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Genes of innate immunity and the biological response to inhaled ozone

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Abstract

Ambient ozone has a significant impact on human health. We have made considerable progress in understanding the fundamental mechanisms that regulate the biological response to ozone. It is increasingly clear that genes of innate immunity play a central role in both infectious and non-infectious lung disease. The biological response to ambient ozone provides a clinically relevant environmental exposure that allows us to better understand the role of innate immunity in non-infectious airways disease. In this brief review, we focus on: (1) specific cell types in the lung modified by ozone; (2) ozone and oxidative stress; (3) the relationship between genes of innate immunity and ozone; (4) the role of extracellular matrix in reactive airways disease; and (5) the effect of ozone on the adaptive immune system. We summarize recent advances in understanding the mechanisms that ozone contributes to environmental airways disease.

Keywords

ozone; oxidative stress; innate immunity; environment; surfactant; toll-like receptor; asthma; extracellular matrix; mindin; hyaluronan

HEALTH EFFECTS OF AMBIENT OZONE

Ozone is a reactive gas consisting of three oxygen atoms. Its highly oxidizing property defines ozone to be a harmful pollutant when existing in the lower atmosphere. Inhalation of ambient ozone is associated with adverse health consequences in vulnerable individuals and can lead to exacerbations of pre-existing respiratory diseases such as asthma and COPD. During days with high concentrations of ambient ozone, asthmatic patients have enhanced rates of emergency room visits and hospitalizations [1–2]. Adverse respiratory consequences are particularly prevalent in individuals participating in outdoor physical exercise[3], children [4–5], and elderly with predisposing cardiopulmonary diseases [6]. Typical ozone-induced pathophysiological manifestations in humans include immediate decrements in lung function, enhanced airways hyperreactivity, enhanced epithelial permeability, and inflammation [7]. Estimates support that for every 10 ppb increase in daily ozone there is an associated 1–4% increase in all-cause mortality [8–9]. However, evidence supports an ozone-induced biological response in human subjects to very low levels of ambient ozone,

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suggesting that adverse health consequences may exist at levels of ambient ozone below current regulatory standards[10–11]. Furthermore, ambient ozone levels are anticipated to rise with climate change [12] and rising surface temperatures are anticipated to enhance the biological consequences of ozone inhalation [13]. Based on predicted changes in climate by 2020, it is estimated that a 7.3% increase in ozone related asthma emergency room visits will occur in children [4]. Because of the ubiquitous exposure, the expected increase in ambient levels with climate change, and the clear health impact of ambient ozone, improved understanding of the molecular mechanisms that regulate the biological response to ozone are of high clinical significance.

MULTIPLE CELL-TYPES CONTRIBUTE TO THE RESPONSE TO OZONE

Inhalation of ambient ozone impacts numerous cell types in the lung and activates specific signaling cascades. Exposure to ozone elicits a variety of responses including: cellular damage, enhanced apoptosis, cytokine production, recruitment of inflammatory cells, and subsequent tissue repair. While many cells in the lung can be modified by inhaled ozone, the dominant cell types implicated in the acute response to ozone include: neutrophils, airway epithelia, and alveolar macrophages (Figure 1).

Neutrophils are frequently the first responder cells recruited into the sites of inflammation and tissue injury. Inhalation of ozone results in an early recruitment of neutrophils into the lung. Depending on the dose and duration of exposure, neutrophil inflammation is present 3–24 hours after inhalation [14–15]. Despite the presence of neutrophils in the lung after ozone, their functional impact in ozone-induced airways disease remains less clear. For example, airway hyperresponsiveness after inhalation to ozone has been demonstrated to be either dependent [16] or independent [17–18] of neutrophils. The reason for these discrepant effects of neutrophils on AHR may be due to the differences in experimental methodology. Independent of the role in AHR, recruited neutrophils can generate neutrophil elastase and matrix metalloproteinases that can contribute to airway injury [19]. Furthermore, exposure to ozone can impair neutrophil function that is associated with impaired pathogen clearance [20–21], a defect which may be related to apoptosis [22]. Together these observations support the notion that neutrophils are early responder cells that may contribute to ozone-induced lung injury and that exposure to ozone may impair the antibacterial function of neutrophils.

A critical aspect of host defense is dependent on intact barrier function, which in the lung is a principle function of epithelium. Airway epithelia are damaged after ozone inhalation resulting in loss of cilliary function [23], increased epithelial permeability [24–25], and defects in mucocilliary clearance [26]. In addition to barrier function, epithelia can also function as a central regulator of pulmonary immune responses [27–28]. After exposure to ozone, epithelia release cytokines and growth factors, such as interleukin 6 (IL-6), interleukin 8 (IL-8), and TNF-α that can result in recruitment of neutrophils and monocytes [29–32]. In addition, there is emerging evidence of cross-talk between epithelia and alveolar macrophages [33]. For example, airway epithelia-derived clara cell secretory protein (CCSP) regulate the response to ozone [34] and CCSP can attenuate macrophage-derived innate immune response [33]. Additionally, macrophages exposed to ozone can release IL-1α that further contributes to the epithelial-derived chemokine response [35]. Therefore, ozone exposure appears to cause a *bi-directional* communication between epithelia and alveolar macrophages. Comprehensive identification of molecules that serve as mediators between epithelia and alveolar macrophages in the biological response to ozone remains an area of considerable interest.

Lung macrophages are a dominant cell type in context of lung injury and repair. Macrophages contribute to the host response to ozone [36]. After inhalation of ozone, the number of macrophages in the lung is increased [37]. However, the regulatory role of macrophages remains incompletely elucidated. Challenges to understanding the role of macrophages in response to ozone are reflected in the fact that macrophages appear to have both pro-inflammatory and anti-inflammatory functions. Macrophage-derived mediators such as TNF-α, IL-1β, IL-6 and IL-8 contribute to the biological response to ozone and likely worsen injury and airway hyperresponsiveness [36, 38–40]. Despite the effect on injury and AHR, other macrophage functions include scavenger functions required for clearance of both apoptotic cells and oxidized lipids, which result in a reduction in inflammatory responses [41–42]. Given the divergent functions of macrophages, we reasoned that unique macrophage subsets were present in the lung with specific functions. Understanding macrophage heterogeneity and subpopulations in various organ systems has significantly expanded our understanding of this pleomorphic population of cells. One approach to understanding these divergent functions in the injured lung has been to understand the source of macrophages. Most macrophages are derived from monocytes in a manner either by recruitment from the blood in the setting of injury or by maturation from local intermediates. Much of this work in the lung has focused on the role of macrophages derived from monocytes in the setting of injury [43–44]. These inflammatory monocyte-derived macrophages have been termed exudate macrophages (ExMac) and are unique from resident alveolar macrophages by cell surface expression (ExMacs are CD11b+, whereas resident alveolar macrophages are CD11b−), morphology, and function [45–46]. We predicted that recruited ExMacs would be present in the lung after exposure to ozone and would account for the discrepancy in responses. To our surprise, ExMacs were not identified in the lung at 24hr after ozone exposure. Instead, we identified maturation of a lung resident macrophage, which were defined by specific cell surface markers by flow cytometry and unique gene expression from other lung macrophages. This population was dependent on CX3CR1 for its development. The loss of CX3CR1 was associated with worsened ozone induced AHR, cellular inflammation, cytokine production, and oxidant stress[47]. This work identified a unique macrophage subpopulation, which appears to protect the lung from ozone-induced pathology. Another approach to understanding divergent functions of macrophages has focused on understanding different phenotypes of macrophages which occur in the setting of injury and repair. This developed out of the observation that *in vitro* stimulation by different cytokines resulted in unique cellular programs. Classically activated macrophages (CAM) can be induced with stimulation by IFN-γ and LPS and are defined by expression of pro-inflammatory mediators such as IL-12 and iNOS. Alternatively activated macrophages (AAM) can be induced by stimulation by IL-4 and IL-13 and are classified based on expression of mannose receptor, arginase-1, Ym1 and FIZZ1[48]. Though *in vitro* stimulation of cytokines on macrophages clearly identifies unique cellular programing, how this works *in vivo* appears to be more complex with combinations of both CAM and AAM programs being evident after injury in several model systems. Recent work by Sunil et al. identified that markers of both classical and alternative activation of macrophages were present after ozone exposure in rats[49]. What is not clear from the present body of literature is how the identification of CAM and AAM in the setting of ozone exposure impact on the pathobiology. Also what remains unclear is how these phenotypic identifications relate to the known subsets of macrophages in the lung (ie alveolar macrophages, ExMacs and interstitial macrophages). Clearly given the central role of macrophages in the host response to ozone further work understanding the function of macrophage subpopulations and phenotypes will provide novel insight into fundamental mechanisms that regulate lung injury and repair.

OZONE AND OXIDANT STRESS

Oxidative stress is a conserved mechanism that contributes to numerous environmental lung injuries. Ozone, as a principle mediator of oxidative stress, is a clinically relevant model to understand the mechanisms that contribute to biological responses to oxidative stress. Oxidation products are either directly toxic and can cause injury to lung tissue or they can function as exogenous ligands *via* binding to cell surface receptors and thereby triggering intracellular inflammatory and/or apoptotic signaling pathways. Ozone-derived oxidative stress can also lead to changes in reactive oxygen species (ROS) in both the intracellular and extracellular compartments. While increased ROS in the setting of oxidant stress can directly induce cell and tissue injury, ROS can also modify innate immune signaling[50]. Ozone-induced oxidant stress modifies several known cell signaling mechanisms: activation of innate immune signaling pathways, upregulation of antioxidant genes, and enhanced release of damage-associated molecular pattern molecules (DAMPs). Tight regulation of oxidant stress is required to resolve tissue injury and maintain homeostasis.

Ozone exposure can directly lead to the development of oxidized intermediates, which are either formed in the epithelial lining fluid or on modified cell surface proteins and lipids (Figure 1). Ozone can result in either lipid peroxidation or lipid ozonation. Lipid peroxidation is a chain reaction between oxidant and polyunsaturated fatty acid, which severely damages the lipid structure of cell membrane [51]. By-products of lipid peroxidation such as 4-hydroxy-2-nonenal and isoprostanes are widely detected in the lung from ozone exposed humans [52–54] and animals [55–57]. 4-hydroxy-2-nonenal was found to induce cell death of alveolar macrophages [58], modulate airway remodeling [59], trigger intracellular ROS generation [60] and generate 4-hydroxy-2-nonenal-protein adducts [61]. Isoprostane can induce contraction of human bronchial smooth muscle *in vitro* [62] and induce airflow obstruction in guinea pigs [63]. Ozone exposure to unsaturated fatty acids can also result in lipid ozonation. Lipid ozonation products (LOPs) are generated by the oxidation of molecules in epithelial lining fluid and cell membranes in the lung by ozone. LOPs exposed *ex vivo* to bronchial epithelial cells leads to the activation of phospholipases A2, C, and D [64–65]. LOPs produced during ozone exposure also appear to direct macrophage and epithelial cell death, as well as induce pro-inflammatory cytokines production, such as IL-6 and IL-8 [65–66]. Oxidized or ozonized intermediates significantly contribute to the biological response to ozone.

Given the dominant role of oxidant stress in response to ozone, it is perhaps not surprising that antioxidant genes contribute to the biological response to ozone. NAD(P)H quinone oxidoreductase (NQO1), heme oxygenase-1 (HMOX1), glutathione-S-transferase isoforms M1 and P1 (GSTM1 and GSTP1) are critical detoxification enzymes in antioxidant defense. In human population studies, polymorphisms of NQO1 [67–68], HMOX1, GSTP1 [69], GSTM1 [68] are tightly associated with host susceptibility to ozone exposure although these associations appear to be complex. Unlike other antioxidant genes where impaired gene function results in decreased lung function after ozone inhalation, GSTM1-null polymorphisms alone had no association with ozone-related decrease in lung function [11, 68]. However, a combination of GSTM1-null and NQO1 major allele was significantly associated with ozone-induced FEV_1 decrease [68]. In seeming contrast to human observations, glutathione (GSH) deficient mice were protected from ozone-induced lung injury, which was resultant from augmented expression of other antioxidant genes [70]. Additionally, ozone-exposed NQO1-deficient mice were protected from ozone-induced airway hyperreactivity and lung inflammation [71]. The level of ozone oxidation product F-isoprostane was lower in BALF from exposed NQO1-deficient mice, when compared with wild type control animals. However, it is recognized that NQO1 can have either pro-oxidant or antioxidant functions depending on the quinone substrate [72–74]. For example, in other

non-infectious models of lung injury, NQO1 protects the lungs from oxidant-induced alveolar destruction [75]. In contrast, NQO1 appears necessary for the pro-oxidant response to ozone. Therefore, the response to ozone appears dependent on a combination of several antioxidant genes. Additionally, exposure to ozone can result in enhanced expression of many antioxidant genes.

SURFACTANT AND RESPONSE TO OZONE

Pulmonary surfactant, originally defined as a lipoprotein complex that aides in reducing surface tension at the air-liquid interface [76–77], has now been redefined to include multiple roles of surfactant proteins in lung host defense [78]. There are four known surfactant proteins: SP-A, SP-B, SP-C and SP-D. Of these, SP-A and SP-D are members of the collectin family of proteins, which have dual functions within the pulmonary innate immune system, acting either as an opsonin to enhance clearance of foreign materials [79] or as a modulator of ROS and pro-inflammatory cytokine production [80].

The surfactant film lining the airway and alveolus is exposed on a regular basis to a variety of oxidizing toxicants, including ozone. Excessive surfactant oxidation can lead to pulmonary complications including decreased lung compliance, impaired gas exchange and eventually airspace collapse. In addition, lipid ozonation products are thought to act as signal transduction messengers that relay response to ozone inhalation to other areas deeper in the lung [51, 81]. Both SP-A and SP-D directly function as effective inhibitors of lipid peroxidation and protect from oxidative cellular injury at physiologic concentrations [82]. Recent studies have found that alterations in SP-A function, either by ablation in the SP-A−/− animal or by reduction in activity by biochemical processes, renders mice more susceptible to the detrimental effects of ozone inhalation [83–84]. While less is known about the importance of SP-D in ozone-induced injury, decreased levels of functional SP-D correlate with increased inflammation after ozone exposure in mice, whereas enhanced SP-D production coincides with enhanced resolution of ozone-induced inflammation [85].

Interestingly, while surfactant proteins play protective roles in mediating the effects of oxidant-induced cellular damage, the function of these proteins themselves can also be impeded by oxidation [86]. Ozone oxidizes the methionine residues in SP-A and SP-B, which likely contributes to respiratory disorders and lung inflammation [87–88]. SP-A normally forms into an octadecamer structure (six trimeric subunits) by self-oligomerizing. Studies have shown that *in vitro* ozone exposure of SP-A results in an inability of SP-A to self-associate, to mediate lipid aggregation and to bind mannose [89]. Tubular myelin, a lipid transport system unique to the lungs, is formed exclusively by the combination of oligomerized SP-A and SP-B, along with phospholipids in the presence of calcium [90]. Therefore, it is not surprising that acute ozone stress leads to decreased tubular myelin organization, and as a result, perturbs the proportion of extracellular surfactant available for absorption into the surface film [91].

In addition to the involvement of SP-A in the biophysical activity of surfactant, several modulatory functions of SP-A in innate immunity are affected by ozone exposure. Ozone inhalation impairs the interactions between SP-A and either alveolar macrophages or the alveolar epithelia [89, 92]. Additionally, pathogen clearance via phagocytic mechanisms is impaired due to the decreased binding affinity of ozonated SP-A to LPS, bacteria, and viruses [93]. It was reported that SP-A exposed to ozone results in a diminished ability of SP-A to stimulate ROS production from macrophages under baseline conditions [92]. Seemingly in contrast, ozonated SP-A can additionally augment ability to modulate the respiratory burst of PMA stimulated macrophages [94]. While the precise mechanisms describing how ozonated SP-A functions differently from normal SP-A remain incompletely

understood, studies strongly suggest that changes in SP-A structure due to ozonation affect multiple facets of SP-A including both physiological and immunological function [95].

Further understanding of the interaction between ozone and innate immunity has been elucidated by studies defining intrinsic connections between surfactant proteins and toll-like receptor 4 (*tlr4*). These interactions may account for the link between surfactant proteins and ozone-induced lung injury which occurs independent of oxidation. For example, both SP-A [96] and SP-D [97] can directly bind to tlr4/MD-2 complex. Such binding reduced the phosphorylation of tlr4 signaling proteins, such as MAPK family and AKT, thereby attenuating LPS derived immune responses in macrophage [98]. In contrast to this protective function, when SP-A is immunostimulatory, *tlr4* is required for a complete inflammatory response [99]. Additionally, the expression of surfactant proteins can be regulated by ozone exposure through *tlr4* signaling pathway [55]. Connor AJ, *et al* reported that the expression of pro-SP-C and SP-D was increased after ozone inhalation; reduced SP-C and SP-D expression in ozone-exposed *tlr4*-deficient animals was associated with reduced airway reactivity and lung inflammation. These findings suggest that surfactant proteins modulate ozone-host immune responses not only through their oxidization products, but also through interactions with other innate immune molecules.

Human SP-A is encoded by two functional genes, *SP-A1* and *SP-A2*, of which several single nucleotide polymorphisms exist and are associated with acute and chronic lung diseases [100–102]. Variability in the susceptibility to ozone exposure has been described among individuals [32, 103–104]. It is possible that these differences are contributed by functional impairment of the different SP-A proteins (SP-A1 versus SP-A2) or by different combinations and/or proportions of SP-A1:SP-A2 and their respective genetic variants. Mikerov *et al* examined several of these possibilities and reported that *in vitro* ozone exposure reduces the ability of SP-A2 to aid in macrophage phagocytosis more than that of SP-A1, indicating that SP-A2 function may be more susceptible to ozone than SP-A1 [93]. This finding suggests that individuals bearing *SP-A2* genetic polymorphisms may be more susceptible to the effects of oxidation and therefore ozone induced pathology.

TOLL-LIKERECEPTORS AND PULMONARY IMMUNE RESPONSE TO OZONE

The discovery of mammalian toll-like receptors (TLRs) [105] substantially advanced our understanding of innate immunity. TLRs were initially identified as pattern recognition receptors (PRR) that recognize pathogen-associated molecular patterns (PAMPs) [106]. Recent evidence support that PRRs additionally recognize damage-associated molecular patterns (DAMPs) including; hyaluronan (HA)[107], fibronectin [108], and fibrinogen [109–110]. We now recognize that TLRs and their downstream signaling pathways are central to the biological response to ambient ozone (Figure 2).

Toll-like receptor 4 was initially discovered to be a susceptible gene for ozone-induced lung hyperpermeability [111–112]. Using genetically modified animals, we identified the role of tlr4 in ozone–induced AHR [113]. Further studies support that tlr4 signaling contributes to ozone-induced cytokine/chemokine production and neutrophil recruitment [114]. We now recognize that tlr4 signaling after exposure to ozone requires the intracellular adaptor proteins myeloid differentiation primary response gene 88 (*MyD88*) and toll-interleukin 1 receptor (TIR) domain containing adaptor protein (*tirap*)[115]. *Tlr4* may additionally contribute to ozone-induced oxidative stress. Comparisons between ozone-exposed C3H/HeJ (*tlr4* mutant) and C3H/HeOuJ mice reveal the levels of lipocalin 24p3 and 4-hydroxynonenal modified protein (markers for oxidative stress and lipid peroxidation) were significantly reduced in C3H/HeJ mice [55]. This observation suggests that *tlr4* is required

for ozone-induced oxidative stress, which is in contrast to previous observations suggesting an antioxidant role of *tlr4* signaling in models of hyperoxia [116] and emphysema [117]. The precise mechanism that *tlr4* signaling regulates oxidant stress remains an area of considerable interest. In addition to *tlr4-MyD88-tirap* signaling pathway, other TLRs including *tlr2* may contribute to the response to ozone [114]. However, the current evidence strongly supports a central role of *tlr4* in the biological response to ambient ozone.

Animal studies support the finding that *tlr4* can regulate mRNA expression of numerous genes after ozone exposure, which has significant functional consequences. Prolonged or higher doses of ozone can enhance the expression of both tlr4 and MyD88 [111, 114, 118–119]. Ozone can also induce *tlr4*-dependent expression of heat-shock protein 70 (Hsp70) that contributes to pro-inflammatory signaling [119]. Macrophage receptor with collagenous structure (*Marco*) is increased on alveolar macrophages after ozone exposure in a manner dependent on *tlr4* and functions to promote the uptake of surfactant-derived oxidation products in the epithelial lining fluid[41, 120]. Together, these findings support that *tlr4* contributes to gene expression patterns after exposure to ozone with important functional consequences.

PRO-INFLAMMATORY FACTORS AND CELL SIGNALING

Previous studies have demonstrated that ozone exposure can induce the release of many pro-inflammatory factors, including: neutrophil elastase, fibronectin, prostaglandins, plasminogen activators, tissue factor, factor VIII, C3a fragment of complement, tumor necrosis factor (TNF)-α, IL-6, IL-8, IL-17, IL-1β, granulocyte macrophage-colony stimulating factor (GM-CSF), keratinocyte-derived chemokines (KC) and monocyte chemoattractant protein-1 (MCP-1)[30, 121–122]. Many of these factors are functionally required for the complete response to ambient ozone [123]. Additionally, many of these pro-inflammatory factors are recognized as downstream products of activated transcription factors required for innate immune response. For example, the p50 subunit of NF-κB is required for ozone-induced production of TNFα and nitric oxide [124]. Cho and colleagues built on this observation and identified that NF-κB and MAPK/AP-1 play key roles in ozone-induced lung inflammation and injury mediated through the TNF receptors [39]. It has been well documented that ozone exposure induces NF-κB activation in many cell types including alveolar macrophages and airway epithelial cells resulting to the production of cytokines and chemokines including; TNF-α, IL-6, and IL-8 [125–126].

EXTRACELLULAR MATRIX AND FUNCTIONAL RESPONSE TO OZONE

The extracellular matrix (ECM) refers to the structural components which comprise the space between tissue cells. Chronic exposure to ozone is known to impact lung structure [10, 127–128]. However, ECM can also contribute to immunological responses in the lung. In the setting of organ injury, degradation or fragmentation of the ECM can generate products that serve as extracellular ligands to TLR receptors that activate the innate immune system. Current evidence from our laboratory supports a role for extracellular matrix proteins including both mindin and hyaluronan (HA) in the functional response to ozone.

Mindin(spondin 2, SPON2) is an extracellular protein abundant in the spleen, lymph node, and the lung [129–130]. Mindin was initially identified as a necessary protein for an intact innate immune response to LPS and bacterial pathogens [129]. We now recognize that mindin additionally contributes to the response to antiviral host defense [131]and allergic airways disease [132–133]. In non-infectious lung injury caused by ozone inhalation, mindin-deficiency protected animals from augmented AHR and pro-inflammatory cytokine production [134]. Mindin contributes to cellular migration, dependent on integrin binding, in many cell types including: macrophages [130], neutrophils [129] and eosinophils [133].

However, the function extends beyond facilitating inflammatory cellular recruitment. After ozone exposure, bronchial rings derived from mindin-deficient mice demonstrated a reduced constriction in response to carbachol [134]. This finding suggests that mindin is required for airway smooth muscle contractility after exposure to ozone. However, the specific underlying mechanism remains unknown. Together these findings support that mindin contributes to innate immunity and inflammatory airways disease through divergent mechanisms. While previous data support a direct interaction between mindin and tlr4 [129], the functional consequences of this interaction in context of ozone remain incompletely defined.

Hyaluronan (HA) is an anionic non-sulfated glycosaminoglycan and a major component of ECM in the lung. The biological function of HA can either be protective or stimulatory depending on the size of the HA molecule [135]. We now recognize that HA can function as an endogenous ligand of tlr4[107]. Oxidative stress can degrade immune-suppressive high molecular weight HA into immunostimulatory low molecular weight HA [136–137]. Hyaluronan is functionally important in additional animal models of oxidative lung injury including; bleomycin [138] and asbestosis [139–140]. Clinically, HA is detected in airspace of patients with asthma [141–142] and COPD [143] implicating its importance in airways disease. Work from our lab demonstrates that hyaluronan fragments are increased in the lungs of mice after ozone exposure. Furthermore, we discovered that hyaluronan was both necessary and sufficient for ozone-induced airways hyperresponsiveness [144]. Interestingly, we were able to demonstrate that hyaluronan fragments function as endogenous ligands to tlr4 and contribute to reactive airways disease through a co-receptor complex with the dominant hyaluronan surface receptor CD44 [145]. We identified that hyaluronan signaling in the lung is dependent on the tlr4-MyD88-tirap signaling pathway, which results in both airway hyperresponsiveness and proinflammatory cytokines production [115]. Together, these observations support the finding that HA can function as an endogenous ligand to tlr4 to mediate ozone–induced reactive airways disease (Figure 2).

OZONE PRIMES PULMONARY INNATE AND ADAPTIVE IMMUNITY

In addition to the direct effects of ozone in the lung, it is important to recognize that exposure to ozone can modify the subsequent biological response to secondary challenges. Ozone inhalation enhances lung injury through modifying the function of both epithelial cells and macrophages. For example, oxidative stress decreases pathogen clearance by impairing anti-pathogen function of effector cells including; suppressing alveolar macrophage phagocytosis [93, 146–147], enhancing macrophage and neutrophil apoptosis [22], and increasing the susceptibility of human epithelial cells to H1N1 influenza infection [148]. While there are numerous mechanisms that could account for the effects of ozone on impaired antimicrobial host defense, we considered whether inhalation of ozone could directly modify subsequent innate immune response and either enhance or suppress the secondary response to pathogens.

To specifically determine whether ozone modifies subsequent innate immune responses, we exposed mice to ozone then subsequently challenged animals to the prototypic ligand to tlr4, bacterial lipopolysaccharide. We identified that pre-exposure to ozone dramatically enhanced innate immune response in the lung [22]. Ozone inhalation resulted in increased response to LPS including; lung injury, cytokine production, airway hyperresponsiveness, and cellular apoptosis. The enhanced response did not appear to be regulated though differences in gene expression, but rather through enhanced trafficking of tlr4 to the surface membrane of alveolar macrophages [22]. We now recognize that ozone results in fragmentation of extracellular matrix hyaluronan, which additionally contributes to ozone priming of the response to LPS [149]. Hyaluronan fragments prime alveolar macrophage for

increased immune response to LPS through inducing tlr4 trafficking to surface lipid rafts on macrophages [149]. Previous observations in models of hemorrhagic shock [50] demonstrate that oxidative stress can alter tlr4 distribution on the cell surface. Recent work demonstrates that HA-induced intracellular ROS production contributes to trafficking of tlr4 to cell surface in both macrophage and dendritic cells [150]. Similar to observations in animal models, inhalation of ozone results in enhanced surface expression of TLR4 in airway macrophages derived from human subjects[151]. We would predict that enhanced surface expression of TLR4 would result in an enhanced response to LPS. The impact of primed innate immunity on health outcomes will certainly depend on the context of the host. Innate immunity is required for effective clearance of live bacterial pathogens, while uncontrolled response could result in enhanced lung injury or exacerbation of underlying airways disease. Understanding the environmental factors and fundamental mechanisms that regulate priming of innate immunity are of high clinical significance and will remain an area of continued investigation.

Previous work supports that exposure to ambient ozone is associated with an increase of asthma related hospitalizations [152–155]. It is well recognized that ozone can exacerbate existing allergic airways disease in human subjects [156–158]. Animal studies demonstrate that combined exposure to both ozone and to OVA challenges primes OVA-sensitized animals for an enhanced Th2 response [159]. These data suggest that ozone inhalation can modify adaptive immunity. Beyond modifying existing disease, ozone exposure has been shown to enhance sensitization to antigens. When rodents are co-exposed to OVA aerosol and ozone, then subsequently challenged to systemic OVA, there was a significant increase in the level of sensitization as supported by enhanced antigen-dependent fatal shock [160–161] and increased IgE-containing cells in the lung [162]. In monkeys, ozone exposure enhances the development of allergy to both house dust mite allergen [163] and inhaled platinum [164]. Cumulatively, these data suggest that ambient ozone promotes airway sensitization to airborne allergens through a previously unrecognized mechanism. Given our previous observations on the role of *tlr4* in the biological response to ozone [113] and the role of *tlr4* as an adjuvant [165], we sought to determine whether *tlr4* contributes to the mechanism by which ozone can act as an adjuvant. We discovered that exposure to ozone during sensitization resulted in activation of dendritic cells in the airway in a *tlr4*-dependent manner resulting in enhanced allergic airways disease [166]. These data suggest that ozone can modulate adaptive immune response to allergen either during sensitization or after challenge to antigen. Ozone-induced priming of immunity could therefore have implications on lung injury, antibacterial host defense, and adaptive immunity.

CONCLUSIONS

Ambient ozone is a commonly encountered urban air pollutant with recognized adverse health effects. Current evidence supports a complex interaction between inhaled ozone and pulmonary innate immunity. Ozone can modify the function of many cell types in the lung required for intact innate immune response. Numerous genes of innate immunity are required for the complete response to ozone. We now recognize that components of the extracellular matrix contribute to activation of innate immunity in context of ozone inhalation and components of extracellular matrix can regulate intensity of subsequent innate immune signaling. Consistent with recognized cross-talk between the innate and adaptive immune systems, ozone activation of innate immunity can modify intensity of adaptive immunity. Given the effects of ozone inhalation on priming of innate immunity, analysis of the health effects of lower levels of ozone will require comprehensive immunological phenotyping for the response to secondary challenges. Improved understanding of the mechanisms that regulate host response to ambient ozone could provide insight into novel therapeutic approaches to reactive airways disease. Overall,

current evidence support a complex relationship between ambient ozone and pulmonary innate immunity.

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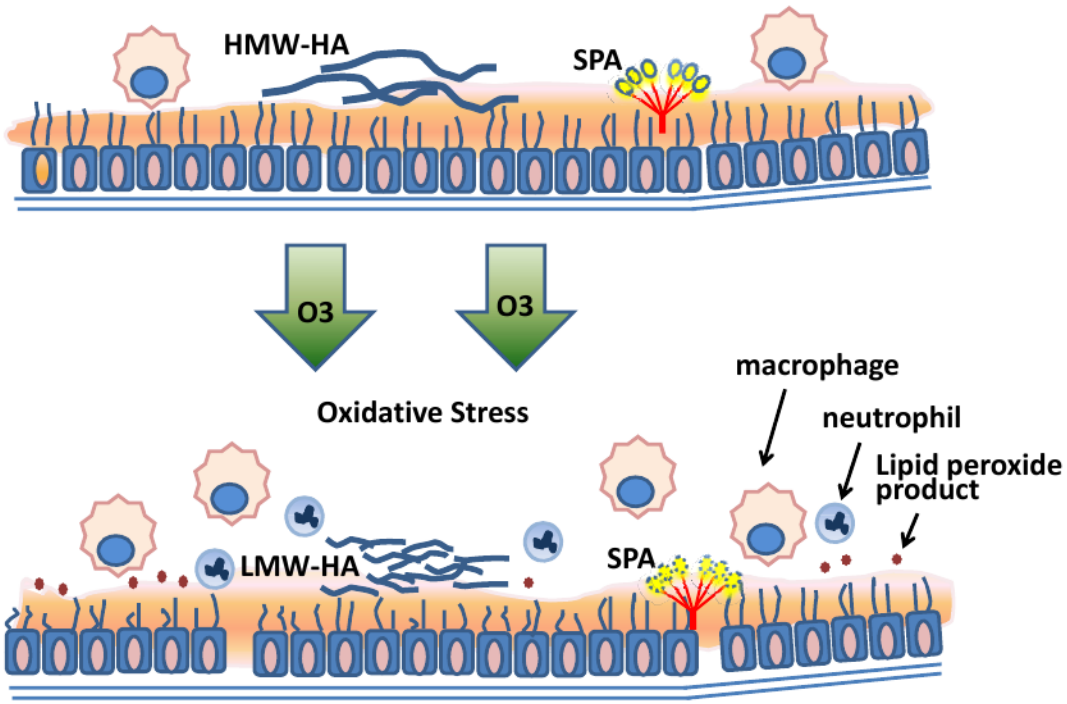

Figure 1. Ozone-induced oxidative stress results in epithelial injury and neutrophil inflammation

Ozone is a strong oxidant that oxidizes components in epithelial lining fluid such as SP-A and lipids producing toxic lipid peroxide products and protein adducts. The integrity of airway epithelium is impaired by ozone resulting in increased epithelial permeability. Ozone–induced oxidative stress induces fragmentation of extracellular matrix HMW-HA into LMW-HA and is associated with recruitment of neutrophils and macrophages into the airspace. (Abbreviation: HMW-HA, high molecular weight hyaluronan; LMWHA, low molecular weight hyaluronan.)

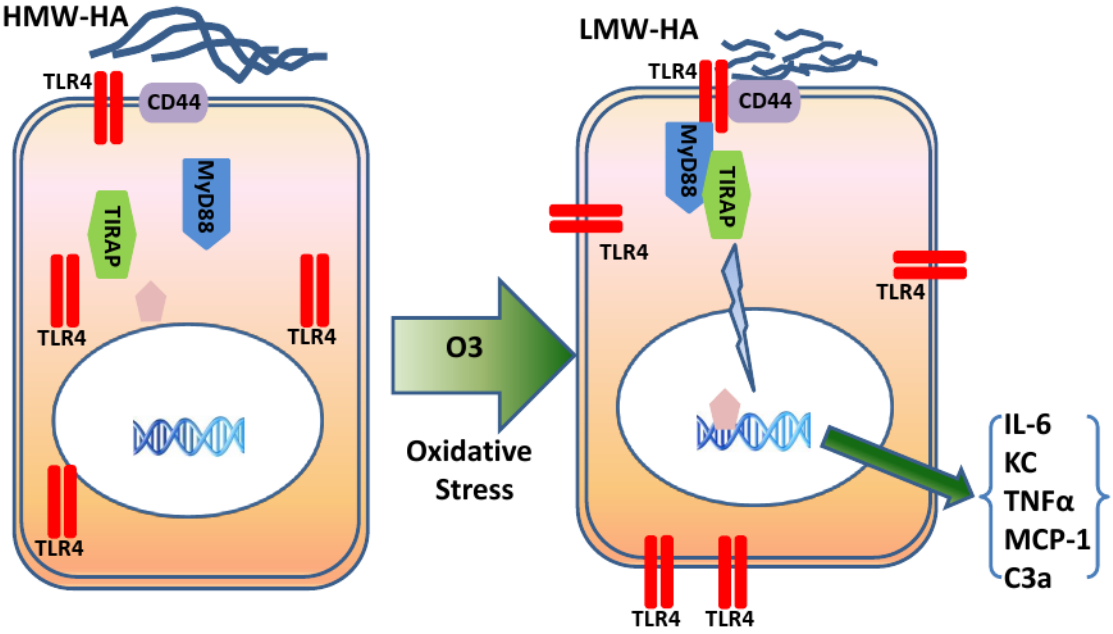

Figure 2. Ozone results in fragmentation of HMW-HA to LMW-HA that stimulates tlr4-dependent innate immune response

Ozone inhalation results in hyaluronan fragments with molecular weight lower than 300KD in the airspace, which is recognized to have immunostimmulatory properties. Both ozone and HA fragments can prime macrophage-derived innate immunity through trafficking oftlr4 to cell surface membrane. Hyaluronan interaction with the tlr4-cd44 complex on cell surface results in activation of the MyD88-tirap signaling pathway resulting in NF-κB activation and both the transcription of pro-inflammatory factors and the development of AHR.