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Review article

Ozone-induced lung injury and inflammation: Pathways and therapeutic targets for pulmonary diseases caused by air pollutants

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ABSTRACT

Exposure to ambient Ozone (O_3) air pollution directly causes by its oxidative properties, respiratory epithelial cell injury, and cell death, which promote inflammation and hyperreactivity, posing a significant public health concern. Recent clinical and experimental studies have made strides in elucidating the mechanisms underlying O_3-induced epithelial cell injury, inflammation, and airway hyperreactivity, which are discussed herein. The current data suggest that O_3-induced oxidative stress is a central event-inducing oxeiptotic cell death pathway. O_3-induced epithelial barrier damage and cell death, triggering the release of alarmins and damage-associated molecular patterns (DAMPs), with subsequent endogenous activation of Toll-like receptors (TLRs), DNA sensing pathways, and inflammasomes, activating interleukin-1-Myd88 inflammatory pathway with the production of a range of chemokines and cytokines. This cascade orchestrates lung tissue-resident cell activation in response to O_3 in leukocyte and non-leukocyte populations, driving sterile innate immune response. Chronic inflammatory response to O_3, by repeated exposures, supports a mixed phenotype combining asthma and emphysema, in which their exacerbation by other particulate pollutants potentially culminates in respiratory failure. We use data from lung single-cell transcriptomics to map genes of O_3-damage sensing and signaling pathways to lung cells and thereby highlight potential hotspots of O_3 responses. Deeper insights into these pathological pathways might be helpful for the identification of novel therapeutic targets and strategies.

1. Background

Ozone (O_3) is a highly oxidative pollutant, and while Earth's upper atmosphere levels are required for temperature and climate balance, ground-level O_3 concentrations are projected to rise due to climate change. Climate dynamics and seasonal variations play a crucial role in shaping ground-level O_3 concentrations, thereby influencing morbidity and mortality rates (Orru et al., 2013; Vicedo-Cabrera et al., 2020). These variations are driven by factors such as changes in temperature, solar radiation, precursor emissions, and meteorological conditions, all of which affect the formation, transport, and dissipation of O_3 (Sicard et al., 2016). Long-term trends indicate a global increase in O_3 concentrations, particularly in urban and suburban areas. This rise is often associated with climate change phenomena, including elevated temperatures and altered wind patterns. O_3 levels are generally higher during warmer months, such as summer, due to increased sunlight and higher temperatures, which intensify photochemical reactions (Sicard et al., 2016).

O_3 at ground level is a harmful and highly reactive air pollutant, inducing oxidative damage that swiftly results in cell injury and death. The resultant oxidative stress on the host likely constitutes a primary mechanism underlying both cell and tissue injury, accompanied by an inflammatory response. Epidemiological modeling comparing the O_3 emission in the decade of 1990–2009 with the baseline period of 1961–1990, showed a most significant increase in O_3-associated mortality and morbidity due to climate change (4–5 %) occurred in Belgium,

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Ireland, the Netherlands, and the UK (Orru et al., 2013). Prolonged exposure to O_3-polluted air is associated with increased morbidity and mortality associated with respiratory diseases (Kasdagli et al., 2024), along with heightened responses to microbial or allergen challenges (Hollingsworth et al., 2010; Last et al., 2004; Liang et al., 2013; Orru et al., 2013; Vicedo-Cabrera et al., 2020).

Recent investigations have unveiled that cellular injury generates damage-associated molecular patterns (DAMPs) such as ATP, uric acid (UA), hyaluronic acid (HA), heat shock protein 70 (HSP70), and others. These DAMPs activate pattern recognition receptors (PRRs) such as NLRs or TLRs (Iwasaki and Medzhitov, 2010; Kawai et al., 2024; Medzhitov, 2001; 2021). Evidence suggests that TLR2 and TLR4 may be implicated in O_3-induced inflammation (Williams et al., 2007). Hyaluronic acid, a degradation product of matrix components, and HSP70, generated by O_3-induced tissue damage, may potentially activate TLR4 (Bauer et al., 2010; Li et al., 2010; Li et al., 2011). Downstream, the TLR adaptor proteins MyD88 and TIRAP are indispensable for the inflammatory response (Li et al., 2011), as they activate transcription factors like NF-κB to regulate cytokine gene expression.

Alarmins, including IL-33 (Interleukin-33), IL-1α (Interleukin-1α), and HMGB1 (high mobility group Box-1 protein), are signaling molecules released by damaged or stressed cells that alert the immune system to potential threats or injury (Bertheloot and Latz, 2017). Unlike traditional cytokines that are often involved in the immune response to pathogens, alarmins are released from the nucleus or cytosol without *de novo* synthesis as a result of cellular stress or damage, and they act as danger signals to prompt the body's defense mechanisms (Bertheloot and Latz, 2017), triggering a cascade of events that lead to increased immune surveillance and repair processes.

In contrast to IL-1α, IL-1β release requires a two-step process, with an initial engagement of pathogen recognition receptors (PRR) to induce *IL1b* gene expression, followed by inflammasome activation for IL-1β protein maturation and secretion. IL-1β is a potent inflammatory mediator induced by bacterial infection and tissue injury (Dinarello, 2009). Recent investigations have elucidated the involvement of the NLRP3 inflammasome complex in the response to O_3 (Michaudel et al., 2016), as further discussed later.

Therefore, the primary objective of this review is to synthesize current evidence on the respiratory health effects of O_3 exposure, focusing on the biological and immunological mechanisms of epithelial barrier cell death and related inflammatory pathways, vulnerable populations, and the role of co-exposure to other pollutants in lung disease development. By providing a comprehensive understanding of O_3-associated respiratory risks, we also highlight gaps in the literature to guide future research and therapeutic strategies.

2. O_3 exposure induces respiratory and systemic toxic effects

O_3 exposure has been shown to induce both respiratory and systemic toxic effects, contributing to significant physiological and pathological changes across multiple organ systems. O_3 exposure is strongly associated with respiratory issues, reduced lung function and increased risk of respiratory infections. It induces oxidative stress and inflammation in the airways, leading to tissue damage and impaired pulmonary function (Kasdagli et al., 2024; Kim et al., 2011; Kim et al., 2020). Inhaled O_3 has a significant causative impact on hospitalization rates for both upper and lower respiratory diseases. Moreover, the harmful effects of ozone on human health vary based on gender and age. Females appear to be more susceptible to inhaled ozone than males, likely due to the influence of estrogen levels and the differential regulation of the lung immune response (Lu and Yao, 2023).

O_3 exposure has extensive multi-organ systemic effects on human health, impacting the cardiovascular, neurological, and renal systems. The underlying mechanisms primarily involve oxidative stress and inflammation, which can contribute to both acute and chronic health conditions. O_3 exposure has been associated with cardiovascular diseases, such as hypertension, myocardial infarction, and stroke, through the promotion of systemic inflammation, which can damage blood vessels and impair cardiac function (Cole and Freeman, 2009; Day et al., 2017; Sun et al., 2024; Wang et al., 2019). Long-term O_3 exposure has recently been associated with an elevated risk of cardiovascular disease incidence in Chinese adults, especially in urban areas (Zhu et al., 2024). Emerging evidence indicates that O_3 exposure may adversely affect the central nervous system, contributing to cognitive decline, neuroinflammation, and an elevated risk of neurodegenerative diseases, such as Alzheimer's disease (Gao et al., 2022; Kilian and Kitazawa, 2018;

Box-1
Putative therapeutic targets for pulmonary inflammation induced by O_3 exposure.

- TNF neutralization presents a potential option; however, it may lead to systemic reductions in host defense and innate immunity.
- The blockade of IL-1 using available neutralizing antibodies and the IL-1 receptor antagonist (Anakinra) has been explored. However, findings from clinical trials suggest limited efficacy.
- Neutralizing antibodies targeting IL-23 and IL-17A are currently available. However, their use may lead to reduced host resistance to fungal infections.
- TLR4 antagonists may be considered (Peri and Piazza 2012; Zhang et al., 2022).
- Dampening of NRP3 inflammasome activation using inhibitors such as MCC950 (Coll et al., 2015; Haag et al., 2018; Hooftman et al., 2020)
- Blockade of nucleic acid sensor activation, notably cGAS/STING, using antagonists (Haag et al., 2018).
- Aryl hydrocarbon receptor activation by microbial tryptophan metabolites and more (Hezaveh et al., 2022)
- Blockades of cholinergic pathways activated based on ChAT reporter and cell-specific KO mice support the beneficial effect of cholinergic pathways (Yamada and Ichinose 2018).
- Blockade of muscarinergic pathways such as Tiotropium and related analogs has been efficacious in COPD patients (Bateman et al., 2009; Kerstjens et al., 2016; Kistemaker et al., 2019; Tashkin et al., 2016; Wollin and Pieper 2010).
- ROS inhibitors such as N-acetyl cysteine and hydrogen disulfide attenuate O_3 inflammation reviewed (Wiegman et al., 2020).
- Microbial metabolites, such as butyrate activating GPR109A/HCAR2, attenuate inflammatory diseases (Correa et al., 2022; Lewis et al., 2019).
- Histone modulators such as histone deacetylases (HDAC) may be another approach using HDAC inhibitors (Fellows and Varga-Weisz 2020).
- DNase treatment, as cell-free DNA is highly inflammatory and released upon cell death. In particle-induced lung injury, enzymatic degradation of DNA by DNase I reduced inflammation (Liu et al., 2023; Yadav et al., 2023).
- Inhibitors suppressing myofibroblast transdifferentiation, such as N23Ps (N-(2-methoxyphenyl)-3-(phenyl)acrylamides) and derivatives are novel compounds suppressing myofibroblast transdifferentiation, collagen deposition, and fibrosis. N23Ps target SMURF2, a SMAD-specific E3 ubiquitin protein ligase2 (Gerckens et al., 2021).

Singh et al., 2022a). Recent studies have identified O_3 as a potential risk factor for kidney disease, inducing systemic inflammation, and may contribute to renal damage and impaired kidney function (Kim et al., 2024; Peng et al., 2024). Therefore, O_3 exposure affects multiple organs and poses a significant health risk.

The respiratory system is the primary route of O_3 exposure, making it the most directly and acutely affected system. As the second largest mucosal area, the lungs play a critical role in immune responses to environmental insults (Hasenberg et al., 2013; Kageyama et al., 2024; Mettelman et al., 2022), such as O_3 (Sokolowska et al., 2019). Substantial evidence links O_3 exposure to respiratory outcomes, including asthma and chronic obstructive pulmonary disease (COPD) exacerbation, and other conditions (Chou et al., 2023; Li et al., 2019; Yang et al., 2024). Revisiting O_3's functional, inflammatory, and morphological effects in an experimental mouse model is essential to verify that it reflects previous studies of human volunteers (Mauderly, 1984). Clinical studies indicate that approximately 50–90 % of inhaled O_3 is deposited in the upper airways due to its high reactivity with airway lining fluid and mucus (Gerrity et al., 1995; Hu et al., 1994). The upper airways predominantly absorb inhaled O_3, particularly during nasal breathing. However, uptake in the lower conducting airways increases with higher respiratory flow rates, while O_3 reaches the alveolar regions at significantly lower concentrations. For ambient O_3 concentrations of 100 parts per billion (ppb), the amount reaching the lower respiratory tract is estimated to range from 10 to 30 ppb, depending on variables such as breathing mode and physical activity level (Gerrity et al., 1995; Hu et al., 1994). Because of its limited water solubility, inhaled O_3 passes the upper airways without much effect and reaches the lower respiratory tract with its fragile alveolar region, where it dissolves in the lipid-rich epithelial lung lining fluid. A single exposure to O_3 causes acute lung injury characterized by damage to the thin respiratory epithelial barrier, resulting in protein leakage and the release of inflammatory mediators, leading to neutrophil recruitment. The respiratory epithelium is the initial structure exposed to O_3, often experiencing cell stress and death as a consequence (Sokolowska et al., 2019).

However, various resident cells that compose the epithelial sheet are potential targets of the O_3-reactive species triggering cell death and inflammation, as depicted in Fig. 1. Data from single-cell RNAseq (scRNAseq) analysis of either healthy human lungs (Fig. 1**A-B**) or mice lungs (Fig. 1**C-D)** enables us to map the expression of the previously discussed receptors, DAMPs, or alarmins to resident cells of different lung niches. For the data selected, the classifications of cell types were better characterized in human lungs, showing increased complexity of cell populations compared to mouse lungs **(**Fig. 1**A-D).** This pool of lung resident cells is composed of leukocytes and stromal cells, including alveolar macrophages, dendritic cells, innate lymphoid cells, T and B cells, as well as underlying Type 1 and 2 epithelial cells, vascular endothelial cells, pericytes, fibroblasts, smooth muscle cells, and other populations **(**Fig. 1**A-D).** Identifying cell types with high expression levels of the correspondingly O_3-induced damage sensing or signaling proteins allows us to nominate candidate cells and hotspots for the underlying pathways.

Damage to alveolar epithelial cells results in cell injury and protein leakage into lung lining fluid, as assessed clinically and experimentally by bronchoalveolar lavage (BALF) and the release of a multitude of mediators such as alarmins (IL-1α, IL-1β, IL-33, HMGB1, DAMPs (ATP, dsDNA, mtDNA), Cytokines (IL-6, IL-17, TNF-α and amphiregulin), leukotrienes, prostaglandins, and chemokines. These mediators attract neutrophils, monocytes, lymphocytes, and other inflammatory cells to the injury site. As we depicted in Fig. 1, by scRNAseq analysis of human samples, a range of lung resident cells from healthy individuals expresses alarmins, DAMP receptors, and inflammasome components, such as IL-33, HMGB1, RAGE, amphiregulin, IL-1β, IL-18 and PYCARD **(**Fig. 1**B),** and the same is observed in mice (Fig. 1**D).** Thus, suggesting that lung-resident cells, including both leukocytes and non-leukocytes, can initiate responses to O_3 and other pollutant-induced insults. Notably, the chronic O_3 activation of these pathways may contribute to lung dysfunction and tissue remodeling.

Most studies investigating the innate immune response to O_3 have predominantly focused on the effects of acute exposure, particularly concerning cell death. However, research examining the impact of chronic O_3 exposure remains comparatively limited. Experimental evidence indicates that acute and chronic O_3 exposure produces distinct functional and morphological alterations in the lungs of mice (Michaudel et al., 2018a). Acute O_3 exposure disrupts respiratory epithelium integrity, resulting in protein leakage and neutrophil recruitment into the bronchoalveolar space. This leads to lung inflammation and airway hyperresponsiveness. These effects are exacerbated with chronic O_3 exposure, which is also associated with collagen deposition (Michaudel et al., 2018a). The structural changes in the airways, as quantified through automated numerical image analysis, reveal significant differences between acute and chronic exposure. Acute O_3 exposure increases bronchial and lumen circularity while decreasing epithelial thickness and area. In contrast, chronic O_3 exposure results in epithelial injury, characterized by reduced epithelial height, distended bronchioles, enlarged alveolar spaces, and increased collagen deposition. These findings suggest the development of peribronchiolar fibrosis and emphysema, as evidenced by a significant increase in airspace density and diameter and a reduction in airspace numbers (Michaudel et al., 2018a).

Recent studies further suggest that chronic O_3 exposure induces significant changes in the gut and lungs' microbial composition. These microbial alterations are associated with elevated lung inflammatory markers, impaired pulmonary function, and an increased risk of lung diseases (Tian et al., 2024). Although there is evidence linking ground-level O_3 exposure to a heightened risk of disease, the effects of low-dose chronic O_3 exposure on diverse tissues remain poorly understood. Ling and colleagues demonstrate that prenatal exposure to environmentally relevant levels of O_3 may exacerbate autism symptoms, potentially through molecular mechanisms that provide novel insights into the pathogenesis of autism, particularly in relation to low-dose pollutant exposure (Ling et al., 2023). Moreover, repeated inhalation of O_3 has been shown to induce oxidative stress, adipose tissue inflammation, and insulin resistance (Zhong et al., 2016), and O_3 exposure tended to aggravate HFD-induced disturbances in lung glycerophospholipid metabolism (Liang et al., 2024). These findings suggest that the toxicity of chronic O_3 exposure extends beyond the lungs, affecting multiple organ systems.

We have investigated the chronic effects of O_3 exposure on lung immune response in mice (Michaudel et al., 2020; Michaudel et al., 2018a; Pinart et al., 2013). Chronic exposure to O_3, such as twice weekly at 1.5 ppm for 90 min in mice, induces repeated episodes of inflammation, leading to the progressive destruction of alveolar epithelial cells and the development of emphysema within six weeks (Michaudel et al., 2020; Michaudel et al., 2018a; Pinart et al., 2013). This pattern closely resembles that found in patients with chronic obstructive pulmonary disease (COPD). Of importance, the expression of these markers (alarmins, DAMPs, and inflammasome) in healthy mouse lungs can define the cell gene signatures, suggesting that in an eventual tissue injury, these cells **(**Fig. 1**E)** can trigger the acute inflammation upon O_3 exposure, and chronicity by repeated exposure to O_3.

Recurrent ozone O_3 exposure can cause significant changes in the immune system, potentially altering responses to subsequent exposures to allergens and pollutants sustaining immune tissue adaptation (Faria et al., 2017), a type of tissue immune activation, differentiation, and memory. These changes are driven by oxidative stress, immune pathway modulation, and epigenetic reprogramming. O_3-induced oxidative stress primes the immune system, particularly alveolar macrophages (Frush et al., 2016), dendritic cells (Hollingsworth et al., 2010), and neutrophils (Rocks et al., 2019), which become hyper-responsive to subsequent stimuli. ROS can enhance antigen-presenting cell activity, increasing sensitization to allergens (Cohen et al., 2001). TLR4 signaling is a key

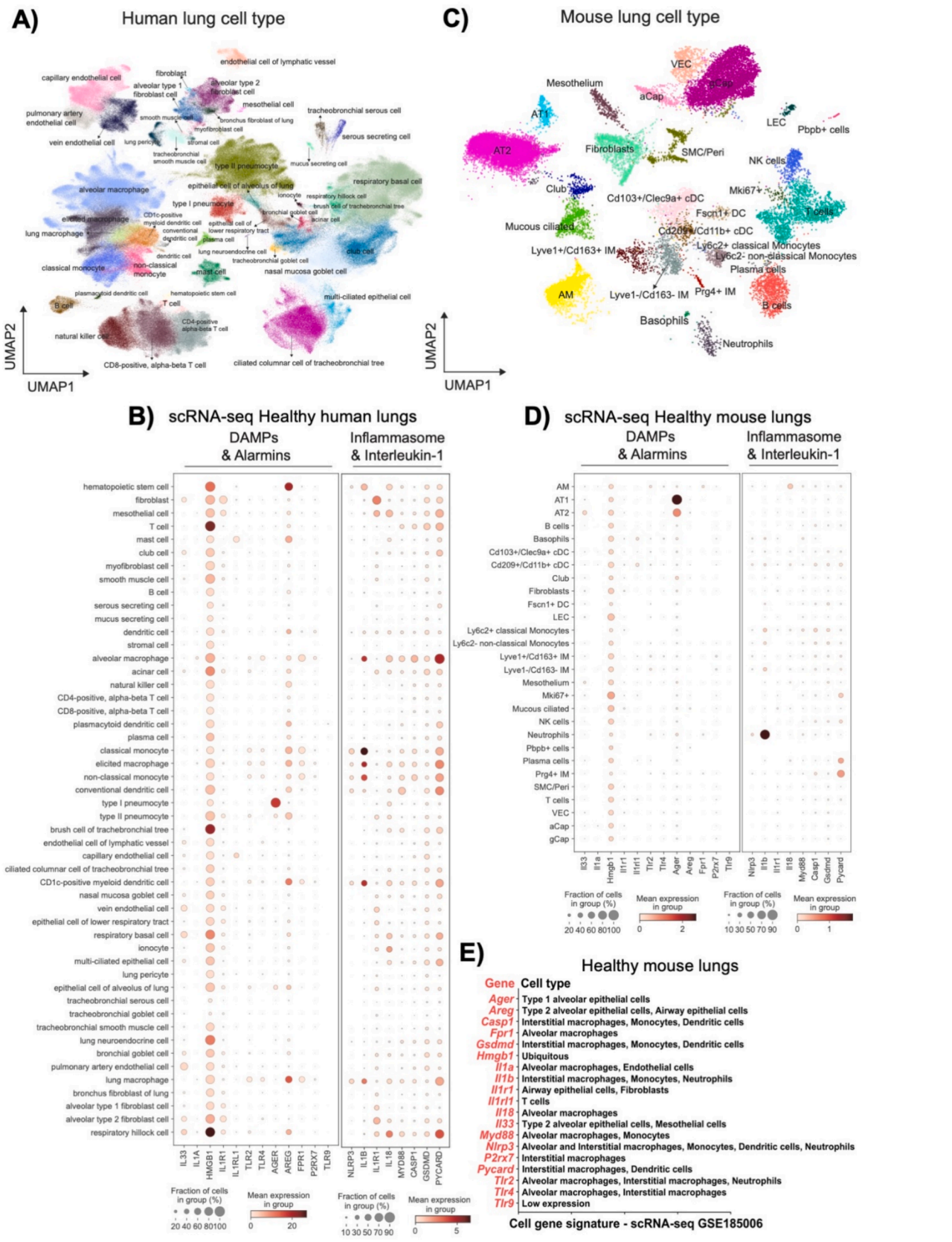

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Fig. 1. Lung-resident cell signatures from healthy humans and mice revealed that they can prompt response to tissue injury by DAMP and Alarmin expression. Visualization of dimension-reduced single-cell transcriptomic data (scRNAseq) by Uniform Manifold Approximation and Projection (UMAP) reveals different annotated cell types in the human lung (A) and in the mouse lung (C). UMAP embedded visualization of "DAMP and Alarmin signaling" and "Inflammasome and Interleukin-1 signaling" related gene expression in human lung cells (B) and in mouse lung cells (D). Cell gene signatures from UMAP embedded visualization of related gene expression in healthy mouse lung cells (E). Single-cell transcriptomic data analysis from both human and mouse healthy lungs was performed in the study to map target genes in the "DAMP & Alarmins pathway" and "Inflammasome & Interleukin1 pathway" to major expressing cell types. Briefly, human lung single-cell data was taken from the integrated Human Lung Cell Atlas (HLCA) core, including data from healthy lung tissue from 107 individuals. The data was downloaded via cellxgene (https://cellxgene.cziscience.com/collections/6f6d381a-7701-4781-935c-db10d30de293). Mouse lung single-cell data is downloaded under GEO accession (GSE185006) containing mice exposed to filtered air (FA) or cigarette smoking for 2 and 4 months to establish a murine COPD model. For our current study, only data from 6 lungs from mice exposed to FA were included to represent healthy lungs (Gunes Gunsel et al., 2022; Sikkema et al., 2023). According to both accessions, a processed data file (h5ad format) was downloaded, which was performed with quality control, graph-based clustering, and cell-type annotation. Data analysis was performed using the Scanpy package (version 1.8.0). To visualize different cell types or cell niches in both human and mouse healthy lungs, the plotting function of "scanpy.pl.umap" was used, shown by a Uniform Manifold Approximation and Projection (UMAP). On each UMAP, clusters with different colors display different cell types with labeled names for either human or mouse healthy lungs. The plotting function of "scanpy.pl.dotplot" was used to visualize target gene expression. The dot size represents the fraction of cells in the treatment group, and the color represents the gene's mean expression in the treatment group.

pathway activated by O_3. This pathway bridges innate and adaptive immunity, promoting the release of IL-1β, IL-6, and TNF-α. TLR4 signaling is critical in the exaggerated inflammatory responses in allergic and pollutant-exposed individuals (Dunigan-Russell et al., 2023; Peden 2011; Tian et al., 2021; Williams et al., 2007). Epigenetic changes, such as modifications in microRNA (miRNA) expression, play a critical role in O_3-induced immune adaptation. MicroRNAs are small non-coding RNAs that regulate gene expression post-transcriptionally, affecting immune cell function and inflammation. O_3 exposure upregulated miRNAs linked to inflammation, such as miR-223 and miR-199a. These miRNAs regulate genes involved in immune cell recruitment, cytokine production, and tissue remodeling (Fry et al., 2014). This suggests that recurrent O_3 exposure may lead to adaptive changes in immune response, potentially altering subsequent reactions to pollutants or allergens. This insight emphasizes the long-term implications of chronic exposure for respiratory health and immune function.

2.1. ROS as a major driver of O_3-induced cell death by oxeiptosis

Reactive oxygen species (ROS) generated by O_3 exposure induce inflammation and cell death in mice or cells, as outlined in the review (Wiegman et al., 2020). O_3 exposure leads to the production of reactive oxygen species (ROS), including superoxide anions ($O_2\bullet-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), which play pivotal roles in oxidative stress and cellular damage. These highly reactive molecules are generated when O_3 reacts with biological macromolecules and lipids in the respiratory tract lining fluid. The resulting oxidative burst contributes to inflammation, tissue injury, and the activation of redox-sensitive signaling pathways, and these ROS species can propagate damage by triggering lipid peroxidation and altering protein structure and function (Byvoet et al., 1995; Kudo et al., 1996; Pryor 1994; Wiegman et al., 2020), thereby amplifying the pathological effects of O_3 exposure.

Oxidized phospholipids (oxPLs) are classified as damage-associated molecular patterns (DAMPs) produced in response to high levels of oxidative stress, such as that induced by O_3-mediated inflammation. These molecules exert their effects by signaling through scavenger receptors (SRs), including SR class B-1 (SR-BI), and toll-like receptors (TLRs). Notably, SR-BI is critical in mitigating oxPAPC-induced lung pathology by maintaining lipid homeostasis (Dunigan-Russell et al., 2023). Chronic O_3 exposure has been linked to elevated levels of chemokines and cytokines across all O_3-exposed groups, highlighting the establishment of a persistent inflammatory environment in the lungs. This inflammatory state is further associated with upregulated gene expression of several HIF-1α target genes, such as *Hdac2* (histone deacetylase 2), *Vegf* (vascular endothelial growth factor), *Keap1* (kelch-like ECH-associated protein 1), and *Mif* (macrophage migration inhibitory factor) (Wiegman et al., 2014). These findings suggest that the disruption of the antioxidative stress response, alongside the activation of the HIF-1α pathway, contributes to the chronic inflammatory response and the development of emphysema observed in O_3-exposed mice (Wiegman et al., 2014). Thus, suggesting that the loss of the antioxidative stress response, coupled with the activation of the HIF-1α pathway (Wiegman et al., 2014), contributes to the inflammatory response and the development of emphysema observed in O_3-exposed mice.

We further investigated the ROS-induced cell-death signaling pathway, elucidating its interactions with the cellular ROS sensor and antioxidant factors NRF2 and KEAP1, the phosphatase PGAM5, and the pro-apoptotic factor AIFM1 (Holze et al., 2018). $Pgam5^{-/-}$ mice exhibited exacerbated lung inflammation and increased levels of proinflammatory cytokines following exposure to O_3, resulting in heightened virus infiltration, lymphocytic bronchiolitis, and reduced survival rates among $Pgam5^{-/-}$ mice. We coined this pathway 'oxeiptosis' as a ROS-sensitive, caspase-independent, non-inflammatory cell-death pathway crucial for inflammation induced by ROS or ROS-generating agents, such as viral pathogens and O_3 (Holze et al., 2018; Scaturro and Pichlmair, 2018). Although low ROS levels are required for homeostatic functions (Scaturro and Pichlmair, 2018), however, elevated ROS levels trigger oxeiptosis, a PGAM5 phosphatase-dependent AIFM1 dephosphorylation triggering chromatin condensation and DNA degradation, with subsequent cell death **(**Fig. 2**A)**. Reanalyzing the public deposited RNAseq datasets from two different protocols **(**Fig. 2**B)**, acute evaluating days 1 and 4 after single O_3 exposure (GSE161538), or chronic by three consecutive weeks of O_3 exposure (GSE156799) **(**Fig. 2**C-F)**, the differential expression highlighted that *Nfe2l2* and *Aimf1* were significative up-regulated 4 days after single O_3 exposure **(**Fig. 2**E)** and repeated O_3 exposure during three weeks **(**Fig. 2**F)**, suggesting that oxeiptosis can be involved lately after a single and chronically by repeated O_3 exposure **(**Fig. 2**G)**. Despite being characterized as non-inflammatory cell death, we need to consider that chronic inflammation by repeated O_3 exposure and oxeiptosis may contribute to chronic tissue inflammation and remodeling, but this needs to be experimentally confirmed.

2.2. Exacerbation of airway diseases by O_3 exposure

Epidemiological and experimental studies provide evidence supporting a correlation between air pollution and heightened incidence and severity of airway diseases. The most affected pathologies are chronic obstructive pulmonary disease, lung cancer, and respiratory infections, including pneumonia, stroke, and heart disease (Bala et al., 2021). The adverse effects of O_3, nitrogen oxides (NOx), sulfur dioxide (SO_2), and particulate matter (PM) are well-documented. Recent investigations, particularly in urban and industrialized regions, have indicated a potential role for pollutants in the pathogenesis of asthma and COPD (Kelly and Fussell, 2011). Exposure to ambient O_3 and household air pollution might be important risk factors for COPD among young adults, and simultaneous exposure to high levels of the two pollutants may intensify their individual effects (Xing et al., 2023). Recent findings suggest a potential causal relationship between long-term

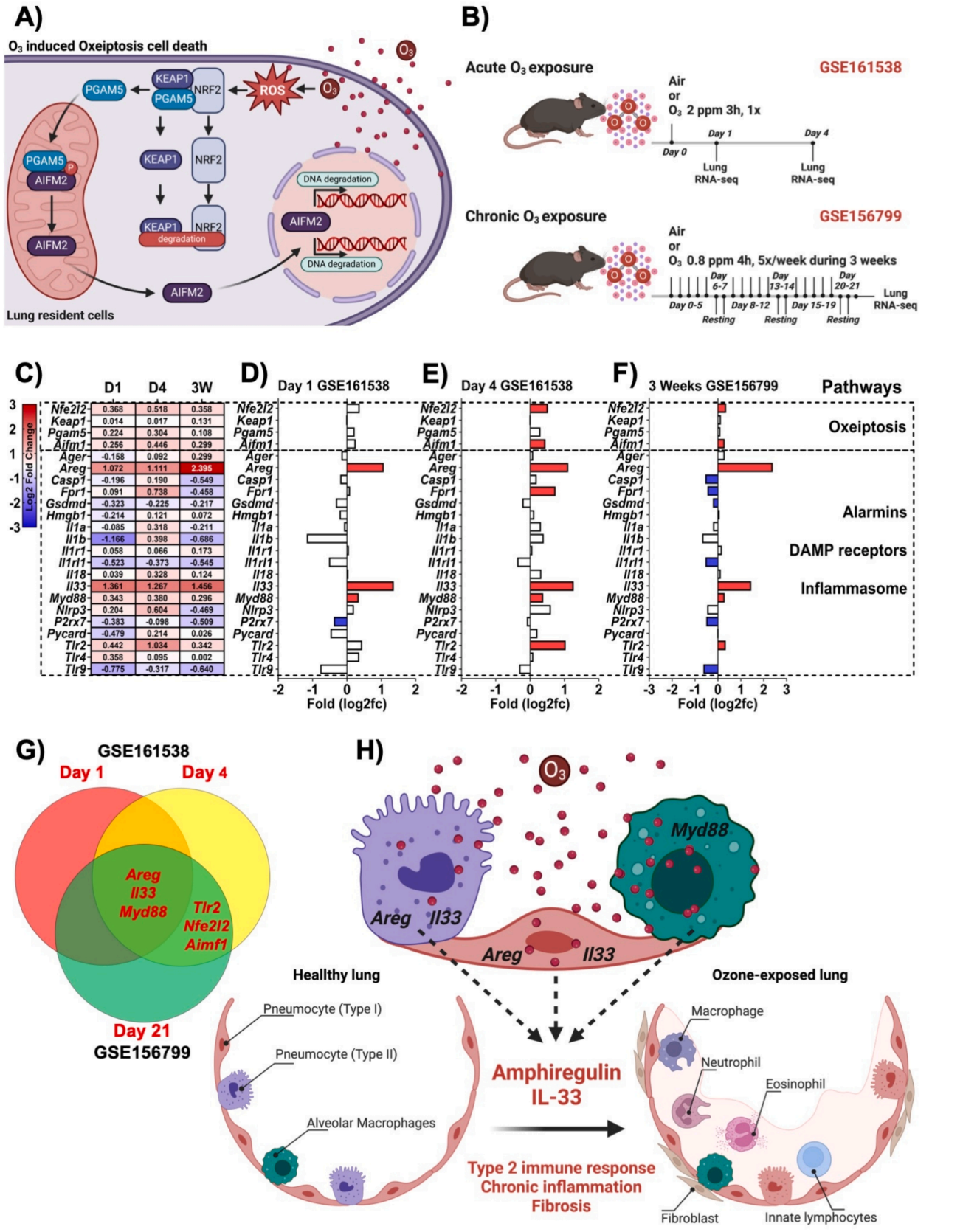

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Fig. 2. Ozone (O_3)-exposure induced acute and chronic alterations in mice lungs: Role of oxeiptosis, alarmins, DAMPs, and inflammasome pathways. O_3-induced oxeiptosis is a caspase-independent, ROS-sensitive cell death pathway distinct from traditional apoptosis (A). This pathway involves the key molecules NRF2, KEAP1, PGAM5, and AIMF1. Oxeiptosis occurs by disrupting the protective antioxidant complex KEAP1/PGAM5/NRF2, releasing NRF2 and the phosphatase PGAM5, which activates AIFM1. PGAM5, in response to oxidative stress induced by O_3, dephosphorylates AIFM1, a pro-apoptotic factor that is a terminal effector protein. Dephosphorylated AIFM1 is translocated from mitochondria to the nucleus, which induces chromatin condensation, DNA fragmentation, and cell death. Experimental design of O_3-induced lung injury and RNAseq of lung tissue (B), characterized as acute (single O_3-exposure, GSE161538) and chronic (multiple O_3-exposure, GSE156799) models. RNAseq analysis showing RNA differential expression comparing O_3-exposure normalized by exposure to filtered air from GSE161538 (Single O_3 2 ppm 3 h, n = 7 filtered air-exposed and 4 O_3-exposed mice) and GSE156799 (O_3 0.8 ppm 4 h/day, during 3 weeks, n = 8 filtered air and 8 O_3-exposed mice) (C-F). Heatmap of RNAseq expression (C), RNAseq differential expression of acute model Day 1 (D) and Day 4 (E) after a single O_3-exposure (GSE161538), and chronic model after 3 weeks (F) of multiple O_3-exposure (GSE156799). RNAseq datasets found in the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) were analyzed by Phantasus (https://genome.ifmo.ru/phantasus) and presented as Log2 Fold Change (Log2FC) (Russo et al., 2023). Differences were considered significant at the Adjusted P-value < 0,05. Red represents significative up-regulated and blue significative down-regulated genes (D-F). Venn diagram illustrating common up-regulated genes induced by O_3-exposure of acute (Day 1 and Day 4) and chronic (3 weeks) models (G). The proposed mechanism of acute and chronic lung injury induced by O_3 exposure shows that Il-33, Areg, and Myd88 may represent possible targets to control lung inflammation (H). Illustration A and H were constructed using Biorender. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

exposure to high-level ambient O_3 and increased risks of adult-onset asthma (AOA) (Su et al., 2024).

Moreover, in mice, O_3 exposure exacerbated acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) induced by endotoxin (Johnston et al., 2005a; Johnston et al., 2002). Hence, the allergen response was examined in O_3-exposed mice, which exhibited heightened allergic asthma characterized by increased airway hyperresponsiveness, elevated recruitment of neutrophils and eosinophils in the BALF, and intensified lung inflammation marked by augmented goblet cells, myofibroblasts, and smooth muscle cells (Jang et al., 2006). Respiratory viral infections have been shown to exacerbate asthma (Kim et al., 2013; Kim et al., 2012). Cumulative research indicates that various ambient air pollutants can exacerbate pulmonary fibrotic processes. Notably, epidemiological studies have revealed a significant association between prolonged exposure to elevated levels of O_3 and nitrogen dioxide and an increased risk of acute exacerbations in individuals with idiopathic pulmonary fibrosis (Johannson et al., 2014).

2.3. Co-exposure of O_3 with other air pollutants

In urban life, exposure to the air pollutant O_3 often coincides with episodes of high particle and toxic gas concentration, and both are associated with increased respiratory symptoms and hospital admissions. Many pollutants are commonly found in combination with O_3, such as particulate matter (PM2.5 and PM10), nitrogen dioxide (NO_2), and sulfur dioxide (SO_2). Positive associations were found between short-term exposure to ambient SO_2 and all-cause and acute respiratory mortality (Orellano et al., 2021). Daily air pollution exposure (mostly NO_2, PM2.5, and O_3) is positively associated with an increase in short-acting beta-agonist (SABA) use in patients with asthma and COPD (Su et al., 2024). Short-term ambient NO_2 and PM2.5 exposure are associated with increased exacerbation in patients with mild to moderate COPD, further heightening the awareness of non-infectious triggers of COPD exacerbations (Ross et al., 2023). Exposure to O_3, NO_2, and SO_2 significantly impacts COPD hospitalization caused by air pollution (Ghanbari Ghozikali et al., 2016). Recent studies suggest that increased exposure to air pollutants may be linked to various health issues in individuals with ILDs, revealing a significant association between the increased risk of AE-IPF induced by PM2.5 (Lan et al., 2023). In the IPF-PRO Registry, long-term exposure to PM2.5 was associated with worse quality of life and lung function of patients, increasing the severity of IPF manifestation (Sack et al., 2024).

Pollutants are commonly found in combination, and the presence of O_3 potentiates the toxic effects on airways, promoting an exacerbated inflammatory response. Co-exposure to O_3 and PM2.5 has been shown to amplify lung inflammatory responses. In animal studies, combined exposure led to increased levels of pro-inflammatory cytokines like TNF-α and interleukin-6, along with more significant lung tissue damage compared to exposure to either pollutant alone (Gangwar et al., 2020). Diesel exhaust particles contain a mix of pollutants, including NO_2 and black carbon. Exposure to diesel exhaust has been linked to increased oxidative stress and inflammation in the respiratory tract, which can be exacerbated by the presence of O_3 (Long and Carlsten, 2022). Experimental studies in rodents have shown that co-exposure to O_3 and diesel exhaust particles (DEP) can synergistically affect lung disease, exacerbating their symptoms (Farraj et al., 2010; Hathaway et al., 2021; Majumder et al., 2023). Apparently, DEP can incorporate O_3 into its soot matrix, thereby activating its reactivity, which might present a mechanism for how ambient O_3 concentrations may increase the toxicological potency of DEP (Madden et al., 2000). Simultaneous inhalation of O_3 and ultrafine carbon black particles has been associated with heightened lung inflammation and functional decline. This co-exposure triggers oxidative stress pathways, leading to epithelial injury and subsequent inflammatory responses (Majumder et al., 2021). Other studies have demonstrated that O_3-oxidized black carbon, a prominent constituent of urban air pollution, is particularly toxic, with a stronger potency to induce IL-6 and IL-33 release and lung damage in mice as compared to black carbon (Chu et al., 2018). While NO_2 alone has mild inflammatory effects at standard ambient concentrations, its presence can enhance the respiratory effects of other pollutants. For instance, NO_2 can act as an adjuvant, increasing the sensitivity of the airways to allergens and other irritants, potentially amplifying the harmful effects of O_3 (Guarnieri and Balmes, 2014). SO_2 exposure primarily causes bronchoconstriction, especially in individuals with asthma. When combined with O_3, there is a potential for increased airway responsiveness and inflammation, leading to more severe respiratory symptoms (Guarnieri and Balmes, 2014). Therefore, the synergistic interactions between O_3 and other air pollutants can significantly enhance lung inflammation and damage, exacerbating lung pathology manifestation. These combined exposures pose a greater risk to respiratory health than individual pollutants alone, underscoring the importance of comprehensive air quality management strategies.

2.4. Chronic obstructive pulmonary disease with inflammation, emphysema, and fibrosis

COPD represents a significant public health concern, which has been investigated by our team and others for over a decade, from cigarette smoke and particulate matter to O_3 exposure. Chronic O_3 exposure induced mixed Th2 and Th17 immune responses. O_3 inhalation during allergic sensitization coalesces in generating a significantly worse Th17 asthmatic phenotype (Hussain et al., 2024). The increased release of IL-17 and IL-1β, and the activation of p38 MAPK in the lungs of ozone-exposed mice are dependent on IL-17R signaling, underlying the increase in airway hyperresponsiveness induced by ozone exposure (Pinart et al., 2013). O_3-induced upregulation of Th2-related cytokines and neutrophil chemoattractants (Bosson et al., 2003). Repetitive ambient O_3 exposure led to early and exaggerated pulmonary inflammation and remodeling of distal and interstitial airspaces and the activation of Th2 inflammatory and profibrotic pathways (Wagner et al.,

2020), providing a mechanistic framework to support the emerging epidemiological associations among air pollution, diabetes, and lung disease. Our preliminary findings on O_3 exposure in mice (1.5 ppm, twice weekly for 6 weeks) indicate the induction of AHR, recruitment of eosinophils and neutrophils, activation of Th2 and Th17 immune responses, injury to respiratory epithelium, and chronic inflammation resulting in disruption of alveolar septae, emphysema, impaired repair, and fibrosis mirroring the characteristics observed in patients with COPD.

Cholinergic signaling contributes to the response, as the muscarinic inhibitor Tiotropium (TTP) (Kistemaker et al., 2015; Kistemaker et al., 2019; Wollin and Pieper, 2010) inhibits airway hyperresponsiveness, IL-5 production, myeloperoxidase (MPO) activity, and eosinophil recruitment. Therefore, chronic O_3-induced lung pathology in mice mimics COPD with AHR. Additionally, six weeks of chronic O_3 exposure in mice induces a persistent inflammatory process, marked by alveolar enlargement and damage linked to epithelial apoptosis, increased protease expression, and collagen deposition, culminating in the loss of epithelial tissue (Triantaphyllopoulos et al., 2011). These observations indicate that chronic O_3 exposure fundamentally alters lung architecture through tissue remodeling and fibrosis processes.

3. O_3 exposure triggers innate immunity pathways

3.1. Role of inflammatory mediators induced by O_3 exposure

The irritant effects of O_3 prompt the release of a wide range of proinflammatory cytokines, chemokines, and mediators, contributing to the inflammatory response. Among these are interleukins such as IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-17A, IL-22, and IL-33, which play critical roles in orchestrating immune responses and recruiting immune cells to the site of inflammation (Borish and Steinke, 2003; Mumby et al., 2019). Additionally, tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and transforming growth factor-beta (TGF-β) are also released, further amplifying the inflammatory cascade (Borish and Steinke, 2003; Mumby et al., 2019). Chemokines such as CC and CXC family recruit neutrophils, monocytes, and lymphocytes to the affected tissue (Borish and Steinke, 2003; Mumby et al., 2019). Furthermore, lipid mediators like leukotrienes and prostaglandins contribute to inflammation and bronchoconstriction (Borish and Steinke, 2003; Mumby et al., 2019).

The differential expression reanalyzing the public deposited RNAseq datasets from days 1 and 4 after single O_3 exposure (GSE161538), or chronic by three consecutive weeks of O_3 exposure (GSE156799) **(**Fig. 2**C-F)** highlighted that *Il-33, Areg,* and *Myd88* were commonly significative up-regulated 1 and 4 days after single O_3 exposure **(**Fig. 2**D-E)** and repeated O_3 exposure during three weeks **(**Fig. 2**F)**, suggesting that Il-33, amphiregulin and Myd88 are pivotal in acute and chronic events triggered by O_3 exposure **(**Fig. 2**G)**. Correlating the differential expression of genes *Il-33, Areg,* and *Myd88* with cell gene signatures **(**Fig. 1**E)**, it is possible that type 1 and 2 alveolar epithelial cells can be a potential source of Il-33 and amphiregulin; meanwhile, increased Myd88 activity induced by acute and chronic O_3 exposure indicating TLR/IL-1 pathway activation **(**Fig. 2**G)**, and thus, contributing to type 2 of immune response progression.

Overall, the release of these inflammatory mediators in response to O_3 exposure underscores the complex and multifaceted nature of the inflammatory response in the airways, which is reviewed shortly here:

Aryl hydrocarbon receptor (AhR) is a protein that functions as a transcription factor, playing a critical role in immune response regulation, cell proliferation, and differentiation. It is implicated in normal cellular functions and allergic diseases (Riaz et al., 2022). We have observed that chronic O_3 exposure activates the Aryl hydrocarbon receptor (AhR), leading to increased production of tryptophan and lipoxin A4 in mice (Michaudel et al., 2020). In $AhR^{-/-}$ mice, we observed increased chronic lung inflammation, airway hyperresponsiveness, and tissue remodeling, accompanied by fibrosis and heightened recruitment of IL-17A and IL-22-expressing cells compared to control mice. Administration of IL-17A- and IL-22-neutralizing antibodies attenuated lung inflammation in both $AhR^{-/-}$ and control mice. Moreover, we observed enhanced lung inflammation and recruitment of ILC2, ILC3, and T cells following T cell-specific AhR depletion using AhR^{CD4cre}-deficient mice (Michaudel et al., 2020). Together, the data demonstrate that O_3 exposure activates AhR, which in turn regulates lung inflammation, airway hyperresponsiveness, and tissue remodeling by modulating IL-22 expression.

Amphiregulin (AREG) is an epidermal growth factor (EGF) family member and regulates cellular proliferation, differentiation, and development. It binds to the EGF receptor (EGFR), initiating signaling pathways that influence cell growth and survival. Amphiregulin is produced by various cell types, including epithelial and immune cells, playing a role in tissue repair and inflammation. Its expression is up-regulated in response to injury and involves wound healing and the development of certain diseases, including cancer and chronic inflammatory conditions (Singh et al., 2022b). Amphiregulin is critical in the T helper type 2 (Th2) immune response, particularly in the lungs. Th2 cells, which are essential for clearing helminth infections, express amphiregulin, enhancing epithelial cell proliferation and repair mechanisms. This cytokine is also produced by other immune cells, such as mast cells, basophils, and group 2 innate lymphoid cells (ILC2s). The interaction between Th2 cells and epithelial cells via amphiregulin contributes to the maintenance and repair of the epithelial barrier, highlighting its role in immune responses and tissue homeostasis. Amphiregulin's involvement in these processes suggests potential therapeutic targets for diseases like asthma and allergic inflammation (Singh et al., 2022b; Zaiss et al., 2015). Exposure to O_3 induces significant oxidative stress and lung inflammation. Amphiregulin has been shown to play a protective role in such scenarios by suppressing epithelial and endothelial cell apoptosis and promoting cell survival and repair (Florentin et al., 2022; Ogata-Suetsugu et al., 2017). Studies on mice indicate that amphiregulin levels increase following O_3 exposure (Michaudel et al., 2018b; Sokolowska et al., 2019). It has been considered that *Areg* is an O_3-sensitive gene (Vasu et al., 2010) and may help mitigate lung damage by enhancing epithelial cell resilience and repair mechanisms. Understanding the O_3 modulation of amphiregulin and its signaling may provide new insights into potential therapeutic strategies to combat O_3-induced lung injury and inflammation.

Inflammasome and Interleukin-1 pathways mediate the innate response, which is part of innate immunity and is initiated by endogenous or environmental danger or injury events, particularly in response to O_3 exposure. The cytosolic multiprotein NLRP3 inflammasome complex is activated in response to signals stemming from tissue injury, metabolic alterations, and infection (Allen et al., 2009; Hise et al., 2009; Lamkanfi and Dixit, 2012; Stienstra et al., 2011; Strowig et al., 2012; Vandanmagsar et al., 2011; Wen et al., 2012). Upon activation of the cytoplasmic NLRP3 protein, a multimeric complex is formed, consisting of the adaptor protein ASC and caspase-1. This complex plays a pivotal role in the processing and secretion of proinflammatory cytokines, such as IL-1β, contributing to the inflammatory response (Agostini et al., 2004; Gombault et al., 2012; Kanneganti et al., 2006). Caspase-1 cleaves inactive pro-IL-1β and pro-IL-18 precursor proteins into their biologically active forms upon activation. Tight regulation of the cytokine IL-1β is crucial, as it plays an indispensable role in systemic inflammation and the recruitment of neutrophils (Dinarello 2009).

It is now widely acknowledged that two signals are necessary for IL-1β production: first, the production of pro-IL-1β and NLRP3 is regulated via TLR ligation, and second, inflammasome oligomerization, caspase-1 recruitment and activation, and caspase-1-dependent cleavage of pro-IL-1β releases biologically active IL-1β. This second signal may be induced by a wide variety of molecules classified either as pathogen-associated molecular patterns (PAMPs) produced by microbes/pathogens or danger-associated molecular patterns (DAMPs), self-components

induced by injury. Upon inhalation, environmental pollutants such as particles, including silica (Cassel et al., 2008; Rabolli et al., 2014), nanoparticles (Jessop and Holian 2015; Yazdi et al., 2010), and fibers like asbestos (Dostert et al., 2008; Hornung et al., 2008), and aluminum salt (alum) adjuvant (Cassel et al., 2008) can cause cell injury with the release of DAMPs, and direct or indirect inflammasome activation leading to IL-1α/β production, and cell death again with subsequent release of DAMPs, and thereby fueling airway inflammation (Reisetter et al., 2011; Unno et al., 2014). Thus, environmental pollutants may present a health hazard due to their capacity to induce IL-1R signaling, with inflammatory amplification provoked by the DAMPs source. Endogenous DAMPs originate from metabolic stressors such as high concentrations of cholesterol, glucose, amyloid-β protein, adenosine triphosphate (ATP), or monosodium urate (MSU) crystals, as recently reviewed (Gombault et al., 2012), can also contribute to exacerbation of tissue inflammation.

NLRP3 inflammasome activation occurs through two primary mechanisms: plasma membrane rupture, as observed with bacterial toxins and ATP, or phagocytosis of particles (Cassel et al., 2009; Pedra et al., 2009). Extracellular ATP (eATP) or toxins from various sources induce cellular K^+ efflux and pore formation (Pelegrin and Surprenant 2007). Particles, including silica, alum, fibrillar amyloid-β protein, or MSU, prompt lysosomal destabilization and rupture, releasing lysosomal proteases such as cathepsin B into the cytoplasm. This process is accompanied by increased K^+ efflux and reactive oxygen species (ROS) production. Our group and others have described mechanistic links between ATP release and particle-mediated inflammasome activation pathways (Riteau et al., 2012; Riteau et al., 2010).

O_3 exposure has been shown to activate the NLRP3 inflammasome complex. All proteins comprising the NLRP3 inflammasome complex, including ASC and caspase-1, gasdermin D (GSDMD) (Tian et al., 2022; Tian et al., 2021), seemed essential for developing airway inflammation and hyperreactivity (AHR) in response to O_3 (Michaudel et al., 2016; Sokolowska et al., 2019; Wiegman et al., 2020). O_3 exposure in mice has been shown to increase the expression of both IL-1α and IL-1β in the lung (Michaudel et al., 2016; Sokolowska et al., 2019; Wiegman et al., 2020). O_3-induced inflammation is mediated by IL-1α, a classical DAMP released from dying cells and thus functions as an alarmin. Its signaling via IL-1R1 depends on the adaptor protein myeloid differentiation factor-88 (MyD88) in epithelial cells (Michaudel et al., 2018c). The blockage of IL-1α through administering anti-IL-1α neutralizing antibodies in $MyD88^{acid}$ mice reduced O_3-induced lung inflammation compared with wild-type mice treated with rmIL-1α, with reduced cell recruitment and inflammation (Michaudel et al., 2018c). Importantly, mice deficient in IL-1R1 exhibit partial protection against O_3-induced inflammation (Johnston et al., 2007). Other members of the IL-1 family, such as IL-18, IL-36, or IL-38, which also possess inflammatory properties, have not yet been investigated in the context of O_3 exposure.

Interleukin-6 (IL-6) plays a crucial role in the pulmonary response to O_3 exposure and is rapidly induced in the lungs in response to O_3 exposure, which contributes to the recruitment of immune cells such as neutrophils and macrophages to the airways, thereby exacerbating inflammation and implicated in respiratory pathology (Yu et al., 2002). Subacute exposure to O_3 (2, 0.5, and 0.3 ppm) in the $IL\text{-}6^{-/-}$ mice or anti-IL-6 antibody treatment resulted in significantly reduced levels of protein, neutrophils, and soluble TNF receptors in the BALF, while airway hyperresponsiveness remained unaffected (Johnston et al., 2005c; Yu et al., 2002). Additionally, IL-6 regulates other cytokines and inflammatory mediators, further amplifying the inflammatory response to O_3. In adiponectin-deficient mice exhibiting enhanced IL-6 levels, an exaggerated inflammatory response to O_3 suggests that IL-6 can modulate the severity of O_3-induced lung damage (Kasahara et al., 2014). A pivotal role of IL-6 in the hyper-inflammatory condition observed in adiponectin-deficient mice following O_3 exposure was elucidated using double $adiponectin^{-/-}$ and $IL\text{-}6^{-/-}$ mice. This involvement of IL-6 was associated with activating SAA3, IL-17A, and G-CSF pathways (Kasahara et al., 2014). Overall, IL-6 is a pivotal mediator in the lung's response to O_3 exposure, driving the recruitment of inflammatory cells and modulating the overall inflammatory milieu in the airways.

Interleukin-10 (IL-10) is known for its anti-inflammatory properties and plays a crucial anti-inflammatory role in the body's response to O_3 exposure, helping to mitigate pulmonary inflammation and injury. IL-10 can attenuate the inflammatory response induced by O_3 by inhibiting the production of proinflammatory cytokines like TNF-α/IL-1 and decreasing the expression of inducible nitric oxide synthase (iNOS) (Reinhart et al., 1999). The IL-10-deficient mice exhibit significantly greater inflammation, indicating the protective role of IL-10 in the context of O_3 exposure. Recent findings from IL to $10^{-/-}$ mice demonstrate increased neutrophil recruitment following low-dose O_3 exposure (0.3 ppm) for 1 to 3 days, accompanied by enhanced activation of NF-κB and upregulation of genes associated with inflammation, including macrophage inflammatory protein 2 (MIP-2), cathepsin E, and serum amyloid A3 (Saa3) (Backus et al., 2010). IL-10 mediates its effects by modulating the activity of the NF-κB inflammatory pathway, thus reducing the severity of the immune response to O_3 (Backus et al., 2010). These findings suggest that enhancing IL-10 levels or activity could be a potential therapeutic strategy to protect against O_3-induced lung injury and inflammation. Therefore, endogenous IL-10 may confer partial protection against O_3-induced lung inflammation.

Interleukin-13 (IL-13) plays a pivotal role in the pulmonary response to O_3 exposure, particularly in enhancing airway hyperresponsiveness (AHR) and inflammation. IL-13 is known to augment the effects of O_3 by increasing the recruitment of neutrophils and macrophages into the bronchoalveolar space, thus intensifying the inflammatory response (Williams et al., 2008). Studies have shown that mice deficient in IL-13 exhibit reduced O_3-induced AHR and inflammation, while IL-13 overexpressing mice display heightened responses (Williams et al., 2008). Furthermore, IL-13 stimulates the production of other cytokines like IL-6 and IL-1β, further promoting inflammatory pathways and tissue damage (Mathews et al., 2017). In obese mice, IL-13 exacerbates O_3-induced pulmonary responses by synergizing with TNF via TNFR2, leading to increased pulmonary mechanics and inflammatory cell recruitment (Williams et al., 2013). These findings underscore the crucial role of IL-13 in mediating the adverse effects of O_3 on respiratory health.

Interleukin-17A (IL-17A) activates Th17 immune responses associated with neutrophilic inflammation (Misra and Agarwal 2022). O_3 exposure increases IL-17A levels in the lungs, contributing to airway inflammation and hyperresponsiveness. IL-17A is increased and is critical in recruiting neutrophils following O_3 exposure (0.3 ppm for 24–72 h) (Mathews et al., 2014). Our study utilizing a chronic 6-week O_3 exposure model showed that pulmonary IL-17A, IL-1β, and p38 MAPK activation were reduced in IL-17RA deficient mice (Pinart et al., 2013). Importantly, airway hyperresponsiveness observed after O_3 exposure depends on IL-17RA signaling, which is mediated by the increased contractility of airway smooth muscle. However, the emphysema and lung inflammation induced by O_3 may be independent of IL-17A (Pinart et al., 2013). Blocking IL-17A with monoclonal antibodies has reduced the chronic effects of O_3-induced airway hyperresponsiveness, neutrophil recruitment, and cytokine production in both lean and obese mice, indicating its critical role in mediating these effects (Zhang et al., 2016). Overall, IL-17A is a crucial cytokine in the pathogenesis of O_3-induced lung inflammation and injury, driving neutrophilic recruitment and exacerbating airway hyperresponsiveness. However, currently, there is a lack of data regarding the Th17 family members IL-17C, IL-17E, IL-17F, and IL-22, which share structural homology with IL-10. Further research is needed to elucidate their potential involvement in these processes.

Interleukin-22 (IL-22) plays a vital role in the response to O_3 exposure by modulating lung inflammation and maintaining epithelial barrier integrity. O_3 exposure activates the aryl hydrocarbon receptor (AhR), which in turn increases IL-22 expression, contributing to the regulation of lung inflammation and airway hyperresponsiveness. In

mice lacking AhR, there is increased lung inflammation, airway hyperresponsiveness, and tissue remodeling, highlighting the protective role of IL-22 in mitigating these adverse effects (Michaudel et al., 2020). IL-22′s protective effects are partly mediated by its ability to reduce epithelial barrier damage and inflammation caused by O_3 exposure. Furthermore, IL-22 has been shown to attenuate antigen-induced eosinophilic airway inflammation, indicating its anti-inflammatory properties in the context of respiratory insults (Takahashi et al., 2011). Therefore, IL-22′s ability to regulate both inflammatory responses and tissue repair mechanisms underscores its importance in mitigating the adverse effects of O_3 on pulmonary health.

Interleukin-33 (IL-33), another released alarmin from dying cells, plays a significant role in the pulmonary response to O_3 exposure, particularly in inflammation and airway hyperresponsiveness. It is expressed at high levels in alveolar epithelial cells and exhibits a biphasic response following a single O_3 exposure (Michaudel et al., 2018b). Studies indicate that O_3 exposure increases IL-33 levels in the lungs, contributing to airway inflammation and hyperresponsiveness, especially in obese mice (Kasahara and Shore 2020; Mathews et al., 2017). Additionally, IL-33 enhances type 2 cytokine production and the activity of innate lymphoid cells (ILC2s), further exacerbating the inflammatory response to O_3 (Kasahara and Shore 2020). After O_3 exposure, there is a rapid disruption of the epithelial barrier within 1 h, followed by a second phase of respiratory barrier injury characterized by increased neutrophil recruitment, reactive oxygen species production, airway hyperresponsiveness, and IL-33 expression in both epithelial and myeloid cells in wild-type mice (Michaudel et al., 2018b). The lack of IL-33 or the IL-33 receptor/ST2 (IL-33$^{-/-}$ and ST2$^{-/-}$ mice), O_3-induced epithelial cell injury with protein leakage, and myeloid cell recruitment and inflammation are further increased. However, the expression of reactive oxygen species in neutrophils and AHR was diminished (Michaudel et al., 2018b). Neutralization of ST2 recapitulated the enhanced O_3-induced neutrophilic inflammation (Michaudel et al., 2018b). Conversely, administration of recombinant mouse IL-33 reduced neutrophil recruitment in IL-33$^{-/-}$ mice (Michaudel et al., 2018b). Thus, playing a protective role against epithelial damage and inflammation. In fact, IL-33 mediates the activation of transforming growth factor-β (TGF-β and epidermal growth factor (EGF) signaling, involved in epithelial cell regeneration by Intestinal Stem Cells (Calafiore et al., 2023; Guan et al., 2023). Thus, IL-33 can regulate regeneration after tissue damage, but further studies are needed to dissect this role in O_3-exposed lungs. Overall, IL-33 is a critical mediator in the pulmonary response to O_3, influencing both inflammatory processes and the maintenance of lung tissue integrity.

Interferon-γ (IFN-γ) modulates the Th1 immune response and is involved in proinflammatory and antiviral activities (Ng et al., 2023). O_3 exposure can induce IFN-γ production in the lungs as part of the body's immune response to oxidative stress and inflammation caused by O_3 inhalation. IFN-γ production is critical for orchestrating innate and adaptive immune responses, mainly by activating macrophages and enhancing their ability to combat intracellular pathogens. Regarding O_3 exposure, IFN-γ has been shown to protect against O_3-induced lung damage by reducing lesion volumes and mitigating alveolar damage. This protective effect is achieved through the induction of IFN-γ, which enhances the immune response and reduces the extent of lung injury (Dziedzic and White 1987). Additionally, exposure to O_3 has been observed to decrease macrophage responsiveness to IFN-γ, leading to compromised immune functions such as phagocytosis and reactive oxygen species production, which underscores the importance of IFN-γ in maintaining pulmonary immune competence under O_3 stress (Cohen et al., 1996). However, it is noteworthy that high doses of O_3 have been shown to reduce IFN-γ production and cytotoxicity of innate lymphoid cells (ILC) ILC1 but not ILC2 (Estrella et al., 2019), potentially shifting towards a Th2 immune response. These findings highlight potential mechanisms by which innate leukocytes, such as macrophages and ILCs, react to air pollution, thereby increasing susceptibility to infections and allergies. Thus, IFN-γ is integral in mediating the immune response and mitigating the adverse effects of O_3 exposure on lung health.

Transforming growth factor-β (TGF-β) plays a critical role in the development of fibrosis, including chemical-induced lung fibrosis (Gasse et al., 2007). TGF-β is a multifunctional cytokine that regulates immune responses, cellular proliferation, differentiation, and tissue remodeling. In response to O_3 exposure, TGF-β is up-regulated, contributing to the lungs' inflammatory and fibrotic processes. This cytokine mediates epithelial-to-mesenchymal transition (EMT), leading to fibrosis and impaired lung function. Additionally, TGF-β modulates the immune response by promoting regulatory T cell differentiation and suppressing excessive inflammation (Prud'homme 2007), thus maintaining tissue homeostasis and mitigating damage caused by O_3-induced oxidative stress. However, chronic exposure to O_3 may lead to dysregulation of TGF-β signaling, exacerbating fibrosis and contributing to chronic obstructive pulmonary disease (COPD) and other chronic pulmonary conditions (Aschner and Downey 2016). O_3-induced emphysema and pulmonary fibrosis in mice may be mediated by TGF-β (Katre et al., 2011). For instance, studies have shown that TGF-β regulates the release of extracellular matrix components and facilitates the healing of injured lung tissues by promoting fibroblast proliferation and differentiation into myofibroblasts (Aschner and Downey 2016). Chronic O_3 exposure (5 days, 0.5 ppm, 8 h/day) for 5 cycles resulted in increased levels of TGF-β protein in BALF and plasminogen activator inhibitor 1 (PAI-1), along with lung fibrosis. The TGF-β signaling pathway blockade with IN-1233 suppressed O_3-induced Smad2/3 phosphorylation, PAI-1 and collagen expression, and α-smooth muscle actin (α-SMA) deposition in the lung. These findings suggest that TGF-β is a key mediator that balances tissue repair and lung inflammation following O_3 exposure, mediating O_3-induced lung fibrotic responses. However, further research is needed to confirm these results using other inhibitors and TGF-β antibodies.

Tumor Necrosis Factor-α (TNF-α) is another fundamental proinflammatory cytokine involved in the biological response to O_3 exposure (Fakhrzadeh et al., 2004; Shore et al., 2011; Zamora et al., 2005). Studies have shown that O_3 exposure leads to the increased expression of TNF-α in lung tissues, which in turn activates various inflammatory pathways, including the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) pathways (Cho et al., 2007). O_3 produces nitric oxide, TNF-α, and tissue injury, which depend on NF-κB p50 (Fakhrzadeh et al., 2004). Blocking TNF-α with specific antibodies has been shown to mitigate O_3-induced neutrophilic inflammation (Bhalla et al., 2002). Furthermore, TNF-neutralizing antibodies reduced protein and neutrophil recruitment in BALF and IL-1α, IL-6, and IL-10 expression in obese mice upon O_3 exposure (Williams et al., 2015). Additionally, TNF-α knockout mice exhibit significantly less O_3-induced inflammation and lung damage than wild-type mice, further underscoring its importance in O_3-induced pulmonary toxicity (Cho et al., 2001). Overall, TNF-α is a critical factor in the pathogenesis of O_3-induced epithelial injury and lung inflammation induced by O_3 exposure, modulating the acute and chronic phases of the response.

Chemokines play a pivotal role in the inflammatory response to O_3 exposure by mediating the recruitment and activation of immune cells in the lungs (Hollingsworth et al., 2007). O_3 inhalation induces the release of chemokines from epithelial cells, macrophages, and other immune cells, orchestrating the recruitment and activation of inflammatory cells such as neutrophils, macrophages, and lymphocytes into the lung tissue (Hollingsworth et al., 2007). These chemotactic proteins act as signaling molecules, guiding immune cells to the site of inflammation and promoting their adhesion to endothelial cells (Russo et al., 2010; Russo et al., 2014), thus exacerbating tissue damage, inflammation, and fibrosis (Russo et al., 2023). Moreover, chemokines regulate the expression of adhesion molecules on endothelial cells, facilitating the transmigration of immune cells across the endothelial barrier into the lung parenchyma (Russo et al., 2010; Russo et al., 2014; Russo et al.,

2023). Consequently, targeting chemokines and their receptors represents a promising therapeutic approach (Russo et al., 2010) for mitigating O_3-induced lung inflammation and injury.

O_3 exposure induces the secretion of various chemokines, including macrophage inflammatory protein-2 (MIP-2) and monocyte chemoattractant protein-1 (MCP-1), which attract neutrophils and monocytes to the site of inflammation (Zhao et al., 1998). These chemokines are critical for developing O_3-induced pulmonary inflammation, as they facilitate the influx of inflammatory cells into the lungs, leading to tissue damage and airway hyperresponsiveness. The expression of cytokine-induced neutrophil chemoattractant (CINC) is significantly increased following O_3 exposure, promoting neutrophil infiltration and contributing to the inflammatory response (Koto et al., 1997). Furthermore, IL-1α released by macrophages in response to O_3 stimulates alveolar epithelial cells to secrete chemokines, enhancing the inflammatory cascade (Manzer et al., 2008).

Human exposure to 0.2 ppm of O_3 for 2 h, twice 3 weeks apart, can induce healthy and asthmatics' chemokine production in lung biopsies (Bosson et al., 2003). Immunostaining for chemokines in the bronchial epithelium revealed significant expression of the neutrophil chemoattractants GRO-a/CXCL1, CXCL5/ENA-78, and CXCL8/IL-8 in the asthmatic group compared to the healthy group, but not for CX3CL1/Fractalkine (Bosson et al., 2003). The chemokine receptor CXCR2 is critically involved in neutrophil chemotaxis induced by murine chemokines CXCL1/KC and CXCL2/MIP-2, which are up-regulated in lungs following O_3 exposure (Johnston et al., 2005b). Mice deficient for CXCR2 had reduced protein and epithelial cells in the BAL, suggesting reduced lung injury and reduced AHR compared to wild-type control mice. Therefore, CXCR2 is essential for maximal neutrophil recruitment, epithelial cell sloughing, and persistent AHR upon O_3 exposure (Johnston et al., 2005b).

Mice exposure to 0.8 ppm O_3 leads to the upregulation of various chemokines including CXCL1/KC, CXCL2/MIP-2, CXCL3/GROalpha, CXCL10/IP-10, CCL3/MIP-1alpha, CCL7/MCP-3, and CCL11/Eotaxin-1 mRNA in the lungs post-exposure, with the airway epithelium identified as a significant source of CCL7 (Michalec et al., 2002). Depletion using anti-mouse antibodies targeting CXCL1/2/3, CCL7, and CXCL10 neutralization effectively inhibited neutrophil recruitment, suggesting that CCL7 and CXCL10 may play a critical role in orchestrating neutrophilic inflammation induced by oxidative stress resulting from O_3 exposure (Michalec et al., 2002). These findings underscore chemokines' importance in mediating the airways' inflammatory response following O_3 exposure and highlight their potential as therapeutic targets for mitigating O_3-induced lung inflammation and injury.

Following the cessation of acute O_3 exposure, mice exhibited elevated chemerin levels in BALF after 24 h. Interestingly, despite the increase in chemerin levels, the receptor for chemerin, Ccrl2, which typically modulates chemerin levels in the epithelial lining fluid of the lungs, was found not to contribute to the development of O_3-induced lung pathology in mice (Malik et al., 2017). This suggests a complex regulatory mechanism for chemerin in the context of O_3 exposure, where its role in lung pathology may be independent of Ccrl2-mediated signaling pathways. Further investigation into the mechanisms underlying chemokine involvement in O_3-induced lung inflammation and injury is warranted to fully understand its potential as a therapeutic target in mitigating respiratory damage caused by O_3 exposure. Overall, chemokines are essential mediators in the inflammatory processes triggered by O_3 exposure, driving immune cell recruitment and contributing to lung injury and dysfunction.

3.2. Role of innate lung leukocytes in response to O_3 exposure

Innate lymphoid cells (ILCs) are critical in the body's response to O_3 exposure. Exposure to O_3 has been shown to induce type 2 immunity in the nasal airways, leading to conditions such as eosinophilic rhinitis, which depends on the presence of ILCs (Kumagai et al., 2016). ILCs play a crucial role in the immune response to O_3 exposure in the lungs. O_3 induces significant airway inflammation, marked by the activation of various immune cells, including ILCs. Specifically, type 2 ILCs (ILC2s) are known to mediate airway inflammation and hyperresponsiveness by producing cytokines such as IL-5 and IL-13, which are crucial for eosinophilic inflammation and asthma-like symptoms (Yang et al., 2016). Additionally, IL-33, a cytokine that can activate ILC2s, has been implicated in exacerbating the inflammatory response to O_3 in obese individuals, indicating that the interplay between ILCs and cytokines significantly impacts the severity of O_3-induced inflammation (Mathews et al., 2017; Michaudel et al., 2018b; Williams et al., 2015). In obese mice, the response to O_3 is even more pronounced, with ILC2s playing a role in enhanced airway hyperresponsiveness (AHR) and inflammation (Mathews et al., 2017). The activation of ILC2s by IL-33, a cytokine released during epithelial cell damage, further exacerbates the inflammatory response, leading to increased production of IL-5 and IL-13, which are critical for the recruitment of inflammatory cells to the lungs (Bauer et al., 2015). This complex interplay of ILCs and cytokines underscores the significant role of ILCs in the pathophysiology of O_3-induced lung inflammation and hyperresponsiveness. These interactions of ILCs modulate the lung's response to O_3, linking environmental pollutants to respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD).

γδ T cells play a significant role in the immune response to O_3 exposure, contributing to pulmonary inflammation and injury. Exposure to O_3 increases the number of γδ T cells in the lungs, producing cytokines such as IL-17A. This cytokine is crucial for recruiting neutrophils and the subsequent inflammatory response. In mice deficient in γδ T cells, the O_3-induced increases in macrophages, neutrophils, and inflammatory cytokines like G-CSF and IL-6 are significantly attenuated, highlighting the pivotal role of γδ T cells in mediating these responses (Mathews et al., 2014). In addition, the deficiency of γδ T cells results in decreased IL-17A production and attenuated inflammatory responses, underscoring these cells' crucial role in orchestrating the immune response to O_3-induced lung damage (Mathews et al., 2014). Furthermore, γδ T cells are involved in the initial stages of the inflammatory response following long-term low-dose O_3 exposure by secreting chemokines that attract macrophages and neutrophils to the lungs. These findings demonstrate that γδ T cells are critical regulators of the pulmonary immune response to O_3, driving the inflammation and recruitment of other immune cells necessary to manage the oxidative stress and damage caused by O_3 exposure. This response is essential for the body's defense mechanisms against environmental pollutants.

Natural killer T (NKT) cells play a significant role in the immune response to O_3 exposure, particularly in developing airway hyperreactivity (AHR). Studies in mice have shown that repeated exposure to O_3 leads to severe AHR, characterized by increased airway NKT cells, neutrophils, and macrophages. Interestingly, mice deficient in NKT cells ($CD1d^{-/-}$ and $Jα18^{-/-}$) did not develop O_3-induced AHR, highlighting the necessity of these cells in the response mechanism (Pichavant et al., 2008). Additionally, blocking NKT cell activation with an anti-CD1d antibody prevented the development of AHR (Pichavant et al., 2008). Severe AHR, accompanied by increased NKT cells, neutrophils, and macrophages in the airway, was absent in NKT cell-deficient, $CD1d^{-/-}$ and $Jα18^{-/-}$ mice and was dependent on IL-17A. Thus, O_3 exposure induces airway hyperreactivity, necessitating the presence of NKT cells and IL-17A production. O_3 exposure has also been shown to reduce natural killer (NK) cell activity in the lungs, which can impair the immune system's ability to combat infections and cancer. Continuous exposure to O_3 at 1.0 ppm for up to 10 days significantly decreased pulmonary NK cell activity, though this suppression was transient and returned to normal after prolonged exposure (Burleson et al., 1989). Furthermore, O_3 exposure in conjunction with surfactant protein D (SP-D) and NK cell interactions influences dendritic cell (DC) homing to lymph nodes, which is critical for initiating adaptive immune responses. O_3 exposure impaired this process, reducing NK cell IFN-γ production

and lung CCL21 mRNA expression, which are vital for effective DC migration (Ge et al., 2016). These findings indicate that NKT cells are crucial for the immune response to O_3 exposure, mediating both airway hyperreactivity and immune regulation, and their dysfunction can lead to impaired immune responses and increased susceptibility to respiratory conditions.

Mucosal-associated invariant T (MAIT) cells, another type of innate immune cells, may also be implicated in O_3-induced lung inflammation (Bugaut et al., 2024). O_3 exposure is known to induce significant immune responses, including the activation of various lymphocyte populations in the lungs. For instance, O_3 exposure can increase the proliferation and activity of lymphocytes in the bronchus-associated lymphoid tissue (BALT) and mediastinal lymph nodes, which are key areas where immune cells, including MAIT cells, reside (Dziedzic et al., 1990). Additionally, O_3 exposure has been shown to suppress the proliferation of various lymphocyte types and reduce IL-2 production, which is crucial for the growth and function of T cells, including MAIT cells (Becker et al., 1989). This suppression could potentially impair the immune surveillance and response capabilities of MAIT cells. Moreover, O_3's impact on the immune system includes altering the response of alveolar macrophages and epithelial cells, leading to the release of chemokines and cytokines that can modulate the activity of MAIT cells (Manzer et al., 2008). These changes suggest that O_3 exposure could affect the function and regulation of MAIT cells in the respiratory system. While direct studies on MAIT cells' response to O_3 are scarce, the available research on related immune responses provides a basis for understanding how these cells might be influenced by O_3 exposure, highlighting the need for further specific research in this area. However, further validation of this hypothesis is required. Additional research is needed to confirm the involvement of MAIT cells in this context and to elucidate their specific role in O_3-induced respiratory inflammation.

4. Therapeutic targets for pharmacological interventions

The existing experimental data offer promising drug targets for mitigating O_3-induced chronic inflammatory lung disease. Nonetheless, the efficacy of therapeutic interventions tested in mouse models necessitates validation through clinical studies. As depicted in Box-1, we outline the potential efficacy of agonists or antagonists that merit consideration for inclusion in clinical trials. While the list of proposed therapeutic targets is not exhaustive, ongoing research to refine inhibitors and gain new mechanistic insights holds promise for developing more efficacious antagonists. Advancements in understanding the complex pathways underlying chronic inflammatory lung diseases may uncover additional targets for intervention. However, it is essential to recognize that while pharmacological approaches offer potential benefits, addressing the root cause of these diseases is paramount. In this regard, reducing airborne pollution with exceptionally high levels of O_3 and smog stands out as the most efficacious measure to prevent the onset and progression of chronic respiratory ailments, such as fibrosis. Implementing comprehensive strategies to curb pollution, including regulatory measures, technological innovations, and public awareness campaigns, could significantly alleviate the burden of these debilitating conditions on global health.

Lung fibrosis involves the excessive deposition of ECM components, mainly collagen, leading to scarring and impaired lung function. The differentiation of fibroblasts into myofibroblasts is a crucial event in this process. This differentiation is driven by profibrotic signals such as TGF-β, increasing collagen synthesis and tissue stiffening, contributing to the pathological remodeling observed in fibrotic lung diseases. Understanding this process is crucial for developing therapeutic strategies to inhibit or reverse fibrosis. N23Ps (N-(2-methoxyphenyl)-3-(phenyl)acrylamides) are a novel class of highly potent class of compounds suppressing myofibroblast transdifferentiation, collagen deposition, cellular contractility, and altered cell shapes with a unique mode of action. Mechanistically, transcriptomics identified the SMURF2, a SMAD-specific E3 ubiquitin protein ligase2, as a potential therapeutic target network. Antifibrotic activity of N23Ps was verified by proteomics in a human ex vivo tissue fibrosis disease model, suppressing profibrotic markers SERPINE1 and CXCL8. N23Ps are highly potent developmental compounds inhibiting organ fibrosis in patients (Gerckens et al., 2021).

O_3-induced signaling pathways are highly complex, involving a web of oxidative stress responses, inflammatory cascades, and cellular signaling mechanisms that collectively contribute to lung injury and inflammation. These pathways interact dynamically, with oxidative stress triggering the activation of transcription factors such as NF-κB and AP-1, which in turn regulate the expression of pro-inflammatory cytokines and chemokines (Manzer et al., 2008; Mathews et al., 2017; Michaudel et al., 2020; Michaudel et al., 2016; Michaudel et al., 2018a; Michaudel et al., 2018b; Michaudel et al., 2018c; Pinart et al., 2013). The multifactorial nature of these processes poses significant challenges for single-target treatment approaches, as targeting a single pathway may fail to address compensatory mechanisms or secondary signaling loops that sustain injury and inflammation. Consequently, multi-target or combination therapies offer potential advantages by concurrently modulating multiple key components of the O_3-induced response. Such strategies could, for example, combine antioxidants to neutralize reactive oxygen species with inhibitors of inflammatory mediators, providing a more comprehensive approach to mitigating the complex, interconnected pathways driving O_3-related lung pathology.

5. Conclusion

O_3 exposure initiates cellular damage, initially causing oxeiptosis of the resident cell lining barrier, including leukocytes and non-leukocytes. Acute O_3 exposure leads to the activation of the NLRP3 inflammasome and subsequent release of mature IL-1α and IL-1β, potent inflammatory mediators. This cytokine recruits and activates neutrophils and macrophages, exacerbating inflammation and causing additional tissue damage. Furthermore, O_3's toxic adducts and endogenous damage-associated molecular patterns (DAMPs) activate receptors (TLR, inflammasomes, and DNA sensors) and secrete alarmins. This cascade releases alarmins, various inflammatory mediators (IL-1β, TNF, IL-6, IL-10, IL-17), and others. *In silico* analysis revealed that O_3 exposure is correlated with the upregulation of *Il-33, Areg,* and *Myd88* in the lungs, as well as the antioxidant *Nfe2l2* (NFR2) and apoptotic protein *Aimf1* genes, suggesting that they are essential in O_3-induced acute and chronic airway inflammation in mice, may be sustained by oxeiptosis and type 2 immune response (Fig. 2). Prolonged exposure to O_3 and other particulate pollutants exacerbates inflammation and may contribute to developing conditions such as emphysema, chronic inflammation, and fibrosis. Further exploring these inflammatory pathways is warranted to better understand their roles in O_3-induced lung pathology and develop targeted therapeutic interventions.

CRediT authorship contribution statement

Remo C. Russo: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Dieudonnée Togbe:** Writing – review & editing, Writing – original draft. **Isabelle Couillin:** Writing – review & editing, Writing – original draft. **Noria Segueni:** Writing – review & editing, Writing – original draft. **Lianyong Han:** Writing – review & editing, Writing – original draft. **Valérie F.J. Quesniaux:** Writing – review & editing, Writing – original draft. **Tobias Stoeger:** Writing – review & editing, Writing – original draft, Conceptualization. **Bernhard Ryffel:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics approval:

Ethical review and approval were waived for this study due to the use of public-deposited data, and Human lung single-cell data was taken from the integrated Human Lung Cell Atlas (HLCA) core https://data.humancellatlas.org/hca-bio-networks/lung/atlases/lung-v1-0, and downloaded via cellxgene (https://cellxgene.cziscience.com/collections/6f6d381a-7701-4781-935c-db10d30de293). The mouse datasets presented in this study are public-deposited data and can be found in online repositories. The name of the repository and accession numbers can be found below: https://www.ncbi.nlm.nih.gov/geo/, GEO accession: GSE185006, GSE161538, and GSE156799.

Consent to participate:

Patient consent was waived due to the use of public-deposited data, found below: https://data.humancellatlas.org/hca-bio-networks/lung/atlases/lung-v1-0, Human lung single-cell data was taken from the integrated Human Lung Cell Atlas (HLCA) core, downloaded via cellxgene (https://cellxgene.cziscience.com/collections/6f6d381a-7701-4781-935c-db10d30de293).

Availability of data and material:

The datasets presented in this study are public-deposited data and can be found in online repositories from the integrated Human Lung Cell Atlas (HLCA) core https://data.humancellatlas.org/hca-bio-networks/lung/atlases/lung-v1-0, and from https://www.ncbi.nlm.nih.gov/geo/ by GEO accession number: GSE185006, GSE161538 and GSE156799.

Data availability

Data will be made available on request.

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