Published in final edited form as:
Curr Opin Allergy Clin Immunol. 2012 April ; 12(2): 133–139. doi:10.1097/ACI.0b013e32835113d6.

The effect of environmental oxidative stress on airway inflammation

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Abstract

Purpose of review—Asthma is an inflammatory respiratory condition with significant associated morbidity and mortality that is increasing in prevalence. Air pollution is an important factor in both the development of asthma and in asthma exacerbations. Oxidative stress as a result of exposure to air pollution and underlying genetic polymorphisms that may play a role in susceptibility to this oxidative stress are the subject of current investigation.

This article reviews the data regarding the effects of air pollution on the innate immune response and potential clinical and treatment implications of how genetic polymorphisms affect this response.

Recent findings—Recent investigation reveals how pollutant-induced oxidative stress impacts airway inflammatory responses. Work by our study group demonstrates that asthmatic patients have an exaggerated inflammatory response to air pollution induced oxidative stress. New trials investigating antioxidants as potential therapeutic interventions may target this specific issue.

Summary—Air pollution plays a critical role in asthma and may affect certain patients more than others. Further investigation into the genetic polymorphisms that affect inflammatory responses may help target patient populations at greatest risk for air pollution induced asthma and may provide new therapeutic options for these patient populations.

Keywords

asthma; oxidative stress; ozone; LPS

Introduction

The increase in prevalence of asthma and other respiratory diseases contributes to patient morbidity and mortality as well as to increased cost in the form of medications, hospitalizations, health care utilization, and school/work absenteeism. While mechanisms behind this increase in prevalence are not fully elucidated, air pollutants generated by industrial progress have been implicated as an important etiologic consideration. Ozone,

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Conflicts of interest
The authors have declared that they have no conflicts of interest.

nitrogen dioxide, and particulate matter are air pollutants that are major constituents of smog and byproducts of fossil fuel combustion.

Ozone is one of the most commonly encountered pollutants, and epidemiologic studies have demonstrated that ozone is a trigger for asthma exacerbations in children, even at levels below the US Environmental Protection Agency (EPA) standards of 120ppb (1 hour average) and 75ppb (8 hour average)[1–4]. Silverman et. al found that among asthmatic children aged six to eighteen, there was a 20% increase in general hospitalizations and a 19% increased risk for ICU admissions for each 22ppb increase in ozone[5]. Particulate matter, such as that from diesel exhaust, is another important environmental exposure that leads to an increased risk for asthma development and exacerbation [6–9]. Endotoxin, also known as LPS, is a component of the outer membrane of gram negative bacteria and is derived from animals and agricultural activities. It has been identified on both large and small particulate matter particles, as well as in tobacco smoke. Exposure to LPS in early childhood has been associated with asthma exacerbations and increased prevalence of asthma [10,11]. Ryan et al. demonstrated that while exposure to traffic related particles increased the risk of wheeze alone, exposure to house dust LPS had a synergistic effect and increased this risk further [12]. A meta-analysis by Mendy et. al demonstrated that LPS exposure was positively correlated with wheeze in infants and toddlers [13].

The purpose of this review is to highlight recent research that has identified a). how oxidative stress responses evoked by pollutant exposure promotes airway inflammation, b). patient populations that are more susceptible to environmental oxidative stress, and c). effects of environmental air pollution.”

Effects of Air Pollution on Innate Immune Responses: Recent Findings with Ozone

The respiratory system is particularly susceptible to air pollution by nature of consistent exposure to the environment, with increased exposure with physical activity. Normally, protection of the host occurs via several pathways involving both the innate and adaptive immune system. Components of the innate immune system may be modified by ozone and LPS in populations with underlying respiratory disease and may contribute to adverse health effects[14] .

The cellular inflammatory response to air pollution in humans is the subject of current investigation and multiple studies have demonstrated that both ozone and LPS exposure augment the influx of neutrophils in the airway [15–17]. The role of neutrophils in airway symptoms, however, is less well defined, though the generation of reactive oxygen species is one possible mechanism by which neutrophils may play a role in airway induced hyper-reactivity[14].

Reactive oxygen species (ROS) have long been known to induce epithelial cell inflammation by directly causing tissue injury. Ozone challenge of epithelial cells results in NFκB activation and production of a variety of pro-inflammatory mediators such as IL-8, IL-6, PGE2, and LTC4 that recruit inflammatory cells [18–22]. Toll like receptors, first line effector molecules in innate immunity, are also believed to play a critical role in airway hyper-reactivity and inflammatory responses induced by air pollution [23,24] through epithelial cell release of substances into the airway lining fluid that activate resident airway macrophages [16,25–31].

A study by Williams et. al in 2007 demonstrated that TLR2 and TLR4 knock-out mice had a decrease in ozone induced airway hyper-reactivity [24]. We and others found similarities in

the airway inflammatory response induced by ozone and LPS [15]. Both ozone and LPS challenge augmented sputum neutrophilia and subjects' responses for each of these pollutants were significantly correlated with each other, suggesting one potential common mechanism to account for similarities in airway neutrophil responses by ozone and LPS.

Because LPS signals through toll-like receptor 4 (TLR4) on the cell surface on macrophages, this signaling pathway is a putative candidate to account for similarities between ozone and LPS airway inflammatory responses. Indeed, mechanistic studies in mice suggest that at least some ozone responses are mediated through TLR4 [32–34]. Toll-like receptors recognize pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) released by injured cells such as heat shock proteins, oxidized lipids, fibrinogen, and low molecular weight hyaluronan (HA) [35] (Figure 1). They are critical in initiating inflammatory responses to a variety of stimuli, characterized by production of pro-inflammatory mediators such as IL-1β, TNF-α, and IL-6.

Hyaluronic acid (HA), a glycosaminoglycan that is a component of the airway epithelial extracellular matrix, has recently emerged as a mediator of ozone-induced airway hyperreactivity & inflammatory responses [26,36]. High molecular weight HA is present on the apical surface of airway epithelial cells; its low molecular weight form is an endogenous ligand for TLR4 [27,31]. In airway epithelium, these low molecular weight fragments can be generated by ROS-induced depolymerization of hyaluronan [25,29], and by hyaluronidase activity associated with upregulation of TNF-α in concert with IL-1 β [30]. Garantziotis et. al demonstrated that TLR4 deficient mice are partially protected from airway hyperreactivity after ozone exposure and after instillation of hyaluronic acid, supporting the idea that TLR4 contributes to the development of ozone-induced airway hyperreactivity [26,36]. Various groups have shown that low molecular weight fragments of HA have pro-inflammatory actions [28]. In rodent models, Hollingsworth and colleagues showed that low molecular weight HA is increased in bronchoalveolar lavage fluid after ozone challenge, and that HA mediates inflammatory responses in the respiratory tract via CD44 [36] and TLR4 [26] signaling mechanisms. We recently reported that normal volunteers, allergic nonasthmatics, and mild allergic asthmatics had increased HA in their respiratory tract lining fluid after ozone exposure [16].

The increased susceptibility of asthmatics to ozone-induced airway disease was previously thought to be secondary to exacerbation of allergic airway inflammation. Ozone has been found to enhance airway eosinophilia [37,38] and to increase the sensitivity to subsequent allergen exposure [39]. Recent evidence has emerged that exacerbations may also be secondary to augmented innate immune inflammatory responses in allergic asthmatics. In controlled chamber studies, both normal volunteers and allergic asthmatics suffer from increased neutrophilic airway inflammation [16,40]. However, in a study of allergic asthmatics and normal volunteers, our group found that only the allergic asthmatics had increased cell surface expression of TLR4 on mature macrophages from induced sputum after 400 ppb ozone exposure, and increased levels of the pro-inflammatory cytokines IL-1β, IL-6, and IL-8 in the respiratory tract lining fluid after ozone exposure [16]. We hypothesized that in addition to exacerbation of underlying allergic inflammation, asthmatics also have enhanced activation of innate immune inflammatory pathways. We recently found that despite similarities in neutrophil and macrophage proportions in induced sputum samples after ozone exposure, gene array profiles were distinctly different. Compared to normal volunteers, allergic asthmatics showed increased immune signaling involving the NFkB network [41], supporting the notion that asthmatics suffer from increased innate immune activation after ozone exposure. Future work will need to focus on mechanisms explaining this enhanced innate immune response in asthmatics.

Oxidative Stress Genes and Susceptibility to Air Pollution

As inhalation of air pollutants promotes the production of radical oxygen species, genetic polymorphisms that result in antioxidant deficiencies may result in increased airway inflammation and hyperreactivity in response to environmental agents. A number of intracellular antioxidant enzymes including NQO1, GSTM1, GSTP1, and HO-1 regulate cellular and mucosal oxidant stress [42–46]. These enzymes are regulated by the master transcription factor NRF2 which translocates to the nucleus after oxidative stress. Cells that encounter oxidative stress activate NRF2 binding to the Antioxidant Response Element (ARE), leading to the transcription of a broad range of antioxidant genes. This cellular response is designed to defend against the harmful effects of oxidative agents.

Polymorphisms of genes of glutathione S-transferases (GSTs) have been investigated because of the important role of these enzymes in antioxidant defense. The GSTM1, or glutathione-S-transferase Mu1, null genotype has been associated with increased response to environmental agents. Studies have demonstrated an increased risk of wheezing in children exposed to tobacco smoke during the perinatal period as well as an increased risk of acute exacerbation of asthma in response to ozone exposure in subjects with the null genotype [47]. Recent investigation by Dillon et. al revealed that subjects with the GSTM1 null genotype have an increased inflammatory response with elevated levels of IL-1β and TNFα in the sputum to inhaled LPS (at 20,000 endotoxin units) [48]. This level of LPS exposure models the amount that a worker would be exposed to during an 8 hour work shift at an animal farming operation and genotypic differences may thus be an important factor in explaining why some people tolerate this level of endotoxin exposure with fewer adverse effects than others.

GSTM1 and GSTP1 have also been found to modify the adjuvant effect of diesel exhaust particles on allergic inflammation. A study by Gilliland et. al in 2004 demonstrated that subjects with the null genotype for GSTM1 and GSTP1 codon 105 variants had enhanced nasal allergic responses in the presence of diesel exhaust particles [49]. Subjects with these genetic variants also demonstrate larger responses to allergens with secondhand tobacco smoke with increases in nasal-allergen specific IgE and histamine [50].

The GSTM1 genotype plays a role in inflammation following ozone exposure as well. Subjects with the GSTMI null genotype have increased neutrophil influx to the airway 24 hours following exposure to ozone at a level of 400 ppb [51]. A study by Wu et. al also demonstrated that GSTM1 is a risk factor for ozone-induced inflammatory responses, as knockdown of GSTM1 lead to enhanced IL-8 production from human bronchial epithelial cells exposed to ozone [52]. IL-8, which is a proinflammatory mediator known to be a potent neutrophil activator, is an important biomarker for ozone-induced airway inflammation.

Potential Therapeutic Interventions against Pollutant-Induced Inflammation

While specific agents targeting pollutant-induced asthma exacerbation are not currently clinically available, understanding the potential mechanisms for pollutant-exacerbated asthma allows investigation into potential therapeutic interventions. As discussed above, air pollutants exert inflammatory effects as well as inducing oxidative stress. For this reason, anti-inflammatory agents and antioxidants have the potential to decrease the effects of pollutants on airway epithelial cells.

While anti-inflammatory agents may help to mitigate the effect of ozone on decrements in lung function, this effect appears to be mediated by analgesic function as opposed to anti-inflammatory function [53–55]. Studies have demonstrated that ozone induced pain-related

symptoms may result in inhibition of maximal inspiration due to the stimulation of airway C fibers [56]. Prior work has demonstrated that non-steroidal anti-inflammatory agents such as ibuprofen and indomethacin inhibit the nociceptive effect of ozone on spirometry concomitantly with inhibition of cyclo-oxygenase products of arachidonic acid. This study by Hazucha et. al did not, however, demonstrate changes in neutrophilic inflammation with ibuprofen administration[54].

Other commonly utilized anti-inflammatory agents have been demonstrated to decrease airway hyperreactivity in response to pollutants. Sodium cromoglycate has been demonstrated to inhibit LPS-induced bronchial obstruction in asthmatic subjects who were pre-treated with this agent, suggesting a possible function in treating asthmatics exposed to LPS [57]. The utilization of corticosteroids for pollution-exacerbated asthma is less well elucidated. Inhaled corticosteroids blunted sputum neutrophilia in response to ozone exposure in asthmatics [58] and in normal volunteers [59], but did not prevent the functional airway response. Novel anti-inflammatory agents tailored specifically to the inflammatory pathways that mediate pollutant-induced effects are currently under investigation. Lazaar et. al recently described a selective CXCR2 antagonist that was able to inhibit CXCL1-induced CD11b expression on peripheral blood neutrophils with subsequent decrease in neutrophil activation and recruitment in ozone-induced airway neutrophilia, suggesting a potential role for this antagonist in neutrophil-predominant diseases[60].

In light of the effect of reactive oxygen species on airway inflammation and the effect of genetic polymorphisms in antioxidant enzymes, antioxidant interventions have become a popular therapeutic target. Different research teams have reported that α-tocopherol and vitamin C combination therapy was effective in mitigating the effect of ozone-induced lung function decrements in asthmatics [61–64], or in normal volunteers after they had undertaken an antioxidant depleted diet for 3 weeks to mimic a state of poor antioxidant nutritional status [63]. However, extracellular antioxidant supplementation had no effect on airway inflammatory cell recruitment after ozone [63]. Oral supplementation with sulforaphane, an antioxidant compound derived from specially bred broccoli, upregulates expression of NRF2-regulated Phase II enzymes (GSTM1, GSTP1, HO1, and NQO1). Application of sulforaphane induced phase II enzymes in B cells and effectively blocked diesel exhaust particle enhancement of IgE-production [65]. In primary bronchial epithelial cells exposed to diesel extract, sulforaphane pre-treatment inhibited pro-inflammatory cytokine production by diesel [66]. It has been shown in vivo that Phase II enzymes in nasal epithelial cells can be induced by 3 days of oral SFN supplementation [67]. Further work is required to determine which antioxidant interventions may be best suited for particular pollutant exposures, the best route of administration, and which populations may benefit the most from particular interventions.

Conclusion

Recent work has highlighted the innate immune inflammatory responses experienced in the airways using both murine and human models of pollutant exposures. Airway inflammation can be caused through oxidative damage to the airway epithelium, as well as through activation of resident airway cells that can then respond to substances released by damaged epithelial cells. Because antioxidant enzymes such as the well studied GSTM1 have been found to influence airway pollutant responses, the role of oxidative stress genes in conferring susceptibility to pollutant-induced inflammation has come to the forefront of research efforts in hopes of targeting susceptible populations.

In addition to studying the effect of these antioxidant genes in normal volunteers, studies are now focused on understanding the effect of antioxidant tone in populations that are

especially susceptible to pollutant-induced inflammation, such as asthmatics and those with COPD. Asthmatic children have been found to have altered glutathione homeostasis (i.e. reduced levels), thought to be secondary to excessive free radical formation that then leads to a state of increased oxidative stress with deranged redox signaling and control [68,69]. Children with severe asthma were found to have decreased NRF2 transcriptional activity in airway cells compared to mild-moderate asthmatic children [69]. It has also been shown that maternal antioxidant gene polymorphisms in NRF2, GSTM1, and GSTT1 can influence their children's risk of asthma and wheezing with a prenatal pharmacologic oxidative stress through acetaminophen consumption [70]. In addition, GSTPi has been found to be decreased in children with asthma, with GSTPi having a role in reducing oxidative stress through cysteine oxidation [71]. What is unknown at present is the interaction between the baseline oxidative stress experienced by asthmatics and their antioxidant response after an environmental pollutant exposure. Such work will delineate the most effective strategies to prevent and treat environmentally-induced asthma exacerbations.

Acknowledgments

Funding Sources: MLH is supported by NIH KL2RR025746. Although the research described in this article has been funded wholly or in part by the United States Environmental Protection Agency through cooperative agreement CR833463-01 with the Center for Environmental Medicine, Asthma, and Lung Biology at the University of North Carolina at Chapel Hill, it has not been subjected to the Agency's required peer and policy review, and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use

Abbreviations

LPS	endotoxin
ROS	reactive oxygen species
HA	hyaluronic acid
TLR4	toll-like receptor 4
ppb	part per billion
IL-8	interleukin-8
IL-6	interleukin-6
PGE2	prostaglandin E2
LTC4	leukotriene C4
PAMPs	pathogen associated molecular patterns
DAMPs	damage associated molecular patterns
NQO1	NAD(P)H dehydrogenase, quinone 1
GSTM1	glutathione S-transferase mu 1
GSTP1	Glutathione S-transferase P1
HO-1	heme oxygenase 1
NRF2	NF-E2-related factor 2

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Key points

- In addition to direct damage of airway epithelium, ozone-induced inflammation is mediated through TLR4 signaling pathways
- Asthmatics have increased innate immune inflammatory responses after a controlled ozone exposure compared to healthy individuals
- Oxidative Stress Genes have been implicated in conferring susceptibility to pollutant-induced airway inflammation
- Antioxidant interventions may show promise against pollutant-induced inflammation.

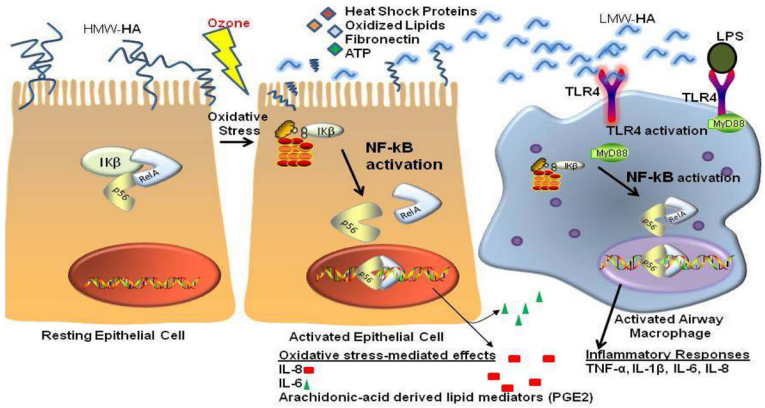

Figure 1. Innate Immune Activation after Pollutant Exposure

Left panels: Ozone activates resting airway epithelial cells via the transcription factor NFkB to produce a variety of pro-inflammatory mediators. It also damages epithelial cells, with release of damage associated molecular patterns (DAMPs) such as heat shock proteins, ATP, fibronectin, and oxidized lipids.

Right panel: Resident airway macrophages are activated through TLR4. TLR4 can be activated by airborne LPS found on particulate matter and tobacco smoke.

High molecular weight hyaluronic acid (HA) can be cleaved into low molecular weight forms by oxidative stress.

It is currently thought that epithelial cells can release DAMPs after experiencing oxidative stress, such as found with ozone exposure. These DAMPS can then activate TLR4 signaling pathways on airway macrophages.