

Genetic susceptibility to ozone-induced lung inflammation in animal models of asthma

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Purpose of review

Epidemiological associations between ozone exposure and allergic responsiveness are well-documented and have been corroborated in animal studies. The complex interaction between ozone and allergen has genetic and environmental components that affect atopic individuals and may increase the incidence of allergy in susceptible individuals. This review describes the advances that have been made in understanding mechanisms of genetic susceptibility to ozone-induced inflammation, and the interaction between ozone and allergen exposure in mice and a non-human primate model.

Recent findings

Antioxidant and innate immune defense genes contribute to ozone-induced inflammation and hyperpermeability in mice and humans. Ozone exposure during the allergic challenge phase induces greater enhancement of allergic responsiveness than the sensitization stage. Ovalbumin-pulsed dendritic cells injected into naïve mice successfully sensitize the mouse to ovalbumin in the absence of adjuvant. Debate continues over the role of T helper 1–T helper 2 immune profile development in mediating the ozone–allergen interaction, and the potential confounding influence of the predominant T helper 2 system most commonly used to study these responses.

Summary

The role of genetic background in susceptibility to ozone-induced lung inflammation has been confirmed, and promising candidate genes have been identified. Descriptive studies confirm that ozone exacerbates allergic responsiveness. Ozone administered during the challenge phase of ovalbumin allergen exposure induces greater responsiveness than during the sensitization phase. Allergen-induced responses enhanced by concurrent ozone exposure warrant further mechanistic research, particularly regarding the influence of susceptibility genes.

Keywords

ozone, ovalbumin, inbred mouse, allergen, genetics, candidate gene, Th1–Th2, dendritic cells, eosinophils

Abbreviations

HDMA	house dust mite allergen
GSTM1	glutathione-S-transferase μ 1
NQO1	NAD(P)H:quinone oxidoreductase 1
Th1	T-helper cell type 1
Th2	T-helper cell type 2

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Introduction

Oxidant air pollutants, including ozone, continue to be a public health concern in industrialized nations [1]. Individuals with cardiopulmonary disease as well as the young and elderly are particularly at risk to the adverse effects of air pollutant exposures [2,3]. Genetic background and various extrinsic factors such as nutritional status may also have important interacting roles in determining responsiveness to air pollutants.

Asthma is a chronic, inflammatory disorder with a complex set of causes consisting of both polygenic and environmental factors such as diet, socioeconomic status, physical activity, exposure to allergens, and air pollutants. Asthma symptoms include wheeze, airway hyper-responsiveness, pulmonary inflammation, and mucus hypersecretion. Approximately 75% of asthmatic patients are atopic and have a T-helper cell type 2 (Th2) immunological profile that includes airway eosinophilia and increases in the production of interleukins 4, 5, 10 and 13, and immunoglobulin E [4].

The incidence and prevalence of asthma and other allergic diseases have increased dramatically worldwide during the last two decades. Because changes in asthma susceptibility gene allele frequencies cannot account for the increased disease incidence, alterations in lifestyle and environment as causative factors have become the focus of recent research [5]. Epidemiological and experimental evidence suggest that air pollutants such as ozone exacerbate asthmatic symptoms (Fig. 1) and may contribute to increased asthma prevalence (Fig. 2). Increased emergency room visits, reduced lung function, increased school absences, and new onset asthma correlate with current (2003) levels of air pollution [6]. Furthermore, strenuous exercise during periods of high ambient ozone levels have been associated with increased prevalence of asthma [7]. Clinical studies corroborate epidemiological associations. For example,

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ozone increased allergic responsiveness by potentiating eosinophilic responses [8], and had a priming effect on allergen-induced responses and intrinsic inflammation in nasal airways of allergic asthmatics [9]. However, the mechanisms underlying the association between ozone and allergy remain unclear.

This review of the recent literature (2002–2004) on ozone and allergic exacerbation initially summarizes the genetic basis of susceptibility to ozone exposure. Discussion of the relevance of murine ovalbumin models, the effect of ozone on allergen sensitization and challenge, and proposed mechanisms follow. Confounding factors in ozone–allergen modeling include

selection of species and strains and the variety of endpoints chosen. We also briefly describe research progress using a non-human primate model of ozone–allergen interaction and lung development.

Genetic susceptibility to the pulmonary effects of ozone exposure

Ozone elicits inflammation, hyperreactivity, and epithelial damage of the airways, as well as altered ventilation and decrements in pulmonary function [3]. Furthermore, suppression or enhancement of immune responsiveness has been demonstrated, and depends on the exposure regimen, genetic background (inbred strains), and preexisting diseases [10]. Genome-wide scans for linkage of the inflammation (polymorphonuclear leukocytes) and hyperpermeability phenotypes in inbred mice have identified significant susceptibility quantitative trait loci on chromosomes 17 and 4, respectively [10]. Tumor necrosis factor- α (*Tnf*) was identified as a functionally relevant candidate gene in the chromosome 17 quantitative trait locus [10], and toll-like receptor 4 (*Tlr4*) on chromosome 4 has been implicated in ozone susceptibility [10]. Evidence also exists for genetic determinants of susceptibility to ozone in human subjects. A number of laboratories have reported inter-individual variation in pulmonary function and inflammatory responses to ozone in otherwise normal, healthy human subjects [3,10]. Moreover, specific gene polymorphisms have been associated with response phenotypes in human subjects exposed to ozone. For example, Corradi *et al.* [11] found that polymorphisms in phase II enzyme genes – *NAD(P)H:quinone oxidoreductase 1* (*NQO1*), *glutathione-S-transferase $\mu 1$* (*GSTM1*) – may have impor-

Figure 1. Ozone exposure may exacerbate asthmatic phenotypes

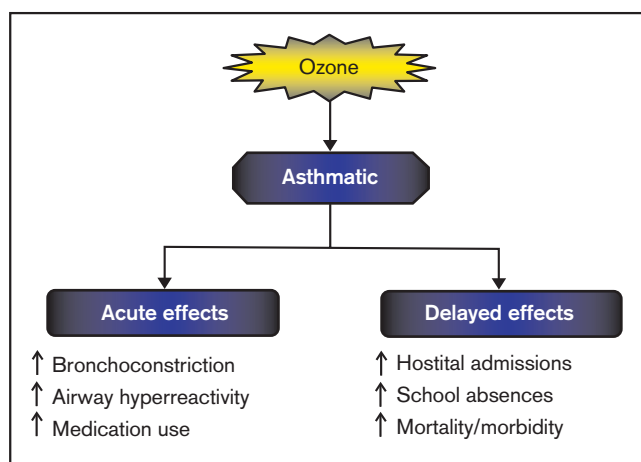
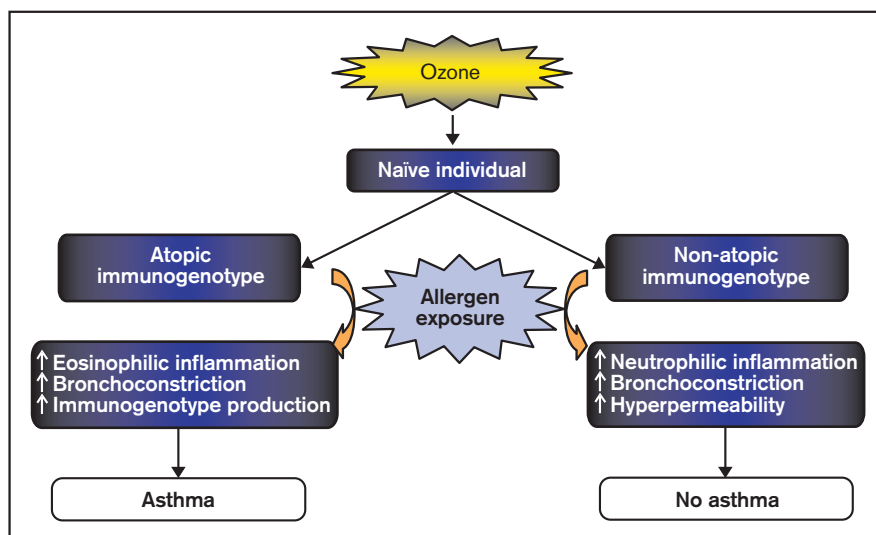


Figure 2. Hypothetical interaction between ozone exposure, innate immunogenotype, and allergen exposure that may prime atopic responses and increase incidence of new onset asthma



tant roles in the pulmonary function and epithelial permeability responses to ozone in non-smoking exercising participants. David *et al.* [12] found an interactive effect of polymorphisms in *NQO1* and *GSTM1* in asthmatic children exposed to ozone. Further, Romieu *et al.* [13] found that *GSTM1* deletion significantly enhanced ozone-related decrements in asthmatic children, and that antioxidant supplementation was more beneficial to children with *GSTM1* deletion than to *GSTM1*-positive children.

The murine model of asthma subphenotypes

The mouse ovalbumin model has been commonly used to study subphenotypes of allergic asthma including antigen-induced bronchoconstriction, immunoglobulin E and Th2 cytokine production, lymphocyte proliferation, hyperpermeability, and eosinophilic inflammation [14]. Mice have been sensitized by one to two intraperitoneal injections of ovalbumin in combination with an adjuvant like aluminum hydroxide (alum) at 1 or 2-week intervals. Fourteen to 35 days after sensitization, animals are challenged (single or multiple doses) with aerosolized ovalbumin by inhalation.

Adjuvants commonly used in animal models do not normally have a role in allergic sensitization. Alum tends to induce production of Th2 cytokines, and may contribute to the underlying Th2 phenotype typical of this model. Depuydt *et al.* [15] bypassed the confounding adjuvant effect by injecting ovalbumin-pulsed dendritic cells into non-sensitized mice.

Parameters of response in ozone-allergen studies

A number of inflammation, function, and histological parameters of response have been utilized in studies of ozone-allergen interaction. Cell differentials recovered from bronchoalveolar lavage fluid [15,16•,17–19] and increases in airway responsiveness or resistance [18–20] have been most commonly reported. Histological changes [15], Th2 and sometimes T helper cell type 1 (Th1) cytokines [21] have also been reported. Less common endpoints include goblet cell hyperplasia [16•], mucus cell metaplasia [17] and arterial blood gases [20]. Investigators have also recently assessed the role of fibrosis in an ovalbumin model [22] and an ozone-ovalbumin model [16•].

Sensitization versus challenge

Several studies have investigated the effect of ozone on allergen sensitization and challenge in the ovalbumin model. Depuydt *et al.* [15] administered ozone concurrently with a sensitizing dose of ovalbumin-pulsed dendritic cells and found no differences in bronchoalveolar lavage fluid cells after ovalbumin challenge relative to air controls. However, compared with ozone or ovalbumin challenge alone, the numbers of eosino-

phils increased significantly in previously sensitized animals that were exposed to 0.1 ppm ozone for 4 h/day for 6 days and challenged with ovalbumin. Others have confirmed the effect of ozone on antigen challenge rather than the sensitization phase [16•,17–20].

To determine if timing of ozone and ovalbumin challenge influences the potential to exacerbate an allergic response, Last *et al.* [16•] exposed presensitized mice to 0.2 or 0.5 ppm ozone either before, after, continuously, or intermittently during ovalbumin aerosol challenge. When ozone was administered for 8 h/day for 2 weeks prior to ovalbumin challenge, the response to allergen was similar to responses observed following ozone exposure (i.e. increased neutrophils and less eosinophilia). In contrast, when ozone was administered daily for 2 weeks after or intermittently in 3-week segments during ovalbumin challenge, the response resembled an allergic response (increased immunoglobulin E and eosinophilia).

Inbred mouse strains (genetic background)

Allergic responses are often characterized as Th2-like (i.e. eosinophilia and increased interleukin 4, 5, 10 and 13 and immunoglobulin E production). In contrast, ozone responses are Th1-like (i.e. neutrophilia and increased interleukin 6 and 8 and tumor necrosis factor- α production). It is generally believed that the Th1–Th2 profile becomes fixed during immune development and genetic background may influence the balance. Certain strains of mice are considered Th2-like (e.g. BALB/c) while others are more Th1-like (e.g. C57BL/6). Not surprisingly, ozone studies that utilized BALB/c mice [16•,18] to investigate ozone-allergen interaction reported prominent Th2 responses following ovalbumin challenge while C57BL/6 mice were resistant to ovalbumin-induced inflammation [19]. A Th2-based model may mask the potential response to ozone, or misrepresent the putative exacerbating effect of ozone on allergic responsiveness. Last *et al.* (2004) [16•] used sensitized BALB/c mice in an ozone-ovalbumin co-exposure model and concluded that ozone administered 2 weeks before or during 6 weeks of ovalbumin challenge antagonizes the inflammatory effects of ovalbumin. In contrast, Neuhaus-Steinmetz *et al.* (2000) [21] reported that ozone exacerbated the allergic response compared with ovalbumin alone in both BALB/c and C57BL/6 mice. These conflicting results may stem partially from differences in experimental design. This between-strain difference in immune response to ozone should be considered when interpreting results of a combined ozone-ovalbumin study that involves Th2-dominant mouse strains in a Th2-dominant model. For example, when investigating the possibility that ozone potentiates responses to ovalbumin (ovalbumin versus ozone plus ovalbumin), an ovalbumin

model in a Th2-type mouse strain may not be appropriate. Conversely, allergens may enhance responses to ozone (ozone versus ozone plus allergen) and could be addressed using a Th1-type mouse strain and an alternate allergic model.

Mechanisms of ozone-enhanced response to allergen

The mechanisms through which ozone induces exacerbation of allergic responses are not well understood. Pollutants injure airway epithelium, impair mucociliary clearance mechanisms or increase permeability for allergens. Alternatively, pollutants may stimulate epithelial cells to release cytokines or adhesion molecules that prime the innate or adaptive immune response upon subsequent challenge [15]. Ozone may also enhance antigen-presenting cell activity in ovalbumin-sensitized mice via upregulation of cell-surface molecule expression in the lung [23]. Th2 cytokines such as interleukins 4 and 13 may stimulate release of eosinophil-derived growth factors that may mediate mucus cell metaplasia [17] and fibrosis [16••]. Further study of the potential mechanisms responsible for these observed responses to ozone and allergen are necessary to better understand this complex disease.

Non-human primate model

Non-human primate models provide an essential and unique opportunity to study asthma and allergic airway disease in association with ozone exposure. The rhesus monkey (*Macaca mulatta*) has proved useful, as their immune system is comparable to that of humans. Furthermore, all of the intrapulmonary conducting airway components that have been shown to be affected in asthmatic patients are present in the respiratory system of the rhesus monkey and appear to be affected in a similar way [24].

There is a paucity of data concerning the effect of ozone on the developing lungs of infant humans who are at risk of atopic airway disease onset. Schelegle *et al.* (2003) [25] confirmed a synergistic allergic and structural remodeling airway response to combined house dust mite allergen (HDMA) and ozone exposure in sensitized rhesus monkeys. Abnormal tracheal basement membrane development [26] and an adaptation of nucleus tractus solitarius neurons regulating respiratory motor responses to episodic ozone exposure were also found in this model [27].

A number of important developmental factors are altered by exposure to ozone in monkeys sensitized to HDMA. For example, following simultaneous exposure to 0.5 ppm ozone and HDMA sensitization, marked increases in airway eosinophilia, plasma histamine, and serum immunoglobulin E were found to be much greater than the additive effects of ozone or HDMA

sensitization exposure alone. However, the potential mechanisms responsible for these responses in infant monkeys have not been studied in detail. Mechanisms relating to changes in plasma histamine have received minor attention. Sensitized monkeys exposed to ozone also required a substantially lower concentration of histamine aerosol to elicit a 150% increase in airway resistance when compared with the control conditions [25]. The mechanism for these higher levels of plasma histamine is not clear. Despite evidence for interleukin-8 mediated increases in histamine release from gastric mucosal cells in canines [28], interleukin-8 does not appear to be a likely mediator of the increase in plasma histamine since the cytokine did not change significantly at ozone concentrations below 1 ppm [29].

Atypical airway maturation and structural remodeling of the airways have also been shown in monkeys exposed to ozone following HDMA sensitization [25]. Increased variability in tracheal basement membrane zone thickness was associated with very low levels or absence of perlecan. Consequently, fibroblast growth factor-2, an important cytokine involved in airway development that normally binds to perlecan, was not stored in the basement membrane zone. These investigators suggest the co-exposures thus impair normal development of the airways that occur prior to adulthood. In addition, changes in the regulation of the respiratory motor response to episodic ozone exposure in infant rhesus monkeys has been demonstrated [27]. Important neurons involved in respiratory regulation extending from the nucleus tractus solitarius exhibited attenuated synaptic transmission during ozone-induced increases in neuron excitability, which potentially affected respiratory motor responses to ozone exposure. These ozone-induced alterations in airway anatomy and physiology may play important roles in the exacerbation of susceptibility to allergic lung inflammation upon exposure to ozone.

Conclusion

The association between oxidant air pollution (ozone) and aggravated asthmatic responses has a 30-year history of epidemiology and basic research. The complex etiology of ozone-induced inflammation and asthma involve multiple environmental factors and genetic predisposition. Recent studies in mouse and non-human primate models have used an array of different exposure protocols and endpoints to confirm the effect of ozone on responses to allergen, and provide some insight on the mechanisms through which this effect is mediated. The Th2-dominant ovalbumin model, while useful, cannot effectively address the contribution of Th1–Th2 cytokine profiles when a Th2 dominant mouse strain is used. Further mechanistic studies are clearly warranted.

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