converted to a selenenic acid in the presence of hydroperoxides (ROOH). GSSG is formed from the reduction of selenenyl sulfide.

Similar associations between selenium and asthma have been observed in mice with allergic airway sensitization induced by ovalbumin sensitization (137, 149). In these studies, mice fed a low-selenium diet for 8 weeks before ovalbumin exposure had significantly lower erythrocyte glutathione peroxidase enzymatic activities than mice fed a high-selenium diet (137). Supplementation with high doses of selenium before ovalbumin sensitization further inhibited airway eosinophil recruitment and the expression of inflammatory proteins in these animals (137, 149), suggesting a potential therapeutic role for selenium in asthma.

In keeping with the animal and human observational findings, one small placebo-controlled trial involving 24 subjects with asthma noted increased serum selenium concentrations in asthmatics treated with daily oral supplementation of 100 mcg of sodium selenite *versus* placebo for 14 weeks (133). Glutathione peroxidase activities were also increased in the selenium-treated *versus* the placebo-treated group and were associated with subjective measures of clinical improvement (133). However, the direct benefit of selenium treatment in asthmatic subjects could not be validated in a larger meta-analysis of these results since other objective parameters of lung function and airway hyperresponsiveness were unchanged (8). Furthermore, in a larger randomized, double-blind, placebo-controlled trial of selenium supplementation in 197 participants with asthma, 100 mcg of selenium per day for a treatment period of 24 weeks increased plasma selenium concentrations but failed to improve quality of life or other secondary measures of lung function, airway hyperreactivity, and symptoms when compared to the placebo-treated group (291). These results are in keeping with those of a more recent case–case control study and meta-analysis that found no overall associations between asthma and plasma selenium in a large sample of subjects (45, 242). While these findings may suggest a limited role for selenium supplementation in asthma, confounding from asthma therapy or other exposures such as cigarette smoking may be responsible. It is also possible that the benefit of selenium supplementation is additive and is only observed when taken in combination with other antioxidant or dietary therapies (81). Alternatively, the outcome indicators may not have been of sufficient sensitivity to reflect changes in GSH/GSSG redox balance.

2. Whey protein. Because oral GSH preparations may not effectively increase GSH tissue concentrations due to limited tissue availability (9), some studies have used whey protein preparations rich in cysteine and γ-glutamyl-glycine

to increase GSH levels *in vivo*. These whey-based products have been shown to increase concentrations of GSH in the liver of supplemented animals (171). In humans, whey products also increase circulating lymphocyte and plasma GSH concentrations in healthy subjects (180, 358) and GSH-deficient subjects with cystic fibrosis (117) and human immunodeficiency virus (218). However, studies in asthma are limited and have shown mixed results. While a small study in atopic children with asthma failed to discern significant differences in systemic GSH concentrations with whey protein supplementation (195), a separate study of subjects with exercise induced bronchospasm noted an attenuation of lung function decline after exercise with whey supplementation as compared to placebo (20). A similar study also found increased endurance and decreased exercise-related dyspnea and fatigue with whey protein *versus* placebo in subjects with chronic obstructive pulmonary disease (181). Because these products contained multiple ingredients, the exact source of the derived benefit is unclear. However, the findings are intriguing and suggest a possible role for whey-based supplemental therapies in asthma. Further studies are needed.

3. Sulfur amino acids. Cysteine is a vital component of GSH. While cysteine is primarily produced from metabolism of the essential sulfur amino acid, methionine, it can also exist independently. Cysteine is released from the breakdown of endogenous proteins (100, 202, 264) and is also ingested from the diet along with methionine. Thus, although methionine serves as the primary source of sulfur for cysteine, dietary cysteine requirements are calculated with the assumption that methionine cannot supply the entire bodily cysteine demand. For healthy adults, the recommended dietary allowance for methionine plus cysteine is 19 mg/kg per day (1140 mg/day for a 60 kg adult) (93). Animal proteins are the richest dietary source (Table 2) (322). These sulfur amino acid requirements are based on classical experiments focused on the minimal intake of methionine for nitrogen balance (68, 281, 355). While total daily sulfur amino acid intake requirements are higher in infants and young children (67, 220), the requirements for older children are similar to adults (321).

Although sulfur amino demands are greater in critically ill subjects or subjects with metabolic stress or cancer-related cachexia (325), whether these demands are similar in subjects with chronic diseases such as asthma is not clear. With low sulfur amino acid intake, cellular methionine is conserved and cysteine stores are utilized to maintain protein synthesis (121, 281). This accumulation of methionine is pro-inflammatory and is associated with inactivation of proteins by homocysteinylation, which is harmful to cardiovascular health (216, 290). Furthermore, in cases of severe protein-energy malnutrition, the reduced sulfur amino acid intake results in decreased GSH synthesis and lower circulating GSH concentrations from decreased cysteine bioavailability (274), which may have detrimental immunomodulatory effects (121). By contrast, consumption of the recommended intake of dietary protein maintains circulating GSH and cysteine pools (147).

Whether the current recommendations for dietary sulfur amino acid intake are sufficient for chronic inflammatory diseases such as asthma is not entirely clear. It is possible that the supply of cysteine from recommended intakes is not sufficient for the maintenance of GSH concentrations and GSH/GSSG redox balance, particularly in cases of severe asthma associated with significant GSH depletion (86, 87). In these individuals and possibly other healthy adults such as athletes with higher protein demands, total sulfur amino acid intakes of up to 25 mg/kg/day may be necessary to maintain nitrogen balance (308, 309, 356). However, excessive intakes should be avoided since high dietary intakes of methionine can also raise plasma homocysteine concentrations (69, 327, 336) and alter immune function (121, 325).

Surprisingly few studies have focused on the dietary composition of subjects with asthma. Given that GSH levels are low in asthma, particularly in cases of severe disease, insufficient methionine intake or alterations of sulfur amino acid metabolism could be responsible. According to the most recent Youth Risk Behavior Surveillance Report (72), 78% of high school students (including students with asthma) had not eaten fruits or vegetables five or more times per day and 82% were not physically active. Furthermore, 48% of children

Table 2. Selected Dietary Sources of Sulfur Amino Acids (Methionine and Cysteine)

Food	*Serving*	*Protein (g)*	*Methionine and Cysteine (mg)*[a]
Tomato	1 medium (123 g)	1.1	18
Banana	1 medium (118 g)	1.3	20
Orange	1 medium (131 g)	1.2	39
Carrot	1 small (50 g)	0.5	52
Kale, cooked, shredded	1 cup (130 g)	2.5	56
Broccoli, cooked, chopped	1 cup (156 g)	3.6	88
Baked potato	1 medium (173 g)	4.3	121
Bread, whole wheat	2 slices (56 g)	7.3	130
Kidney beans, cooked	½ cup (89 g)	7.7	198
Peanuts, dry roasted	1/3 cup (48 g)	11.4	286
Oatmeal, boiled	1 cup (234 g)	5.9	335
Egg, hard boiled	1 large (50 g)	6.3	342
Milk, 2% fat	1 cup (244 g)	8.1	464
Chicken, roasted	1 leg (52 g)	14.1	562
Ground beef, 15% fat, pan-broiled	3 ounces (85 g)	20.9	745
Tuna	3 ounces (85 g)	21.7	874

[a]The recommended dietary allowance is 19 mg/kg/day for adults (93). Data were obtained from reference (322).

10 to 12 years of age had salty snack consumption at least one time per week that was primarily associated with television or video game viewing (13). In these children, consumption of salty snacks more than three times per week was associated with a nearly fivefold increased likelihood of asthma symptoms and increased sedentary behavior (13). By contrast, children more adherent to a Mediterranean-style diet characterized by a high intake of fruit, vegetables, and lean proteins had a decreased likelihood of asthma symptoms (13). The benefits of the Mediterranean diet in asthma have also been supported in other large cross-sectional studies spanning several countries (102, 239, 326) and in a recent meta-analysis focused on asthma prevention (242). While it is difficult to determine whether single or multiple nutrients are responsible for these results, a recent study observed fewer asthma symptoms in children with increased fish and meat intake (12), suggesting that dietary sulfur amino acids may play an important role. Studies focused on the role dietary sulfur amino acids in asthma are ultimately needed.

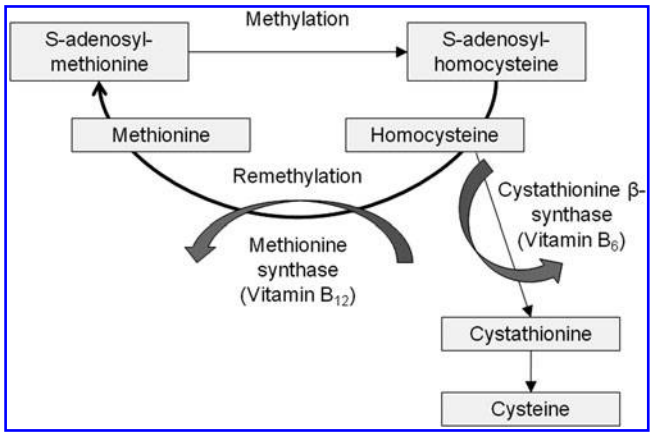

FIG. 15. Role of vitamin B_6 and vitamin B_{12} in maintaining homeocysteine balance and cysteine availability. Vitamin B_6 is essential for the conversion of homocysteine to cystathionine. Vitamin B_{12} likewise functions as a cofactor for the re-methylation of homocysteine to methionine.

4. B vitamins. Of the B vitamins, vitamin B_2 (riboflavin), B_6, and B_{12} are relevant to GSH synthesis and function. Riboflavin is an essential cofactor of flavin adenine dinucleotide (FAD), which carries high-energy electrons for oxidative phosphorylation. The glutathione reductase enzyme, which is necessary for reduction of GSSG to GSH, forms a FAD-bound heterodimer. Glutathione reductase functions through reduction of FAD by NAPDH. Thus, glutathione reductase activity reflected by the erythrocyte glutathione reductase activity coefficient is frequently used as a measure of riboflavin status (135, 287).

Although riboflavin deficiency is rare in Western societies, riboflavin status may be suboptimal in certain populations, such as the elderly (38, 104). Riboflavin supplementation improves riboflavin status in these individuals and others with marginal deficiency (104, 141, 259). While the functional relevance of suboptimal riboflavin status in otherwise healthy individuals is not clear, *in vitro* studies have shown increased protein carbonylation, DNA strand breaks, and cellular stress and apoptosis with riboflavin deficiency associated with little to no glutathione reductase activity (211, 340). These effects are similar to findings observed in subjects with severe asthma and GSH depletion (87). Whether riboflavin status is similarly altered in subjects with asthma is not clear.

Vitamin B_6 and vitamin B_{12} intake may also be relevant to GSH/GSSG redox balance, particularly in terms of methionine metabolism and cysteine synthesis. Vitamin B_6 functions as a cofactor for cystathionine β-synthase and is therefore essential for the conversion of homocysteine to cystathionine. Vitamin B_{12} likewise functions as a cofactor for methionine synthase (also referred to as 5-methyltetrahydrofolate-homocysteine methyltransferase), which is essential for re-methylation of homocysteine to methionine (Fig. 15). Deficiencies of either vitamin B6 or vitamin B12 result in altered homocysteine metabolism and increased homocysteine concentrations (136, 323), which are detrimental to cardiovascular health (2, 216, 331).

Vitamin B_6 and vitamin B_{12} have not been well studied in asthma. Although vitamin B_{12} levels can vary by genotype, in a recent population-based study, neither vitamin B_{12} serum levels nor genotype was associated with lung function or asthma (318). However, folate deficiency was associated with an increased prevalence of physician-diagnosed asthma and asthma symptoms, suggesting a possible role of B vitamins in the disorder (318). In keeping with this notion, older studies of children with asthma have observed alternations in tryptophan metabolism that were reversed by vitamin B_6 supplementation (50, 51). While one study found no evidence of obvious vitamin B_6 deficiency in asthmatic children (125), others have shown decreased plasma and erythrocyte vitamin B_6 levels in asthmatics *versus* controls that were not entirely normalized with vitamin B_6 supplementation (65, 277). However, the subjects in these studies were likely treated with the bronchodilator, theophylline, which is a vitamin B_6 antagonist and results in decreased circulating vitamin B_6 levels in treated subjects (294, 324). Because theophylline is no longer widely used or recommended for the treatment of asthma given its potential for toxicity, additional studies in asthma are needed. In a more recent study of otherwise healthy volunteers, administration of a cereal fortified with recommended intakes of folic acid, vitamin B_6, and vitamin B_{12} led to significant increases in plasma vitamin B_6 and vitamin B_{12} concentrations and beneficial reductions in homocysteine (320). These findings suggest that vitamin B_6 and vitamin B_{12} status may have important implications for inflammation and health even in absence of obvious deficiency.

5. Glutamine and glycine. Like cysteine, glycine and glutamate are also essential for GSH synthesis and ultimately GSH/GSSG redox balance. While few studies have examined glycine and glutamate in subjects with asthma, a case–control study of adult subjects 18–65 years of age found significantly lower concentrations of glycine in the plasma of asthmatics *versus* controls (92). Furthermore, the odds of asthma diagnosis were significantly reduced for subjects with the highest plasma glycine concentrations (odds ratio 0.30) (92). A trend toward asthma protection was also noted for subjects with higher concentrations of glutamine (odds ratio 0.44), which is synthesized from glutamate and ammonia (92). These observations were supported by a separate study in children, which also found significantly lower plasma concentrations of glycine and glutamate in mild asthmatics than in healthy controls

(284). These findings may have important clinical ramifications. *In vitro* studies have shown that glycine inhibits production of superoxide and tumor necrosis alpha from alveolar macrophages (341) and decreases the contraction of airway smooth muscle (354). Other studies have also demonstrated inhibition of Th2-mediated pro-inflammatory cytokine expression and airway hyperresponsiveness in murine models of allergic asthma induced by ovalbumin sensitization (174, 182). Taken together, these findings suggest a beneficial role of glutamate and glycine in asthma and further study of these amino acids is warranted.

X. Clinical Implications and Future Directions

Despite advances in the understanding and treatment of asthma over the past decade, asthma remains a challenging disorder associated with a significant degree of respiratory morbidity. While the specific mechanisms underlying asthma have yet to be identified, there is accumulating evidence that oxidative stress from altered GSH/GSSG redox status may play an important role in the modulation and severity of the disorder. In more severe patients in whom airway GSH reserves are depleted, this may result in a detrimental cascade with a shift toward GSSG formation and oxidation of thioredoxins, leading to post-translational protein modifications through oxidation of protein-methionine residues and formation of protein disulfides from glutathionylation or other mechanisms (Fig. 16). Ultimately, these disturbances may render the functional activity of other glutathione-related enzymes insufficient and increase susceptibility to oxidant-induced airway injury. However, although GSH plays an essential role of GSH in redox signaling, detoxification, and other vital cellular processes, the functional and clinical ramifications of GSH/GSSG redox alterations in subjects with asthma are not clear and additional studies are clearly warranted in this population. There is a particular need for human studies focused on the following: (i) validation of airway and systemic GSH/GSSG redox balance as a biomarker of asthma susceptibility, (ii) the natural history of GSH/GSSG redox balance in asthma across the lifespan, (iii) the specific role of GSH/GSSG redox balance in clinical manifestations of the disorder, including asthma severity and the response to secondary insults, (iv) the utility of GSH/GSSG redox balance as a predictor and indictor of asthma treatment responsiveness, and (v) therapeutic approaches to restore the GSH/GSSG redox balance in asthma and modify the course of the disorder. The latter may be particularly relevant in subpopulations of asthmatics given the marked clinical heterogeneity of the disorder.

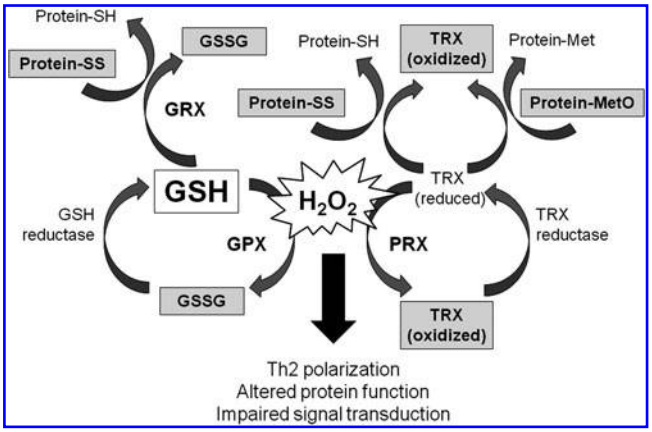

FIG. 16. Molecular modulation of airway thiol redox balance and possible role in asthma. In subjects with asthma, decreased airway-reduced GSH reserves may result in a detrimental cascade whereby concentrations of GSSG and oxidized thioredoxin (TRX) are increased and significant post-translational modification of proteins occurs (with excessive oxidation of protein-methionine residues [protein-MetO] and protein disulfide [protein-SS] formation from glutathionylation or other mechanisms). These effects may ultimately overwhelm the functional activities of GPX, glutathione reductases, peroxiredoxins (PRX), thioredoxin reductases, and glutaredoxins (GRX), resulting in insufficient oxidant defenses and characteristic features of asthma.

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Abbreviations Used

BAL = bronchoalveolar lavage
CySS = cystine
DNTB = 5,5′-dithiobis-(2-nitrobenzoic acid)
ELF = epithelial lining fluid
FAD = flavin adenine dinucleotide
GPX = glutathione peroxidases
GR = glutathione reductase
GRX = glutaredoxins
GSH = glutathione, reduced (thiol) form
GSNO = nitrosoglutathione
GSSG = glutathione disulfide
GST = glutathione-S-transferase
H_2O_2 = hydrogen peroxide
HPLC = high-performance liquid chromatography
IL = interleukin
MetO = methionine sulfoxide
MMA = mild-to-moderate asthma
NF-κB = nuclear factor kappa B
$^{\bullet}NO$ = nitric oxide radical
NO_2^- = nitrite
NO_3^- = nitrate
Nrf2 = nuclear factor (erythroid-derived 2)-like 2
$O^{\bullet}{}_2^-$ = superoxide anion
ONOOH = peroxynitrous acid
OONO- = peroxynitrite
PRX = peroxiredoxins
RANTES = regulated on activation, normal T-cell expressed and secreted
RNS = reactive nitrogen species
ROS = reactive oxygen species
SA = severe asthma
-SeH = selenocysteine (selenol) residue
SeOH = selenenic acid
SeSG = selenenyl sulfide adduct
-SG = reactive glutathione
-SH = sulfhydryl
SOD = superoxide dismutase
TGFβ1 = transforming growth factor beta-1
Th2 = T helper type 2
TRX = thioredoxin

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