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Commentary

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# Commentary: Pathways and Therapeutic Targets of Ozone induced Lung Disease

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## Abstract

Chronic exposure to ambient Ozone ($O_3$) air pollution induces respiratory inflammation and hyper reactivity, emphysema and interstitial lung fibrosis. $O_3$-inducedoxidative stress causes epithelial barrier injury and cell death activating Toll-like receptors, DNA sensing pathways and inflammasomes with production of a range of inflammatory chemokines with a mixed phenotype of COPD and asthma. $O_3$ exposure is often associated with other pollutants causing exacerbation leading to severe respiratory disease. Here, we review mechanisms and therapeutic targets to control $O_3$-induced COPD-like disease.

**Keywords**: Pollution; $O_3$, DAMPs; Inflammasome; Alarmin; IL-33

## Pathways

Ozone ($O_3$) is a 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 causing an inflammatory response. Prolonged exposure to $O_3$-polluted air is associated with increased morbidity and mortality, along with heightened responses to microbial or allergen challenges **[1-3].** Hyaluronic Acid (HA), a degradation product of matrix components, and HSP70, generated by O3-induced tissue damage, may potentially activate TLR4 **[4-6].**

Furthermore, the TLR adaptor proteins MyD88 and TIRAP are indispensable for the inflammatory response [5], as they activate NF-κB and regulate cytokine gene expression. IL-1β is a potent inflammatory mediator induced by bacterial infection and tissue injury [7] involving the activation of the inflammasome complex in the response to $O_3$ [8].

## Therapeutic targets for pharmacological interventions

The existing experimental data offer promising drug targets mitigating $O_3$-induced chronic inflammatory lung disease. Nonetheless, the efficacy of therapeutic interventions tested in mouse models necessitates validation through clinical studies. Below, we outline the potential efficacy of agonists or antagonists that merit consideration for inclusion in clinical trials:

- TNF neutralization presents a potential option, but reduces host innate immunity.
- IL-1β neutralizing antibodies or IL-1 receptor antagonist (Anakinra).
- Neutralizing antibodies targeting IL-23 and IL-17A are currently available.
- Dampening of NRP3inflammasome activation using inhibitors such as MCC950 [9-11].
- Blockade of nucleic acid sensor activation, notably cGAS/STING, using antagonists [11].
- Aryl hydrocarbon receptor activation by microbial tryptophan metabolites and more [12]
- Blockade of cholinergic pathway has a beneficial effect [13].
- Muscarinergic inhibitors such as Tiotropium are efficacious in COPD patients [14-18].
- ROS inhibitors such as N-acetyl cysteine attenuate $O_3$ inflammation [19].
- Microbial metabolites, such as butyrate activating HCAR2, attenuate inflammatory diseases [20,21].
- Histone modulators of Histone Deacetylases (HDAC) using HDAC inhibitors [22].
- DNase degrades inflammatory cell-free DNA upon cell death degrading DNA and lung inflammation [23,24].
- Inhibitors suppressing myofibroblast transdifferentiation, such as by N23Ps(N-(2-methoxyphenyl)-3-(phenyl) acrylamides) inhibit fibrosis [25].

## Novel compounds suppressing myofibroblast transdifferentiation

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 and results in increased 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. Thus, N23Ps are highly potent developmental compounds inhibiting organ fibrosis in patients **[25].** 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). 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 COPD model. Only 6 lungs from mice exposed to FA were included in the study to represent healthy lungs **[26,27].** Lianyong, please explain shortly how you analyzed these data, once that, the method will be mentioned here.

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. 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. Ozone-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 the disruption of the protective antioxidant complex KEAP1/PGAM5/NRF2, releasing NRF2 and the phosphatase PGAM5, which activates AIFM1. PGAM5, in response to oxidative stress induced by O3, 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 O3-exposure, GSE156799) models. RNAseq analysis showing RNA differential expression comparing $O_3$-exposure normalized by exposure to filtered air, from GSE161538 (Single ozone 2ppm 3h, n= 7 filtered air-exposed and 4 O3-exposed mice) and GSE156799 (Ozone 0.8 ppm 4h/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

O3-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) **[28].** 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.

## 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 ROS activating the NLRP3 inflammasome and release of mature IL-1α/β, a potent inflammatory mediator, activating neutrophils and macrophages causing additional tissue damage. Furthermore, ROS activate TLR, inflammasomes and DNA sensors triggering 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. 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 role in $O_3$-induced lung injury and develop targeted therapeutic interventions.


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