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Forum Review

Role of the Nrf2-Mediated Signaling Pathway as a Negative Regulator of Inflammation: Implications for the Impact of Particulate Pollutants on Asthma

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ABSTRACT

Particulate matter (PM) is an environmental factor that may contribute to the exacerbation and possibly the development of asthma. PM contain redox-active chemicals and transition metals which generate reactive oxygen species (ROS). Excessive ROS can induce oxidative stress, which proceeds in hierarchical fashion to generate cellular responses. The most sensitive cellular response to mild oxidative stress is the activation of antioxidant and phase II enzymes (tier 1). If this protection fails, further increase of oxidative stress can induce inflammation (tier 2) and cell death (tier 3). Tier 1 antioxidant defenses are critical for protecting against airway inflammation and asthma. The expression of these antioxidant enzymes is regulated by the transcription factor, Nrf2. In response to oxidative stress, Nrf2 escapes from Keap1-mediated proteasomal degradation resulting in prolonged protein half-life and its nuclear accumulation. Nrf2 interacts with the antioxidant response element (ARE) in the promoters of phase II enzyme genes, leading to their transcriptional activation. Several phase II expression polymorphisms are associated with an increased risk of asthma. The indispensable role of Nrf2 in tier-1 oxidative stress response suggests that polymorphisms of Nrf2-regulated genes may be useful susceptibility markers for asthma. Moreover, chemopreventive Nrf2 inducers may be used for treating PM-exacerbated asthma. *Antioxid. Redox Signal.* 8, 88–98.

INTRODUCTION

ASTHMA IS A COMPLEX genetic disorder characterized by chronic airway inflammation (14, 19). Although the disease pathogenesis is still poorly understood, it is clear that allergen presentation by antigen presenting cells (APC) is the first important step in the development of atopic asthma (63). While the involvement of dendritic cells, T-helper 2 (Th2) lymphocytes, IgE-secreting plasma cells, mast cells, eosinophils, neutrophils, as well as proinflammatory cytokines and chemokines is well recognized, it is less appreciated that asthma is also a disease of oxidative stress (19, 68). Oxidative stress can be an afferent as well as an efferent component of airway inflammation (68). Genetic variations in the pathways that regulate oxidative stress in the lung may constitute a new avenue of understanding why only some people with an allergy go on to develop airway inflammation and asthma (68).

Among the many environmental factors that can induce asthma exacerbation, exposure to air pollutants is attracting greater attention. Particulate matter (PM) is considered a major air pollutant that can contribute to asthma exacerbation and possibly also disease prevalence (26, 80, 83, 88, 102). A number of epidemiological studies have demonstrated an association between ambient PM levels and increased prevalence of asthma (26, 80, 83, 86, 88, 102). How exactly these particulate pollutants lead to asthma is uncertain, but PM contain organic chemicals that are capable of participating in redox cycling reactions that generate ROS and oxidative stress in the

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lung (7, 10, 33, 34, 42, 43, 59, 65–68, 112). One of the targets in the lung are cells from the immune system, as demonstrated by the fact that diesel exhaust particles (DEP) act as an adjuvant for IgE production and allergic inflammation in response to exposure to common environmental allergens. Redox homeostasis in immune cells is important for a wide range of immune functions ranging from APC maturation, antigen presentation, to cytokine production. Oxidative stress interferes with the maturation and antigen presenting capability of APC such as dendritic cells (DC) (55, 92). Reduced glutathione (GSH) levels have been suggested as an important factor that determines T-helper 1 (Th1) versus T-helper-2 (Th2) response patterns (78, 79, 87).

To counteract the deleterious effects of oxidative stress generated by electrophilic and redox cycling chemicals, which are the major PM components, cells have developed an elaborate defense to maintain redox homeostasis (68). This system includes a series of phase II and antioxidant enzymes that metabolize redox cycling chemicals, eliminate excessive ROS, and protect cells against the damaging effects of electrophiles and oxidants (18, 20, 40, 65, 68, 70, 75, 112). Decreased expression or altered function of these protective enzymes (e.g., due to genetic polymorphisms) may render individuals more prone to PM-induced disease processes such as asthma. Several recent studies demonstrate that genetic polymorphisms of the genes that encode for phase II enzymes can affect asthma susceptibility, including occupational asthma that is induced by toluene diisocyanate (38, 73, 100, 113).

The coordinated response of these phase II and antioxidant enzymes are regulated through a *cis* element located in the promoter of their genes; this element is known as antioxidant response element (ARE) or electrophile response element (68, 70, 90). Activation of ARE is regulated by the bZIP transcription factor, Nrf2 (1). The importance of Nrf2 is illustrated by the failure to induce antioxidant and phase II enzyme expression in Nrf2 knock-out mice rendering these animals more susceptible to oxidative stress (8, 16, 17, 20, 32).

In this communication, we will outline the role of antioxidant defense pathways in asthma with particular reference to the role of Nrf2. We will discuss the regulation of antioxidant and phase II enzymes by Nrf2 in response to PM-induced oxidative stress. We will also discuss the importance of Nrf2 in protecting against pulmonary inflammation. Finally we will explore the possibility of using polymorphisms in Nrf2-regulated genes as susceptibility markers for PM-related asthma.

GENERATION OF OXIDATIVE STRESS BY REDOX ACTIVE PM CHEMICALS

In recent years, studies on the adverse biological effects of PM have focused on the components of PM important for the biological outcome. Using DEP as a model pollutant, it has been established that the organic chemicals capable of undergoing redox cycling and generating oxidative stress play a major role in the biological effects of PM (7, 42, 59, 65, 66). DEP as well as ambient PM contain a carbon core, which is coated by hundreds of organic chemicals and transition metals (44, 97). As a first step in characterizing the mechanisms by which organic DEP constituents exert their effects, a dichloromethane extract of DEP was fractionated by silica gel chromatography (65). Using elution solvents of increasing polarity, the crude DEP extract was separated into three fractions, namely aliphatic, aromatic, and polar (65). Whereas the aliphatic fraction had little effect, aromatic and polar fractions were capable of creating a pro-oxidant state within cells by altering the ratio of reduced to oxidized glutathione (GSH/GSSG) (65, 67, 68). Chemical analyses revealed that the aromatic fraction was enriched with PAH, while the polar fraction contained a large amount of quinones (65, 70). Many PAHs can be metabolized to redox active quinones by cytochrome P450 1A1, expoxide hydrolase, and dihydrodiol dehydrogenase (84). Quinones play important roles in PM toxicity due to their ability to undergo redox cycling, often in the presence of transition metals (69, 75). Redox active quinones are metabolized to semi-quinones via one-electron reductions catalyzed by NADPH cytochrome P450 reductase (69, 75). In the process of being converted to the original quinones, the semiquinones produce superoxide ($O_2^{\bullet -}$), which can proceed to generate further ROS. Consistent with this reaction, addition of an organic DEP extract to mouse lung microsomes induced $O_2^{\bullet -}$ formation in a cytochrome P450-dependent manner (59). In addition to the quinones actually deposited on the PM, several quinones are generated from PAH by enzymatic conversion intracellularly (68). The possibility that PAH may participate in the generation of oxidative stress was demonstrated by the correlation between PAH content of ambient PM and the induction of a tier 1 (HO-1 expression) response in macrophages (69). Ambient ultrafine particles (UFP), which have the largest surface area and highest PAH content, are much more potent in their ability to generate ROS and activate tier 1 responses compared to coarse and fine PM (69).

Taken together, one of the current major hypotheses for the role of DEP and ambient PM in inducing oxidative stress and inflammation is that these particles contain redox cycling compounds such as PAH and quinones (59, 68).

THE CELLULAR RESPONSE TO PM INVOLVES A HIERARCHICAL OXIDATIVE STRESS RESPONSE

Maintenance of intracellular redox homeostasis is essential to cellular function and survival. Oxidative stress is generated when ROS production overwhelms the antioxidant defense (68). Oxidative stress can elicit a variety of cellular responses ranging from protective to injurious, depending on the level of oxidative stress. Using DEP as a model pollutant, we have demonstrated that incremental PM doses induce a hierarchical oxidative stress response (Fig. 1) (4, 112). Mild oxidative stress (tier 1) generated by small quantities of PM induces the expression of a battery of phase II enzymes that detoxify ROS and electrophilic chemicals. These include HO-1, NAD(P)H-quinone oxidoreductase1 (NQO1), glutathione-S-transferase (GST), glucuronosyltransferase-1a6 (UGT1a6), γ-glutamate cysteine ligase regulatory subunit (γ-GCS), superoxide dismutase (SOD), and glutathione peroxidase (GPx) (70). The activation of these cytoprotective enzymes is the

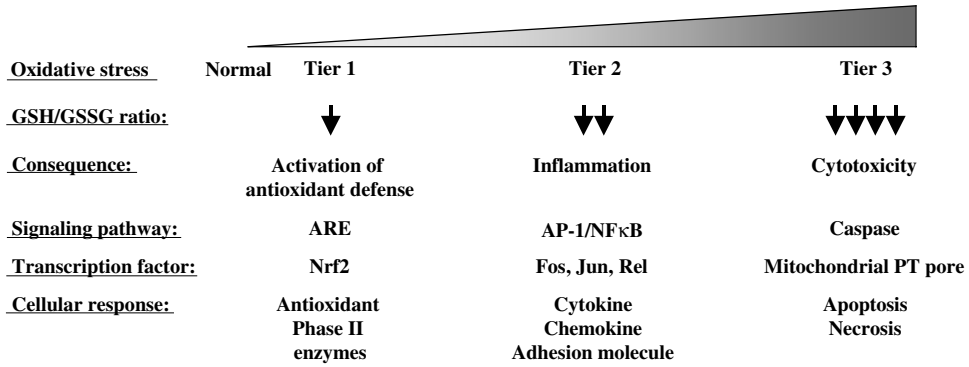

FIG. 1. PM-induced hierarchical oxidative stress model and three tiers of cellular responses. Three tiers of oxidative stress induced by PM are defined by the ratio of intracellular GSH/GSSG ratio. Mild oxidative stress (tier 1) activates antioxidant and phase II enzymes via ARE/Nrf2 signaling pathway. Intermediate level of oxidative stress (tier 2) elicits cellular pro-inflammatory responses through the activation of AP-1 and NFκB. Severe oxidative stress (tier 3) depletes cellular GSH, perturbs mitochondrial PT pore, and leads to cell death.

most sensitive cellular response to oxidative stress. The failure of these antioxidant and detoxification mechanisms to correct the redox disequilibrium could lead to the escalation of oxidative stress to tier 2, which has more damaging effects. Tier 2 cellular responses are characterized by the activation of cellular signaling cascades such as MAPK and NFκB, leading to the transcriptional activation of cytokines, chemokines, and adhesion molecules that can contribute to airway inflammation (81, 82, 106). These pro-inflammatory effects could be involved in the adjuvant effects of PM, including the promotion of Th2 responsiveness (68, 80). This effect may be the result of oxidative stress altering APC function, such that the APC characteristics favor Th2 skewing during interaction with T-helper lymphocytes (80). The third tier of oxidative stress involves cytotoxic effects and mitochondrial damage that may result in the shedding of bronchial epithelial cells and contribute to asthma exacerbation following a sudden rise in ambient PM levels (36, 103, 104).

Nrf2 AS A KEY TRANSCRIPTION FACTOR IN PM-INDUCED TIER 1 OXIDATIVE STRESS RESPONSES

The coordinated response of antioxidant and phase II enzymes is regulated through a *cis* element, ARE, located in the promoter of their genes (68, 70, 90). ARE is required for the induction of these enzymes in response to oxidative and electrophilic stress (1, 65). Activation of the ARE is regulated primarily by the bZIP transcription factor, Nrf2, in association with transcription factors from Maf and AP-1 families (1, 50, 52, 62, 76, 100). Nrf2 is crucial to the induction of ARE-mediated cytoprotective enzymes. Not only does Nrf2 deficiency fail to induce phase II and antioxidant enzyme expression, but it also renders animals more susceptible to oxidative stress (5, 16, 17, 32, 91).

Nrf2 is a member of basic leucine zipper (bZIP) protein family. While its C-terminus is responsible for DNA binding, its N-terminus is responsible for transcriptional activation (1). Nuclear access of Nrf2 is regulated, in part, by a cytoplasmic protein, Keap1 (1, 6, 50, 52, 61, 62, 74, 76, 91, 98, 101). Under basal conditions, Nrf2 is sequestered in the cytoplasm through the direct interaction between its Neh2 domain and the Kelch domain located at the C-terminal of Keap1 (49, 74). This association between Nrf2 and Keap1 represses nuclear translocation and transcriptional activity of Nrf2 (6, 50, 52, 62, 74, 76). In addition to sequestering Nrf2 in the cytosol, Keap1 also regulates the proteasomal degradation of Nrf2 (6, 74, 98, 101). Although the exact mechanism by which Keap1 targets Nrf2 for ubiquitin-mediated proteasome degradation is still unclear, very recent studies have suggested that Keap1 interacts with Cullin3 (Cul3) and Rbx1 to form a Cul3-based ubiquitin ligase complex that target Nrf2 for ubiquitin conjugation both in vivo and in vitro (23, 37, 58, 118). In this case the BTB domain of Keap1 binds to Cul3, while its Kelch domain binds to Nrf2 (67). Upon cellular exposure to oxidants, Nrf2 escapes from Keap1 repression, accumulates in the nucleus, heterodimerizes with small Maf as well as AP-1 proteins such as JunD, and activates ARE (1, 50, 52, 62, 76).

Although it is widely accepted that the dissociation of Nrf2 from Keap1 and its nuclear translocation is required for transcriptional activation of phase II and antioxidant enzyme genes, the mechanism by which electrophilic chemicals and oxidative stress regulate Nrf2 dissociation from Keap1 remains poorly understood. While several hypotheses have been proposed to explain the escape of Nrf2 from Keap1 (9, 46, 52, 61), the most likely hypothesis is that electrophilic chemicals associated with DEP and ambient PM covalently modify one or more sulfhydryl groups in Keap1 (117). Keap1 is a thiol-rich protein with 25 cysteines among its 624 amino acids (76). These sulfhydryl groups in Keap1 are sensitive to oxidative stress. Dinkova-Kostova and co-workers demonstrated that the ability of the structurally diverse chemicals to activate Nrf2-mediated gene expression correlates with their thiol reactivity (28, 29). These phase II enzyme inducers covalently react with cysteine thiol groups on Keap1 at rates closely related to their interactions with C251, C273, C288, and C297 as the most reactive residues (28, 29). As a result of this covalent modification, inducers disrupt cytoplasmic Keap1/Nrf2 complexes,

leading to Nrf2 release to the nucleus, and increase ARE-mediated gene transcription (29). This may explain the effect of DEP chemicals on the activation of Nrf2-regulated antioxidant and phase II enzymes.

DEP and ambient PM contain a large number of organic chemicals including PAH and quinones, which are antioxidant and phase II enzyme inducers (68). A number of PAHs can be metabolized to redox-active quinones at cellular level. It is possible that these PAH-derived quinones along with the quinones originally present on the PM, covalently interact with the Keap1 cysteine residues to allow Nrf2 release from Keap1 inhibition (28, 29, 64). Zhang *et al.* have identified two critical cysteine residues in Keap1, C273 and C288, which are required for Nrf2 ubiquitination (117). This is particularly important for Nrf2 protein accumulation since under homeostatic conditions Nrf2 ubiquitination is responsible for proteasomal degradation and a short half-life (≈ 15 min) of the protein (6, 70, 74, 98, 101). The importance of C273 and C288 was confirmed by a recent study from Wakabayashi *et al.* (110), which showed that modification of C273 and C288 by phase II enzyme inducers are critical for the dissociation of Nrf2 from Keap1. It is possible that oxidants and electrophiles in the aromatic and polar fractions of DEP and PM interfere with Nrf2 ubiquitination by covalent modification of these critical cysteines, leading to increased Nrf2 protein stability and nuclear accumulation (70). This was supported by the observation that aromatic and polar DEP chemicals increase Nrf2 protein stability in murine macrophages, while aliphatic materials of DEP had no effect on Nrf2 protein half-life (70). Use of the thiol antioxidant, NAC, provides further evidence of this model (70). NAC interferes with Nrf2 nuclear accumulation and inhibits DEP-induced HO-1 expression (70). NAC exerts its antioxidant activity through several actions. In addition to its direct ROS scavenging effect and acting as a precursor for GSH synthesis, NAC also directly binds to electrophilic compounds in DEP and PM (112, 70). The direct binding of NAC to PAH and quinones could prevent DEP from covalently modifying the key thiol groups in Keap1 (112, 70).

Although C273 and C288 are critical for Nrf2 protein stability, a third Keap1 cysteine residue in Keap1, C151, may be a target for electrophilic PM compounds (70, 117). Zhang *et al.* (117) suggested that C151 acts as a redox sensitive switch that is independently targeted by electrophilic compounds. It is also possible that electrophilic compounds and quinones in PM target this residue (70). Another study from the same group demonstrated that C151 is required for inhibition of Keap1-dependent Nrf2 ubiquitination by *t*-butylhydroquinone and sulforaphane (107). A single mutation of C151 to serine renders Keap1 molecule resistant to electrophilic chemicals (118). Although the above-mentioned mechanisms may explain PM-induced Nrf2 signaling, there is an additional mechanism that may contribute to Nrf2 accumulation in the nucleus. This observation is derived from the fact that the total cellular and nuclear Nrf2 abundance is increased in cells exposed to DEP chemicals. Thus, there has to be a mechanism that is responsible for this increased Nrf2 abundance, in addition to its release from Keap1. This suggests that there is also *de novo* synthesis of Nrf2 protein (6, 101). It is possible, therefore, that pro-oxidative PM chemicals may also prevent newly synthesized Nrf2 from associating with Keap1. In the absence of such association, Nrf2 will not be ubiquitinated and by default be directed to the nucleus (70). The observation made by Zhang and Hannink that there was minimal release of Nrf2 from Keap1 in response to electrophiles is consistent with this hypothesis (117).

ASTHMA AS A DISEASE OF OXIDATIVE STRESS IS FURTHER ENHANCED BY PM EXPOSURE

Asthma involves a number of cell types that are capable of ROS generation (68). This includes a role for macrophages, neutrophils, eosinophils, and epithelial cells (68). However, while ROS production may be the consequence of airway inflammation, a great body of evidence has also demonstrated that ROS-producing cells play an afferent role in generating pulmonary inflammation (2, 12, 13, 15, 68, 95). These findings are summarized in Table 1. $O_2^{\bullet -}$ generation has been demonstrated at sites of allergen challenge in the human lungs (47, 95). Moreover, oxidative damage to the airway epithelium produces airway hyperreactivity (AHR) in humans (47). This is further supported by the demonstration of increased peroxidation (94) and nitrotyrosine products in the lungs of asthmatic subjects (53). Increased peroxidation products, including 8-iso-PGF2α, can also be detected in the blood and urine of asthmatics (30). Neutrophils and mononuclear cells from asthmatic patients generate proportionately more $O_2^{\bullet -}$ and H_2O_2 than cells of matched healthy subjects; this activity correlates with metacholine-induced AHR (3, 4, 54, 71). There is also a positive correlation between increased CO in the sputum of asthmatics and elevated eosinophil counts (114, 116). The principal source of CO in the lung is heme oxygenase-1 (HO-1), a phase II enzyme that is induced by oxidative stress and plays a role in heme catabolism to Fe^{2+}, biliverdin, and CO (72). The final evidence for the importance of oxidative stress in asthma is altered antioxidant enzymes in the lung as well as a decrease in ascorbate and α-tocopherol levels in lung lining fluid of asthmatic (Table 1; 22, 56, 57, 72, 89).

TABLE 1. ASTHMA IS A DISEASE OF OXIDATIVE STRESS

Evidence for involvement of oxidative stress in asthma	*References*
$O_2^{\bullet -}$ generation at the site of allergen challenge	47, 95
Oxidative damage to airway epithelium produces airway hyperreactivity	47
Increased peroxidation and nitrotyrosine products in the lungs of asthmatics	30, 53, 94
Increased ROS in neutrophils and mononuclear cells in asthmatic patients	3, 4, 54, 71
Increased CO in the sputum of asthmatics correlates with elevated eosinophil counts	114, 116
Altered antioxidant enzymes and decreased nonenzymatic antioxidants in lungs of asthmatics	22, 56, 57, 89

PM leads to asthma exacerbation, as exemplified by an increase in asthma attacks following a sudden surge in ambient PM levels (22, 72, 85, 96, 109). In addition to this short-term effect, there is also an increased prevalence of asthma in polluted urban environments (86). A possible explanation for this long-term effect is the ability of PM to act as an adjuvant for allergic sensitization to common environmental allergens (25–27, 48, 77, 80, 93, 105, 108). This adjuvant effect of PM has been linked to the ability of these particles to generate oxidative stress, which in turn, can be ascribed to their content of redox cycling chemicals, organic chemicals, and transition metals as described before (26, 68, 80). This notion is supported by the observation that thiol antioxidants such as NAC and bucillamine are effective in inhibiting the adjuvant effect of DEP in an ovalbumin-induced animal asthma model (68, 111).

ROLE OF Nrf2-MEDIATED CYTOPROTECTIVE ENZYMES IN PREVENTING AIRWAY INFLAMMATION AND ASTHMA

From our prediction that the tier 1 response protects against cellular inflammation and cytotoxic damage, it follows that antioxidant and phase II enzymes protect inflamed airways against the pro-oxidative effects of electrophilic PM chemicals. Accordingly decreased activation of phase II enzymes may lead to increased susceptibility to pro-oxidative environmental chemicals in the exacerbation of airway inflammation and asthma (12, 21, 22, 24, 31, 35, 38, 41, 45, 51, 60, 73, 89, 99, 100, 107, 113).

Multiple antioxidant and phase II enzyme genes that are under the regulation of Nrf2 theoretically are able to protect against environmental electrophiles and oxidative stress (50, 52, 62, 76). In human airway epithelial cells, organic DEP compounds induce the expression of NQO1 and HO-1 (8, 67). In murine macrophages and bronchial epithelial cells, DEP chemicals induce the gene expression of HO-1, SOD3, NQO-1, GST-Ya, UGT1a6, and γ-GCS (39, 70). These Nrf2-ARE regulated enzymes could play an important role in preventing asthma by their abilities to detoxify PM chemicals, maintain cellular redox homeostasis, and prevent airway inflammation (20, 50, 52, 62, 68, 76). Nrf2-deficient mice exhibit substantially more sensitivity to oxidative stress by environmental chemicals. For example, $Nrf2^{-/-}$ mice have significantly reduced levels of antioxidant and phase II enzymes, rendering these animals more susceptible to the effects of hyperoxia, pro-oxidative chemicals, and drugs (5, 16, 17, 20, 32, 70, 91). Cho *et al.* reported that $Nrf2^{-/-}$ mice had significantly lower levels of NQO1, GST-Ya, UGT, GPx2, and HO-1 mRNA and were more susceptible to hyperoxic pulmonary injury (20). HO-1 expression in response to organic DEP chemicals was significantly reduced in $Nrf2^{-/-}$ peritoneal macrophages (70). Induction of HO-1 expression is important in protecting cells against DEP-induced apoptosis (65). Therefore impaired HO-1 expression due to Nrf2 deficiency renders cells prone to DEP-generated oxidative stress. In addition, studies by Aoki *et al.* demonstrated accelerated xenobiotic-DNA adduct formation in $Nrf2^{-/-}$ mice exposed to diesel exhaust (5).

Although Nrf2 *per se* has not been shown to be directly involved in asthma, Nrf2-mediated antioxidant and phase II enzymes play an important role in preventing airway inflammation and airway hyperreactivity through their ability to suppress inflammation. Growing evidence suggests that these Nrf2-regulated genes can serve as susceptible markers for asthma pathogenesis (35, 38, 41, 51, 60, 62, 68, 73, 100, 113). Several studies conducted in asthmatic patients have shown changes in antioxidant enzyme activity or expression (Table 2). For instance, SOD activity is generally reduced in the bronchial epithelial cells and lung lining fluid of asthmatics (6, 12, 21, 24, 99). GPx activity is decreased in pediatric asthmatics (89). Also, the level of CO in expired breath is increased in the expired breath of asthmatics (45). CO is derived from HO-1 activity in the lung, correlates with the extent of airway inflammation (45). In addition, alterations in phase II enzyme genes such as GST have also been linked to the susceptibility to asthma pathogenesis (35, 38, 73, 100). Individuals who are homozygous for the GSTM1 (null) genotype are at higher risk for asthma development or nasal allergic effects (38), whereas GSTP1 (Val) expression confers protection on toluene di-isocyanate-induced asthma (73). The HO-1 gene exhibit a number of polymorphisms that are based on poly-$(GT)_n$ repeats in its promoter (113). The number of -$(GT)_n$ repeats determines the inducibility of this gene. Japanese male smokers with a short poly-$(GT)_n$ polymorphism and a poorly inducible HO-1 gene have a statistically higher incidence of emphysema than smokers with a long poly-$(GT)_n$ repeat and a better inducible gene (113). Ragasamy *et al.* recently demonstrated that disruption of the Nrf2 gene in mice led to an earlier onset and extensive emphysema in response to cigarette smoke characterized by pronounced bronchial alveolar inflammation and enhanced expression of oxidative stress marker, 8-oxo-7,8-dihydro-2′-deoxyguanosine (91). Taken together, the evidence suggests that there is a possibility to use Nrf2-regulated antioxidant and phase II enzyme genes as susceptibility markers for asthma.

Table 2. Importance of Nrf2-Regulated Antioxidant and Phase II Enzymes in Asthma

Changes in antioxidant and phase II enzymes	*References*
Decreased SOD activity in lung cells or lung lining fluid in asthmatics	12, 21, 24, 99, 107
Decreased GSH and GPx activity in adults and children with asthma	89
Increased risk for asthma in individuals homozygous for GST M1 (null) polymorphism	35
Decreased susceptibility to asthma in individuals with homozygous expression of GST P1(Val)	38, 41, 51, 73
Increased risk for emphysema due to poor induction of HO-1 in individuals with short poly-$(GT)_n$ polymorphism	113

CONCLUDING REMARKS

The association between ambient PM and asthma has been clearly established. PM participate in asthma exacerbation by either exerting direct effects or by acting as an adjuvant. PM-associated redox-active chemicals induce three tiers of oxidative stress. At tier 1, low levels of oxidative stress activate antioxidant and phase II enzymes that provide protection against further oxidative damage. Failure to elicit tier 1 response will result in increased level of oxidative stress leading to inflammation (tier 2). Severe oxidative stress will lead to mitochondrial damage and cell death. A large number of antioxidant and phase II enzymes are regulated by the Nrf2-ARE signaling pathway. The central role of Nrf2 in providing protection against environmental electrophilic and oxidative stress has been clearly established. Impaired activation of Nrf2-mediated enzymes will lead to increased susceptibility to environmental oxidative stress and the development of airway inflammation and asthma. Therefore, the responsiveness of the Nrf2 signaling pathway is a major factor in determining susceptibility to PM-exacerbated asthma through the induction of antioxidant and phase II enzymes. In addition, by up-regulating

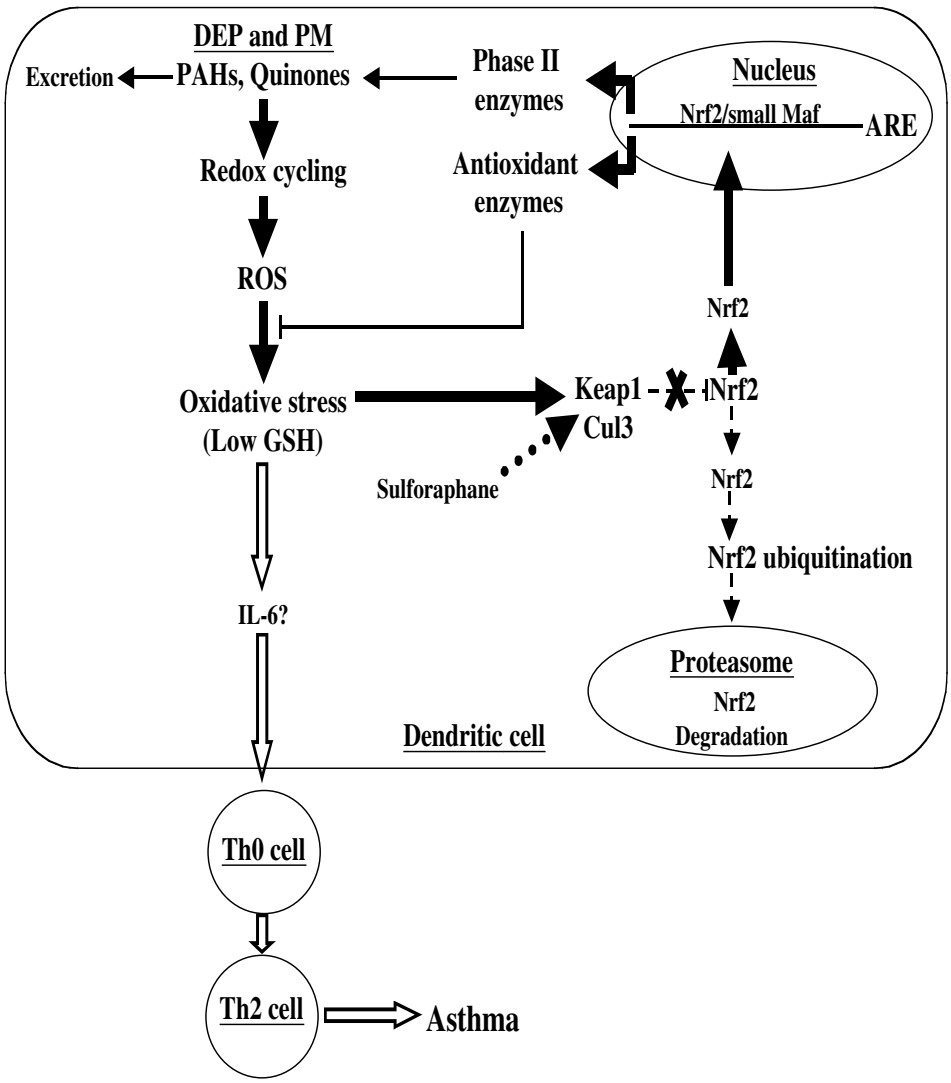

FIG. 2. Role of the Nrf2-mediated signaling pathway in PM-induced asthma development. Under basal conditions Nrf2 is constantly targeted for proteasomal degradation by its inhibitor, Keap1 (*broken arrows*). Organic PM chemicals such as PAH and quinones induce oxidative stress by undergoing redox cycling. Oxidative stress generated by PM modifies the thiol residues on Keap1 resulting in the release of Nrf2 from Keap1 inhibition and nuclear translocation. In the nucleus, Nrf2 heterodimerizes with small Maf and other transcription factors and binds to the ARE resulting in activation of a battery of antioxidant and phase II enzyme genes (thick black arrows). When the Nrf2/ARE-mediated defense pathway is functional, activated antioxidant and phase II enzyme will metabolize PM chemicals and remove excessive ROS (*fine black arrows*). However, if this defense mechanism fails, oxidative stress escalates to tier 2 cellular response leading to alterations in DC maturation and co-stimulatory activity, which polarizes Th0 cells towards Th2 differentiation (*white arrows*). Nrf2-inducers such as sulforaphane may be used as a chemopreventive agent for the treatment and prevention of asthma (*dotted arrow*).

antioxidant and phase II enzymes and restoring redox homeostasis in immune cells, the Nrf2 signaling pathway may act as a negative regulator for Th2 skewing and PM-exacerbated asthma (Fig. 2). We suggest two possibilities for exploiting the Nrf2-mediated signaling pathway for the treatment and prevention of PM-induced asthma development: 1) polymorphisms of Nrf2-regulated antioxidant and phase II enzyme genes in DC may be used as potential markers to determine PM susceptibility in asthmatics; and 2) use of Nrf2 inducers such as sulforaphane in asthmatics to prevent PM-induced asthma exacerbation.

ABBREVIATIONS

APC, antigen presenting cell; ARE, antioxidant response element; DC, dendritic cells; DEP, diesel exhaust particles; GSH, reduced glutathione; GSSG, oxidized glutathione; γ-GCS, γ-glutamate cysteine ligase regulatory subunit; GPx, glutathione peroxidase; GST, glutathione-S-transferase; HO-1, heme oxygenase-1; NQO1, NAD(P)H-quinone oxidoreductase1; Nrf2, NF-E2-related factor 2; PAH, polycyclic aromatic hydrocarbon; PKC, protein kinase C; PM, particulate matter; ROS, reactive oxygen species; SOD, superoxide dismutase; UFP, ultrafine particles; UGT1a6, UDP-glucuronosyltransferase-1a6.

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