Mechanistic Basis for Obesity-related Increases in Ozone-induced Airway Hyperresponsiveness in Mice

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Abstract

Obesity is a risk factor for asthma, especially nonallergic asthma. Ozone, a common air pollutant, is a nonallergic asthma trigger. Importantly, ozone-induced decrements in lung function are greater in obese and overweight human subjects than in lean individuals. Obese mice also exhibit exaggerated pulmonary responses to ozone. Ozone causes greater increases in pulmonary resistance, in bronchoalveolar lavage neutrophils, and in airway hyperresponsiveness in obese than in lean mice. Our data indicate that IL-33 plays a role in mediating these events. Ozone causes greater release of IL-33 into bronchoalveolar lavage fluid in obese than in lean mice. Furthermore, an antibody blocking the IL-33 receptor, ST2, attenuates ozone-induced airway hyperresponsiveness in obese but not in lean mice. Our data also indicate a complex role for tumor necrosis factor (TNF)- α in obesity-related effects on the response to ozone. In obese mice,

genetic deficiency in either TNF- α or TNF- α receptor 2 augments ozone-induced airway hyperresponsiveness, whereas TNF- α receptor 2 deficiency virtually abolishes ozone-induced airway hyperresponsiveness in lean mice. Finally, obesity is known to alter the gut microbiome. In female mice, antibiotics attenuate obesity-related increases in the effect of ozone on airway hyperresponsiveness, possibly by altering microbial production of short-chain fatty acids. Asthma control is often difficult to achieve in obese patients with asthma. Our data suggest that therapeutics directed against IL-33 may ultimately prove effective in these patients. The data also suggest that dietary manipulations and other strategies (prebiotics, probiotics) that alter the microbiome and/or its metabolic products may represent a new frontier for treating asthma in obese individuals.

Keywords: asthma; IL-33; tumor necrosis factor- α ; microbiome; IL-13

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Obesity is a risk factor for asthma. Both the prevalence and the incidence of asthma are increased in obese individuals (1–3). Furthermore, both surgically induced and diet-induced weight loss reduce asthma symptoms in obese patients with asthma (4, 5). It is increasingly well established that asthma is a highly pleiotropic disease with multiple phenotypes (6). Even within the subcategory of “obese asthma,” at least two different phenotypes exist. One phenotype includes mostly female, nonatopic subjects with late-onset asthma. In this group, obesity appears to be a causative factor for asthma, and weight loss reduces airway hyperresponsiveness (AHR) (5). The other

obese asthma phenotype includes subjects with atopic asthma, usually early-onset. Obesity appears to worsen asthma symptoms in this group of patients with asthma, but it does not appear to cause their asthma: weight loss improves asthma control but does not reduce AHR (5).

Compared with nonobese patients with asthma, obese patients with asthma do not respond as well to standard asthma control therapeutics (7–9). Better understanding of the mechanistic basis for asthma in obese patients is needed for the development of therapeutics that are effective in this population. Such understanding will require appropriate animal models.

Nonatopic asthma is more common in obese than in lean individuals (10–12). Furthermore, whereas obesity is a risk factor for asthma, it does not impact other atopic diseases, at least not in adults (13). Consequently, our research group has been focusing on models in which obese mice are exposed to a nonallergic asthma trigger.

The air pollutant ozone (O_3) is one such trigger. O_3 causes asthma symptoms, reduces lung function, and causes AHR (14–16). Emergency room visits and hospital admissions for asthma are more common following days of high ambient O_3 (15, 16). Importantly, O_3 -induced asthma symptoms are greater in obese than in lean

children (17), and O₃-induced decrements in lung function are greater in obese and overweight adults than in lean adults, especially when those subjects have preexisting AHR (18, 19).

Obese mice also exhibit exaggerated pulmonary responses to O₃. O₃ causes increases in pulmonary resistance (R_L) in obese but not in lean mice, and O₃-induced AHR is greater in obese than in lean mice (20–27) (Figure 1). Moreover, obesity-related increases in the magnitude of O₃-induced AHR are observed, regardless of the cause of murine obesity. The following mice all have augmented responses to O₃: obese *ob/ob* and *db/db* mice lacking the satiety hormone leptin or its receptor; *Cpe^{fat}* mice genetically deficient in carboxypeptidase E (Cpe), an enzyme that regulates neuropeptides involved in eating behavior; and mice with diet-induced obesity (28).

O₃ reacts with lipids in lung and airway lining fluid to generate lipid ozonation products (29) that injure epithelial cells (30). The ensuing inflammatory response includes increases in bronchoalveolar lavage (BAL) concentrations of acute-phase cytokines and chemokines, such as IL-6, chemokine (C-X-C motif) ligand 1 (CXCL1), tumor necrosis factor (TNF)- α , and IL-33, as well as neutrophil recruitment to the lungs (20, 23, 24, 26, 31). Compared with lean mice, obese mice have greater increases in these cytokines and chemokines, as well as greater increases in BAL neutrophils (20–27, 32), after O₃ exposure. In this article, I describe the roles of IL-33 and TNF- α in

the augmented responses to O₃ observed in obese mice. Because obesity has profound effects on the gut microbiome (33–39), and because the microbiome is known to impact allergic airway responses (summarized in [40]), I also describe data indicating a role for the microbiome in responses to O₃.

Role of IL-33

IL-33 is an IL-1 family cytokine that signals via a complex composed of ST2, the primary IL-33 binding receptor, and a coreceptor, IL-1 receptor accessory protein. Importantly, both IL-33 and ST2 are genetically associated with asthma (41). IL-33 is expressed in lung epithelial cells and is released upon O₃ exposure (24, 42, 43), consistent with the known role of cell injury in IL-33 release (44). Furthermore, in mice, pulmonary administration of IL-33 causes AHR and increases in BAL neutrophils (45, 46), events also observed after O₃ exposure. Consequently, we examined the hypothesis that IL-33 contributes to obesity-related increases in the response to O₃ (24). We treated both obese *db/db* mice and their lean wild-type (WT) controls with either an ST2-blocking or isotype antibody prior to acute O₃ exposure (2 parts per million for 3 h). O₃-induced increases in BAL IL-33 and neutrophils, in baseline R_L, and in the magnitude of AHR were each greater in *db/db* than in WT mice. In O₃-exposed *db/db* mice, anti-ST2 treatment reduced AHR and baseline R_L toward, but not to, levels observed in WT mice, whereas

anti-ST2 had no effect in WT mice (Figure 2). Anti-ST2 also reduced BAL neutrophils in *db/db* but not in WT mice. Taken together, the results indicate a role for IL-33 in the augmented responses of obese mice to O₃.

Exogenous administration of IL-33 promotes airway narrowing, causes AHR, and increases BAL neutrophils by inducing the release of (1) type 2 cytokines such as IL-13 and IL-5; (2) chemokines that use C-X-C motif chemokine receptor 2, such as CXCL1; and (3) IL-6 (45–47). Consistent with the greater increases in BAL IL-33 observed in obese than in lean mice after O₃ exposure, we also observed greater increases in BAL IL-13, IL-5, CXCL1, and IL-6 after O₃ exposure in obese than in lean mice (20, 21, 23, 24, 26, 27, 48). Furthermore, anti-ST2 attenuated but did not abolish O₃-induced increases in BAL concentrations of each of these cytokines and chemokines in obese mice (24).

IL-33-dependent increases in IL-13 likely contribute to the ST2-dependent effects of O₃ on baseline R_L in obese mice because anti-IL-13 antibodies also attenuate O₃-induced increases in R_L in these mice. IL-33-dependent increases in IL-13 may also contribute to the augmented neutrophil recruitment observed in obese versus lean mice after O₃ exposure because anti-IL-13 also reduces BAL neutrophils in obese O₃-exposed mice (26), likely as a result of changes in chemokine (C-C motif) ligand 20. However, IL-33-driven factors other than IL-13 must be responsible for O₃-induced AHR in obese mice because blocking IL-13 does not attenuate O₃-induced AHR in obese mice (26), whereas blocking ST2 does (24). CXCL1 and IL-6 could be among these factors because BAL CXCL1 and IL-6 are reduced by anti-ST2 treatment in obese O₃-exposed mice (24). Furthermore, blocking CXCL1 signaling reduces AHR induced by exogenous IL-33 (46). IL-6 contributes to allergic airway inflammation in mice (49), and recent data derived from human patients with asthma suggest a link between serum IL-6 and asthma severity, especially in obese individuals (50). Effects of IL-33 on CXCL1 and IL-6 may also contribute to the role of IL-33 in neutrophil recruitment because both IL-6 and CXCL1 are required for O₃-induced increases in BAL neutrophils, including in obese mice (32, 51, 52). The proposed role of IL-33 is represented schematically in Figure 3.

Figure 1. Obesity increases the magnitude of airway hyperresponsiveness following ozone exposure. Shown are changes in pulmonary resistance (R_L) induced by inhaled methacholine in lean wild-type (WT) and obese *db/db* female mice exposed to air or ozone (2 parts per million for 3 h) and studied 24 hours after exposure. Results are expressed as the mean $\pm$ SE of four to eight mice per group. **P* < 0.05 versus air; #*P* < 0.05 versus lean mice with the same exposure. Reproduced by permission from Reference 24. PBS = phosphate-buffered saline.

Figure 2. Blocking IL-33 signaling attenuates ozone (O_3)-induced airway hyperresponsiveness in obese mice but not in lean mice. Shown are changes in pulmonary resistance (R_L) induced by inhaled aerosolized methacholine in lean wild-type (WT) and obese *db/db* female mice exposed to O_3 . Mice were treated with either isotype or anti-ST2 (the IL-33 receptor) antibody prior to O_3 exposure. Results are expressed as the mean $\pm$ SE of four to eight mice per group. $^{\#}P < 0.05$ versus lean mice with same exposure; $^{\dagger}P < 0.05$ versus isotype-treated mice of same genotype. Reproduced by permission from Reference 24. PBS = phosphate-buffered saline.

Airway epithelial cells and macrophages are likely to be the cells responsible for IL-33-dependent increases in IL-6 and CXCL1 after O_3 exposure. These cells are known to produce IL-6 and CXCL1 after O_3 exposure (53, 54) and are capable of responding to IL-33 (44, 55, 56). In contrast, IL-33-dependent changes in type 2 cytokines appear to involve effects of IL-33 on type 2 innate lymphoid cells (ILC2s) and on $\gamma\delta$ T cells. Data supporting a role for ILCs include the demonstration that pulmonary IL-13 $^{+}$ and IL-5 $^{+}$ ILC2s increase after O_3 exposure in obese mice (24). In addition, mice deficient in lymphoid cells have reduced type 2

cytokine release in the nose and lungs after O_3 exposure, and adoptive transfer of ILC2s is sufficient to restore these responses (42, 43). There are also data supporting a role for $\gamma\delta$ T cells in the type 2 cytokine release observed in obese mice after O_3 exposure. O_3 increases pulmonary IL-13 $^{+}$ $\gamma\delta$ T cells in obese mice, and $\gamma\delta$ T cells express ST2, the receptor for IL-33, especially after O_3 exposure (24). Furthermore, BAL IL-5 and BAL IL-13 are both lower in obese T-cell receptor δ -chain-knockout (*TCR $\delta^{-/-}$*) mice, which lack $\gamma\delta$ T cells, than in obese WT mice (24). Finally, after O_3 exposure, both airway responsiveness and BAL neutrophils are lower in obese *TCR $\delta^{-/-}$* than in obese WT mice (24). In summary, IL-33 contributes to the augmented responses to O_3 observed in obese mice, at least in part via its ability to induce type 2 cytokine production in ILC2s and $\gamma\delta$ T cells, though other cellular targets of IL-33, such as airway epithelial cells and macrophages, may also be involved.

Role of TNF- α

Circulating concentrations of TNF- α are elevated in obesity (20, 57), and data derived from animal models indicate that TNF- α contributes to the etiology of some obesity-related conditions, including insulin resistance (58–62). TNF- α may also be relevant for asthma in obesity because obesity confers a greater risk for asthma in subjects with TNF- α polymorphisms that promote TNF- α expression, especially

among nonatopic patients with asthma (63). Exogenous administration of TNF- α can induce AHR (64), and TNF- α is known to contribute to O_3 -induced AHR in lean mice (65, 66). Taken together, these data suggest that TNF- α may contribute to obesity-related increases in the response to O_3 . To address this hypothesis, we bred obese *Cpe^{fat}* mice that were deficient in TNF- α (*Cpe^{fat}/TNF- $\alpha^{-/-}$*) and compared their responses with responses to acute O_3 exposure in obese TNF- α -sufficient *Cpe^{fat}* mice and lean TNF- $\alpha^{-/-}$ or WT mice (27). Similar experiments were performed in *Cpe^{fat}* mice deficient in TNF receptor 2 (TNFR2; *Cpe^{fat}/TNFR2 $^{-/-}$* mice) (26).

The results indicate a complex role for TNF- α in the augmented responses to O_3 characteristic of obese mice. O_3 -induced AHR and O_3 -induced increases in BAL neutrophils were greater in *Cpe^{fat}* than in WT mice (26, 27), as discussed above. Both TNF- α and TNFR2 deficiency attenuated O_3 -induced increases in BAL neutrophils in obese but not in lean mice and effectively abolished obesity-related differences in neutrophil recruitment (26, 27). The data indicate that TNF- α is required for the augmented effects of acute O_3 exposure on pulmonary inflammatory cell recruitment in obese mice. In contrast, TNF- α and TNFR2 deficiency augmented O_3 -induced AHR in *Cpe^{fat}* mice, even though deficiencies in TNF- α signaling reduced O_3 -induced AHR in lean control mice (26, 27). These results indicate that TNF- α protects against O_3 -induced AHR in obese mice. They also emphasize the differing roles of TNF- α in the O_3 responses of lean and obese mice. Finally, these results indicate that O_3 -induced AHR and inflammation are mechanistically dissociated: Neutrophils and/or their products do not contribute to O_3 -induced AHR in obese mice.

Given the importance of IL-33 for O_3 -induced AHR in obese mice (see above), we considered the possibility that TNF- α , acting via TNFR2, might protect obese mice against O_3 -induced AHR by affecting IL-33 expression. We used type 2 cytokine expression to assess the impact of IL-33 in these TNF- α - or TNFR2-deficient mice. BAL IL-13 levels were significantly greater in O_3 -exposed *Cpe^{fat}/TNF- $\alpha^{-/-}$* than in *Cpe^{fat}* mice (Figure 4), and a similar trend was observed in *Cpe^{fat}/TNFR2 $^{-/-}$* versus *Cpe^{fat}* mice (26, 27). BAL IL-5 levels were also significantly greater in O_3 -exposed

Figure 3. Schematic representation of proposed role of IL-33 in responses of obese mice to ozone. AHR = airway hyperresponsiveness; CXCL1 = chemokine (C-X-C motif) ligand 1; ILC2 = type 2 innate lymphoid cells.

Figure 4. Tumor necrosis factor (TNF)- α deficiency augments obesity-related increases in the effects of ozone on bronchoalveolar lavage (BAL) IL-13. Results are expressed as the mean $\pm$ SE of data from five to eight mice per group. * $P < 0.05$ versus genotype matched unexposed mice; # $P < 0.05$ versus TNF- α genotype-matched carboxypeptidase E (*Cpe*)-sufficient mice; % $P < 0.05$ versus *Cpe* genotype-matched TNF- α -sufficient mice. Reproduced by permission from Reference 27. WT = wild type.

Cpe^{fat}/TNFR2^{-/-} than in *Cpe^{fat}* mice (26). Taken together with the roles of IL-33 and type 2 cytokines described above, the data are consistent with a role for TNF- α in suppressing IL-33 release and/or suppressing IL-33-dependent effects on target cells in obese mice. How might this effect of TNF- α be mediated? The observation that O₃-induced increases in BAL protein carbonyls, markers of oxidative stress, were augmented in *Cpe^{fat}/TNF- α ^{-/-}* versus *Cpe^{fat}* mice (26, 27) indicates greater oxidative stress in *Cpe^{fat}/TNF- α ^{-/-}* than in *Cpe^{fat}* mice. Greater oxidative stress would be expected to promote greater epithelial injury, IL-33 release, and subsequent induction of type 2 cytokines, as observed previously (26, 27). Consequently, it is possible that TNF- α acts to protect against O₃-induced AHR in obese mice by inducing the expression of antioxidant enzymes. Though TNF- α has the capacity to induce the antioxidants heme oxygenase 1 (*Hmox1*), metallothionein 1 (*Mt1*), and metallothionein 2 (*Mt2*), we were unable to demonstrate a role for TNF- α in

the induction of these antioxidants by O₃ in obese mice (26, 27). However, it is possible that TNF- α contributes to the induction of other antioxidant enzymes, such as the enzyme glutamate-cysteine ligase catalytic subunit that catalyzes the rate-limiting step in the synthesis of the potent antioxidant glutathione.

Role of the Microbiome

An increasing body of evidence supports a role for the microbiome and/or its metabolic products in models of allergic airway disease (40). A series of preliminary reports from our laboratory suggest that the microbiome also impacts responses to O₃, including the augmented O₃-induced AHR of obese mice. Obesity alters the gut microbiome (33–39), and there are data indicating a role for the gut microbiome in the insulin resistance and systemic inflammation associated with obesity (33, 67–70), as well as in the nonalcoholic fatty liver disease that often accompanies obesity (71).

We performed a metabolomic profiling experiment in lungs of obese and lean mice exposed to air or O₃ (72). Many of the identified metabolites were impacted by obesity or O₃ exposure. Among these metabolites were several that are known to require bacteria for their generation. Remarkably, each of these latter metabolites was significantly affected either by obesity, by O₃, or by the combination of these two factors. These results suggest a role for the microbiome in obesity-related alterations in the response to O₃. Indeed, in lean mice, we demonstrated that antibiotics attenuated O₃-induced AHR (73). Similar results were obtained in obese female mice, in which antibiotics nearly abolished obesity-related differences in O₃-induced AHR (74).

In lean mice, treatment with a variety of antibiotics allowed me to identify bacteria taxa that were associated with the magnitude of O₃-induced AHR. These bacteria were notable plant degraders and producers of short-chain fatty acids (SCFAs). Subsequent analysis of serum indicated reductions in SCFAs only in mice

treated with those antibiotics that attenuated responses to O₃. Furthermore, exogenous administration of SCFAs via the drinking water augmented O₃-induced AHR in lean mice (75). Taken together, the data support a role for the microbiome obesity-related increases in the response to O₃ and suggest that microbiota-dependent metabolism of SCFAs may be contributing to this role. Given the importance of genetic factors in affecting the microbiome (76) and reports that TNFR2 deficiency alters gut microbial communities (77), the data also suggest that the protective role of TNF- α /TNFR2 against obesity-related increases in the response to O₃ described above might be mediated via changes in the gut microbiome. Whether obesity-related changes in the microbiome also account for obesity-related differences in the impact of O₃ on the lungs remains to be established.

Conclusions

Our data indicate that IL-33 contributes to the augmented responses to O₃ observed in obese mice, whereas TNF- α exerts a protective effect, at least for O₃-induced AHR. What are the clinical implications of these observations? Asthma control is often difficult to achieve in obese patients with asthma (78), and therapeutics that are effective in these patients are desperately needed. Various therapeutics directed against IL-33 are in development and may ultimately prove effective in the treatment of obese patients with asthma. In contrast, the present data suggest that TNF- α inhibitors might actually worsen symptoms in obese patients with asthma. Data supporting a role for the microbiome in this nonallergic model of obese AHR also suggest that dietary manipulations and other strategies (prebiotics, probiotics) that alter the gut microbiome and/or its metabolic products may represent a new frontier for treating asthma in obese individuals. Greater understanding of the bacteria and bacterial products involved, especially in human subjects, is required for the development of such strategies. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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