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*Review Article*

# Inflammasome, IL-1 and inflammation in ozone-induced lung injury

Chloé Michaudel[1,2], Aurélie Couturier-Maillard[2], Pauline Chenuet[2], Isabelle Maillet[2], Catherine Mura[2], Isabelle Couillin[2], Aurélie Gombault[2], Valérie F Quesniaux[2], François Huaux[4], Bernhard Ryffel[2,3]

*[1]ArtImmune SAS, 13 Avenue Buffon, Orleans, France; [2]CNRS, UMR7355, Experimental and Molecular Immunology and Neurogenetics, 45071 Orleans, France; [3]IDM, University of Cape Town, RSA; [4]ULB, Bruxelles Louvain Centre for Toxicology and Applied Pharmacology (LTAP), Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, Brussels, Belgium*

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**Abstract:** Exposure to ambient ozone causes airway hyperreactivity and lung inflammation, which represent an important health concern in humans. Recent clinical and experimental studies contributed to the understanding of the mechanisms of epithelial injury, inflammation and airway hyperreactivity, which is reviewed here. The present data suggest that ozone induced oxidative stress causes inflammasome activation with the release of IL-1, other cytokines and proteases driving lung inflammation leading to the destruction of alveolar epithelia with emphysema and respiratory failure. Insights in the pathogenic pathway may allow to identify novel biomarkers of ozone-induced lung disease and therapeutic targets.

**Keywords:** ROS, NLRP3 inflammasome, IL-1, IL-17, DAMP, airway hyperreactivity

## Background

Ozone ($O_3$) is a highly reactive molecule causing oxidative damage leading to cell death. Oxidative stress is likely a major mechanism causing cell and tissue injury with an acute respiratory inflammatory response. Long term exposure to ozone due to environmental pollution causes chronic morbidity and mortality with enhanced responses to microbial or allergen challenges [18, 33, 37].

Recent investigations revealed that cellular injury generate danger associated molecular patterns (DAMPs) such as adenosine triphosphate (ATP), uric acid (UA) crystals, hyaluronic acid (HA), heat shock protein (hsp) 70 and other DAMPs which activate PPRs such as NLRs or TLRs, see recent reviews on DAMPs, PPRs such as NLRs and TLRs.

TLR2 and TLR4 signalling appear to be involved in ozone-induced inflammation [58]. TLR4 activation might be mediated by HA, a degradation product of matrix components and hsp70, known TLR4 agonists [5, 35, 36]. Downstream, the TLR adaptor proteins MyD88 and TIRAP are required for the inflammatory response [35] activating NFκB-dependent inflammatory gene expression.

IL-1β is a potent inflammatory mediator which is induced by bacterial infection and tissue injury including ozone [10]. IL-1β production relies on activation of the inflammasome complex, which may be promoted by ozone directly or via injury-induced endogenous NLRP3 activators. Activation of caspase-1 and mature IL-1β production and airway hyperreactivity has been reported to be NLRP3 dependent but however not confirmed [13].

## Ozone-induced respiratory effects in rodents

It may be important to revisit the functional, inflammatory and morphological effects of ozone in the experimental mouse model, reflecting what may happen in man [39].

Acute exposure leads to lung injury affecting the respiratory epithelial barrier with release of inflammatory mediators and neutrophil recruitment [41]. The claudin protein family are

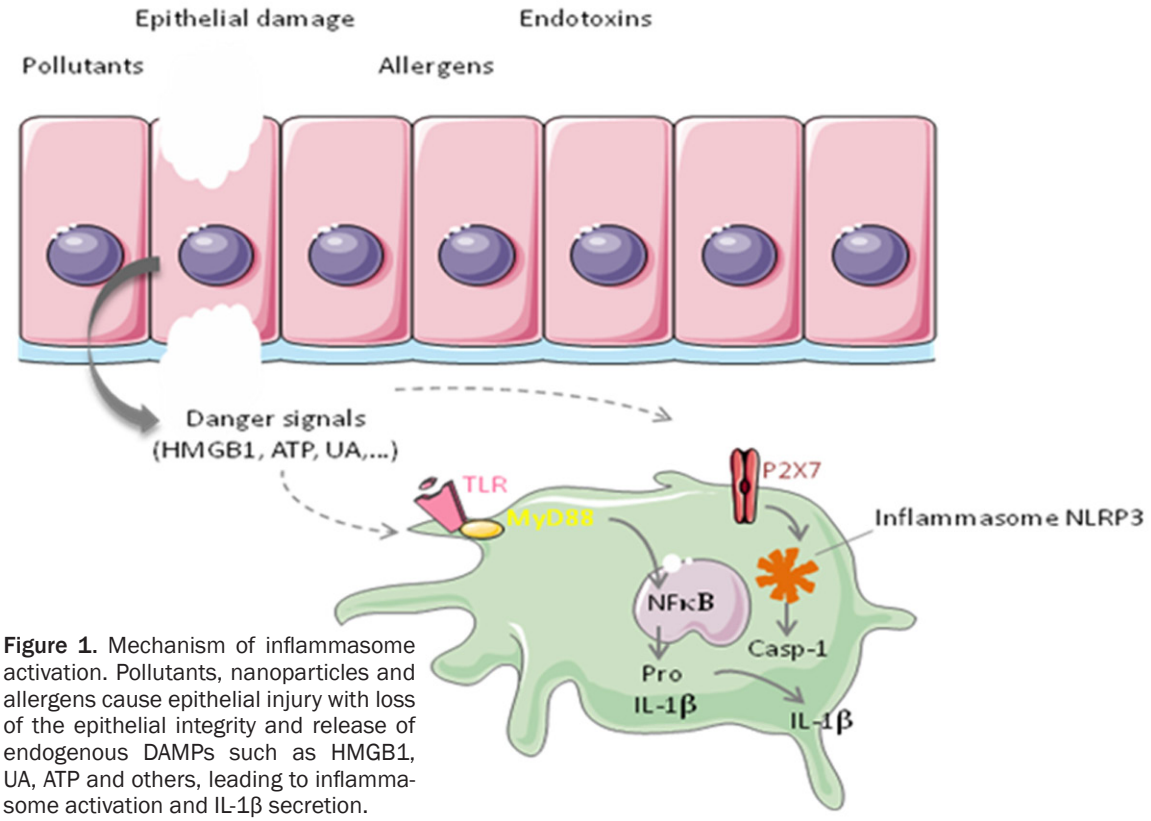


**Figure 1.** Mechanism of inflammasome activation. Pollutants, nanoparticles and allergens cause epithelial injury with loss of the epithelial integrity and release of endogenous DAMPs such as HMGB1, UA, ATP and others, leading to inflammasome activation and IL-1β secretion.

involved in the formation of the intercellular tight junctions controlling paracellular fluid exchanges. Claudin 18, exclusively expressed in alveolar epithelium, and claudin 4 are critical for homeostasis and involved in lung inflammation [51].

The respiratory epithelium represents the first structure exposed to ozone, but other cells in the lamina propria such as macrophages, dendritic cells, innate lymphoid cells and T cells, vascular endothelium, fibroblasts and smooth muscle cells are likely targets of the reactive species generated by ozone. Epithelial cell damage with disruption of the tight junctions [42] causes protein leak into BALF, the release of a variety of mediators such as IL-1α and IL-1β, IL-25, IL-33, TSLP, leukotrienes and prostaglandins, and chemokines which attract neutrophils, monocytes, lymphocytes and other cells.

Chronic ozone exposure such as twice weekly 2-3 part per billion (ppm) for 3 h causes repeated bouts of inflammation with progressive destruction of alveolar epithelial cells and emphysema within 6 weeks, resembling in part to that found in COPD [47, 55].

## Inflammasome and interleukin-1 - key players in inflammatory response

The immediate immune response, known as innate immunity, is triggered by endogenous or environmental dangers or injury events through the NLRP3 inflammasome complex. The NLRP3 inflammasome is a cytosolic multiprotein complex which is activated in response to signals derived from tissue injury, metabolic changes and infection [2, 17, 32, 53, 54, 56, 57]. Activation of the cytoplasmic NLRP3 protein induces the formation of a multimeric complex containing the adaptor protein ASC and the effector protein caspase-1 [1, 16, 27]. Activated caspase-1 cleaves inactive pro-IL-1β and pro-IL-18 precursor proteins to their biologically active forms. The regulation of the cytokine IL-1β needs a tight control as it plays an essential role in systemic inflammation and neutrophil recruitment [10, 34]. It is now accepted that two signals are necessary for IL-1β production (**Figure 1**): 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 releas-

es biologically active IL-1β. This second signal may be induced by a broad variety of molecules classified either as pathogen-associated molecular patterns (PAMPs) produced by microbes/pathogens or DAMPs induced by injury. Environmental pollutants such as silica and other particles including nanoparticles and fibres such as asbestos [11, 19], aluminum salt (alum) adjuvant [8] represent exogenous DAMPs. Endogenous DAMPs originate from metabolic stress such as high concentration of cholesterol, glucose, amyloid-β protein, ATP, or UA crystals as reviewed recently [16].

NLRP3 inflammasome activation occurs through two major mechanisms: plasma membrane rupture (for bacterial toxins and ATP) or phagocytosis of particles [9, 44]. Extracellular ATP (eATP) or toxins from different sources cause cellular $K^+$ efflux and pore formation [45]. Particles including silica, alum, fibrillar amyloid-β protein, or UA crystals cause lysosomal destabilization and rupture with the release of the lysosomal proteases such as cathepsin B into the cytoplasm with increased $K^+$ efflux and reactive oxygen species (ROS) production. Recently, we described mechanistic links between ATP release and particle-mediated inflammasome activation pathways [49, 50].

### Role of reactive oxygen species (ROS)

Since ozone generates reactive oxygen species, a direct activation of the inflammasome by ozone is likely to occur. However endogenous danger signals released upon oxidative cell injury such as eATP and UA crystals may contribute to NLRP3 inflammasome activation. The cascade of events resulting in NLRP3 activation needs further investigations.

To elucidate the role of ROS, the scavenger N-acetylcysteine (NAC) has been tested in the experimental mouse model. The therapeutic administration of NAC conferred a partial amelioration of ozone-induced inflammation and airway hyperresponsiveness (AHR) [34].

Increased IL-1β expression in the lung upon ozone exposure has been established [43]. Absence of IL-1R1 [24] or neutralisation of IL-1α and IL-1β by the IL-1R antagonist anakinra partially protected from ozone-induced inflammation [43]. However the contribution of IL-1α in ozone-induced lung inflammation has not been established.

Altered functions of human alveolar macrophages upon *in vitro* exposure to ozone (0.1-1.0 ppm for 2-4) were reported with increased release of prostaglandin E2 (Becker et al. 1991). Alveolar macrophages obtained from guinea pigs and humans exposed to ozone significantly secreted higher levels of cytokines with a peak value at 0.4 ppm for 1 h in the absence of cytotoxicity. IL-1β, IL-6, TNF-α, and IL-8 were increased within 1 h ozone exposure *in vitro* [3].

Alveolar macrophages accumulate lipids upon cigarette smoke exposure resembling foamy macrophages and release spontaneously the IL-1α and IL-1β cytokines [40]. This was not investigated upon ozone exposure.

Ozone induction of other members of the IL-1 family proteins such as IL-18, IL-33, IL-36 or IL-38 with inflammatory properties have so far not been investigated.

### Other inflammatory mediators

The irritant effects of ozone causes the release of a variety of other pro-inflammatory cytokines, chemokines and mediators, which is shortly discussed.

IL-6 is another inflammatory cytokine which is involved in ozone-induced respiratory pathology [26]. Subacute (72 h) exposure to 0.3 ppm ozone with increased protein leak, neutrophils, soluble TNF receptors in BALF were significantly reduced in IL-6- deficient mice, while AHR was not affected.

A recent study on ozone exposure (0.3 ppm for 24-72 h) showed increased neutrophilic inflammation and IL-6 in adiponectin-deficient mice. In adiponectin x IL-6 double deficient mice exposed to ozone, the hyperinflammation was reduced with lower IL-17A and G-CSF expression [28].

IL-10 has known anti-inflammatory properties. Recent data from IL-10 deficient mice suggested increased neutrophil recruitment after low dose ozone (0.3 ppm) at 1 to 3 day with enhanced NF-kB activation and MIP-2, cathepsin E, and serum amyloid A3 gene expression [4]. Therefore, endogenous IL-10 confers partial protection from ozone-induced lung inflammation [4].

TGF-β, transforming growth factor β plays a critical role for the development of fibrosis

including chemical induced lung fibrosis [14]. Ozone-induced emphysema and pulmonary fibrosis may be mediated by TGF-β in ozone exposed mice [29]. Chronic ozone exposure (5 day, 0.5 ppm, 8 h/day) for 5 cycles increased TGF-β protein levels in BALF, plasminogen activator inhibitor 1 and lung fibrosis. Blockade of the TGF-β signalling pathway with IN-1233 suppressed ozone-induced Smad2/3 phosphorylation, PAI-1 and collagen expression and α-SMA deposition in the lung. These data suggest that TGF-β signalling mediates ozone-induced lung fibrotic responses. The results are interesting and need to be confirmed using other inhibitors and TGF-β antibodies.

IL-17A is a pro-inflammatory cytokine which is dependent on IL-1R and IL-23R signalling [6, 15]. In a 6 weeks ozone exposure model we found increased production of IL-17A and IL-1β, and the activation of p38 MAPK in the lungs which was reduced in IL-17RA deficient mice [48]. Importantly AHR seen after ozone exposure relies on IL-17RA signalling mediated by the increased contractility of airway smooth muscles. The emphysema and lung inflammation induced by ozone however were independent of IL-17RA signalling [48]. By contrast another recent study showed that IL-17A antibody neutralisation reduced the recruitment of neutrophils after subacute ozone exposure (0.3 ppm for 24-72 h) [38]. γδ T cells are an important source of IL-17A. Ozone-induced increases in BAL macrophages, neutrophils and IL-17 were diminished in TCRδ deficient mice. The data indicate that pulmonary inflammation induced by subacute ozone exposure requires γδ T cells and TNFα-dependent recruitment of IL-17A+ γδ T cells to the lung [38].

The role of other IL-17 family members in AHR and lung inflammation is presently unknown. Our preliminary data suggest a protective effect for the related Th17 member, IL-22 which has structural homology with IL-10.

NKT cells: Pichavant et al. demonstrated that ozone induces a form of asthma that occurs in the absence of adaptive immunity characterized essentially by airway neutrophilia, but not eosinophilia, associated with AHR, which is a cardinal feature of asthma [46]. Repeated ozone exposure induced severe AHR associated with an increase of natural killer T (NKT) cells, neutrophils, and macrophages in the airway, which was absent in NKT cell-deficient CD1d(-/-) and Jα18(-/-) mice and was IL-17-dependent [46]. Thus, ozone exposure-induced AHR requires the presence of NKT cells and IL-17 production. Therefore NKT cells are required for the development of two very disparate forms of AHR (ozone- and allergen-induced) and more investigations on the role of NKT cells in ozone pathology may be necessary.

TNF is another fundamental proinflammatory cytokine involved in the biologic response to ozone [12, 52, 59]. Ozone induces the production of nitric oxide, TNF-α and tissue injury, which is dependent on NF-kB p50 [12].

Furthermore, neutralising TNF-α antibodies reduced protein and neutrophil recruitment in BALF and IL-1α, IL-6 and IL-10 expression upon ozone exposure [7] in animals exposed to $O_3$. Therefore TNF-α is involved in lung inflammation and epithelial injury produced by ozone exposure which is modulated by other cytokines.

CXCR2 is a critical receptor for the neutrophil chemokines KC and macrophage inflammatory protein-2 (MIP-2), which are upregulated in lungs following ozone exposure [25]. Mice deficient for CXCR2 had less protein leak in the BALF and epithelial cell damage suggesting reduced lung injury with diminished AHR as compared to wild-type control mice. Therefore, the role of CXCR2 for maximal neutrophil recruitment, epithelial cell sloughing, and persistent AHR upon ozone exposure should be further explored [25].

### Enhanced airway hyperreactivity by ozone resulting in chronic lung disease

Data from epidemiological and experimental studies support a link between air pollution and an increased incidence and/or severity of airway disease. Detrimental effects of ozone, nitrogen dioxide and particulate matter are documented. Recent studies, particularly in urban areas, have suggested a role for pollutants in the development of both asthma and COPD [30].

Preclinical studies may predict and provide supportive data that air environmental pollution by generation of ozone may cause exacerbation of allergic asthma. However as reported before ozone alone causes AHR, a special form of asthma with neutrophilic asthma [46].

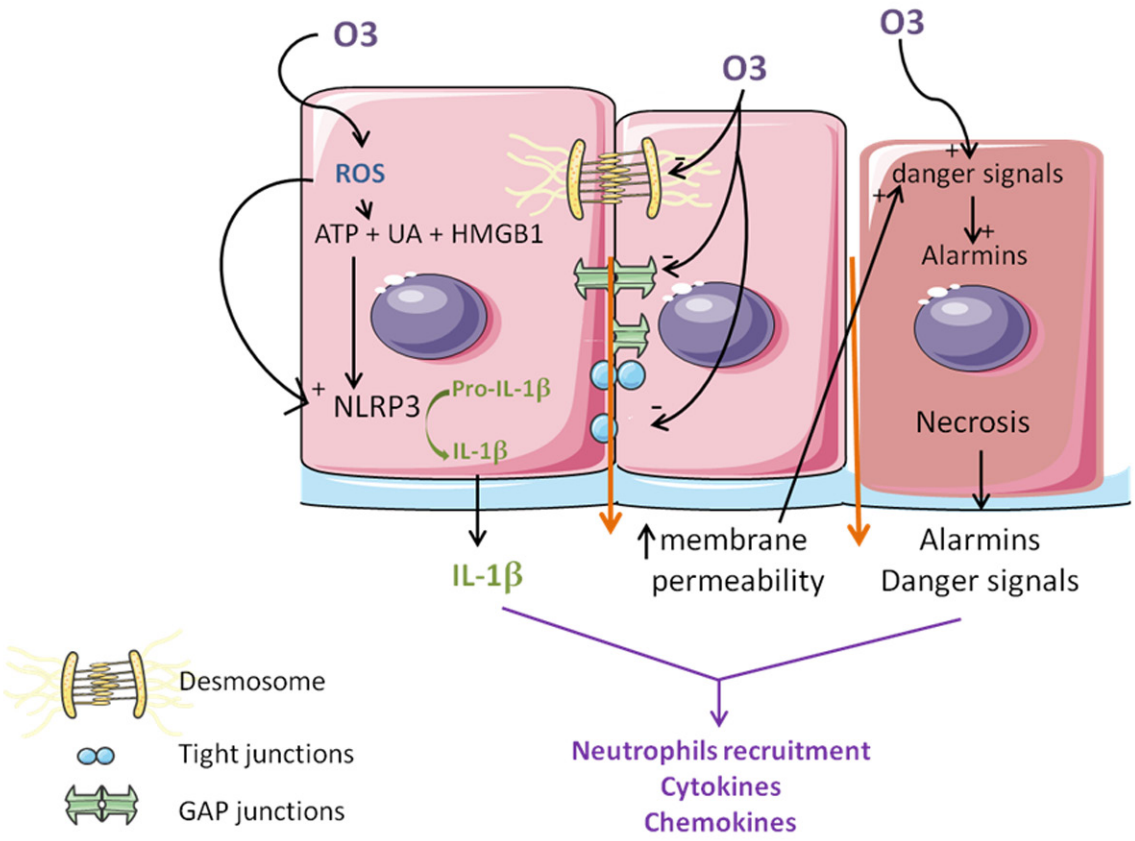


**Figure 2.** Mechanism of ozone-induced injury, hyperreactivity and inflammation. Ozone generates ROS which damage the lung epithelial cells, activating the innate immune response notably the NLRP3 inflammasome with release of IL-1β. Ozone causes increased permeability by down regulating tight junctions and release of alarmins which are released upon cell necrosis. These events induce chemokines/cytokines production and neutrophils recruitment and inflammation.

Pre-exposure to low dose of endotoxin enhanced the inflammatory response to ozone suggesting sequential and concomitant low doses of injury may result in an exacerbated response [22, 23].

The allergic response was investigated in ozone exposed mice [20]. Ozone exposure enhanced allergic asthma with augmented AHR, increased neutrophils and eosinophils in the BALF and lung inflammation with increased numbers of goblet cells, myofibroblasts, and smooth muscle cells [20].

Influenza infection induces AHR through a pathway that required the interleukin 13 (IL-13)-IL-33 axis via innate lymphoid cells 2 (ILC2). Influenza A virus infection activates the NLRP3 inflammasome, resulting in IL-33 production by alveolar macrophages, which in turn activated ILC2 producing substantial IL-13 and enhanced asthma [31]. The data show that respiratory virus infection enhance AHR and whether ozone enhances viral-induced responses needs to be further explored.

There is evidence that several ambient air pollutants enhance the pulmonary fibrotic processes. A recent epidemiological study demonstrated increased ozone and nitrogen dioxide exposure over the preceding 6 weeks was associated with an increased risk of acute exacerbation in patients with idiopathic pulmonary fibrosis [21]. This is clearly an area which needs to be further explored. No studies investigating the role of ozone in exacerbating pulmonary fibrosis are available and this could be tested in the bleomycin model induced lung fibrosis.

## Mechanisms and perspective

The present view on the mechanisms of ozone induced lung pathologies can be summarized

as shown in **Figure 2**. Ozone generates ROS which damages the lung epithelial cells, releasing endogenous danger signals, activating the innate immune response notably the NLRP3 inflammasome with release of IL-1. Additional factors including proteases and chemokines are released recruiting inflammatory cells augmenting the local injury response.

In conclusion, this review shows our limited knowledge on the mechanisms of ozone-induced lung inflammation and pathology and its contribution to exacerbation of allergic asthma, viral infection, COPD, pulmonary fibrosis and other respiratory diseases necessitating more mechanistic investigations in addition to a strict control of ozone pollution of the air. Furthermore epidemiological studies are critical to understand the relevance of the preclinical studies.

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## Disclosure of conflict of interest

None.

## Abbreviations

LPS, endotoxin; AHR, airway hyperresponsiveness; ROS, reactive oxygen species; HA, hyaluronic acid; TLR4, toll-like receptor 4; ppb, part per billion; PGE2, prostaglandin E2; LTC4, leukotriene C4; PAMP, pathogen associated molecular patterns; DAMP, damage associated molecular patterns.

**Address correspondence to:** Bernhard Ryffel, CNRS UMR7355, 45071 Orleans, France. Tel: 0033 238 25 54 39; Fax: 0033 238 25 79 79; E-mail: bryffel@cnrs-orleans.fr

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