Effect of obesity on pulmonary inflammation induced by acute ozone exposure: role of interleukin-6

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Lang JE, Williams ES, Mizgerd JP, Shore SA. Effect of obesity on pulmonary inflammation induced by acute ozone exposure: role of interleukin-6. Am J Physiol Lung Cell Mol Physiol 294: L1013–L1020, 2008. First published March 21, 2008; doi:10.1152/ajplung.00122.2007.—To determine the role of interleukin (IL)-6 in the increased ozone (O3)-induced inflammation and injury observed in obese vs. lean mice, lean wild-type and leptin-deficient obese (ob/ob) mice were injected with anti-IL-6 antibody (Ab) or isotype control Ab 24 h before exposure to either O3 (2 ppm for 3 h) or room air. Four or 24 h after O3 exposure, bronchoalveolar lavage (BAL) was performed, and the lungs were harvested for Western blotting. Anti-IL-6 Ab caused substantial reductions in O3-induced increases in BAL IL-6 in mice of both genotypes. Four hours following O3, ob/ob mice had increased BAL neutrophils compared with controls, and anti-IL-6-Ab virtually abolished this difference. At 24 h, O3-induced increases in BAL protein and BAL serum albumin were augmented in ob/ob vs. wild-type mice, and anti-IL-6 Ab ablated these obesity-related differences in epithelial barrier injury. O3 increased tyrosine phosphorylation of STAT-3 and STAT-1. There was no effect of obesity on STAT-3 phosphorylation, whereas obesity decreased STAT-1 expression, resulting in reduced STAT-1 phosphorylation. IL-6 neutralization did not alter STAT-3 or STAT-1 phosphorylation in ob/ob or wild-type mice. O3 increased BAL leukemia inhibitory factor (LIF) to a greater extent in obese than in lean mice, and LIF may account for effects on STAT phosphorylation. Our results suggest that IL-6 plays a complex role in pulmonary inflammation and injury observed in obese vs. lean mice. Moreover, obesity-related differences in activation of STAT proteins may contribute to some of the differences in the response of obese vs. lean mice.

neutrophil; airway; chemokine; STAT-3; STAT-1; leukemia inhibitory factor

Obesity is an increasing public health problem that elevates the risk for atherosclerosis, type 2 diabetes, hypertension, and some forms of cancer (14). Accumulating data also suggest that obesity enhances the risk for new-onset asthma (5, 7, 17, 18, 45). More than 13 prospective studies involving multiple age groups and ethnic backgrounds report an increased risk for incident asthma in the overweight or obese and show that obesity antedates asthma (see recent reviews in Refs. 15, 43, 45). In addition, obese asthmatic patients who lose weight experience fewer asthma symptoms, increased airflow rates, and reduced peak flow variability (11, 19). Data from animal models also support a relationship between obesity and asthma. Regardless of the modality of obesity, obese mice exhibit innate airway hyperresponsiveness (26, 34, 41, 46). Obese mice also have exaggerated pulmonary responses following acute exposure to ozone (O3).

Ozone is a common air pollutant that causes oxidant injury to epithelial cells in the lung. Emergency room visits for asthma, asthma symptoms, and asthma medication use increase on days of high ambient O3 concentrations, possibly as a result of interactions with copollutants (16, 51). The deleterious effects of O3 include disruption of the epithelial barrier leading to increased permeability [often assessed by increases in total bronchoalveolar lavage (BAL) protein or BAL serum albumin], an influx of neutrophils into the lungs, and generation of cytokines and chemokines (3). Each of these effects is augmented in obese mice regardless of the modality of obesity (26, 34, 44, 46).

Interleukin-6 (IL-6) is a pleiotropic cytokine whose serum levels increase with adiposity and appear to be associated with the development of other obesity-related sequelae, including atherosclerosis and diabetes mellitus (2, 37, 47, 50, 52). IL-6 is also associated with endothelial dysfunction and decreased flow-mediated vasodilation (33). Elevated serum IL-6 is associated with an increased incidence of type 2 diabetes and myocardial infarction in previously healthy adults (38, 39) and predicts coronary events in patients with unstable angina (4). Animal models also support a role for IL-6 in obesity-related conditions including hypertension (32) and early atherosclerosis (22).

IL-6 is also expressed in the lung following acute O3 exposure (10, 12, 24, 25, 56), likely from bronchial epithelial cells and macrophages (12). Indeed, IL-6 is among the genes most strongly induced by acute O3 exposure (55). Experiments using anti-IL-6 antibodies (Ab) and IL-6-deficient mice indicate that IL-6 also contributes to O3-induced pulmonary injury and inflammation (25, 56). Hence, the purpose of this study was to examine the hypothesis that IL-6 mediates the exaggerated inflammatory responses to O3 observed in obese mice. To test this hypothesis, we injected obese ob/ob mice and lean controls with either anti-IL-6 or control Ab before acute O3 exposure (2 ppm for 3 h) and collected BAL fluid to assess inflammation and injury 4 or 24 h later.

Many of the downstream effects of IL-6 cytokine family activation are mediated through activation of signal transducer and activation of transcription 3 (STAT-3) (20). Activation of STAT-3 is initiated by IL-6-dependent homodimerization of gp130 receptors resulting in STAT-3 phosphorylation at Tyr705. STAT-3 then dimerizes with other phosphorylated STAT-3 molecules, and translocates to the nucleus, where it...
activates target genes. STAT-1 is also activated by IL-6 family members (20), albeit to a lesser extent than STAT-3, and STAT-1 can mediate some effects of IL-6 (9). Accordingly, we also measured STAT-3 and STAT-1 activation by Western blotting of lung homogenates from wild-type and ob/ob mice exposed to air or O₃.

MATERIALS AND METHODS

Animals. This study was approved by the Harvard Medical Area Standing Committee on Animals. Obese male and female ob/ob mice were studied at 8–13 wk of age. Ob/ob mice have a single base pair mutation in codon 105 of the leptin gene that results in a premature stop codon (57). In the absence of leptin, mice eat excessively and become massively obese. The increased body weight results entirely from an increase in body fat. Because the ob/ob mice were on a C57BL/6 background, age- and gender-matched lean C57BL/6 mice were used as controls. All mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were fed identical standard mouse chow diets (Pico Mouse Diet 5058, Pharmaserve).

O₃ exposure. Conscious mice were placed in individual wire mesh cages inside a stainless steel and plexiglass exposure chamber (total volume ~145 l). Mice were exposed to O₃ (2 ppm for 3 h) by passing dry 100% oxygen through ultraviolet light and mixing it with filtered room air in the chamber or to filtered room air within a separate but identical chamber. The chamber’s atmosphere was drawn continuously via a sampling port, and O₃ concentration was measured by an O₃ chemiluminescent analyzer (model 49; ThermoElectron Instruments, Hopkinton, MA), which was calibrated by an ultraviolet photometric O₃ calibrator (model 49PS, Thermo-Electron Instruments). The temperature in these chambers ranges between 70 and 78°C and the humidity between 40 and 58% depending on the ambient conditions on the exposure day.

Protocol. Ob/ob and wild-type mice were administered either rat anti-mouse IL-6 antibody (2.5 mg/kg MP5-20F3; BD-Biosciences, San Jose, CA) or isotype control antibody (R3-34, Rat IgG1 anti-mouse IL-6 antibody (2.5 mg/kg MP5-20F3; BD-Biosciences) via tail vein injection. The concentration of anti-IL-6 Ab used was chosen based on reports of its ability to markedly reduce serum IL-6 in a mouse model of sepsis induced by cecal ligation and puncture (40). Twenty-four hours after injection, mice were exposed to either O₃ (2 ppm for 3 h) or to filtered air. The O₃ exposure protocol was chosen to allow for comparison with data of other investigators studying acute O₃-induced inflammation in mice (6, 8, 21, 23, 36, 42, 49, 58), including obese mice (26, 34, 46), and because we had previously demonstrated a role for IL-6 in the neutrophil influx into the lungs that occurred in response to this O₃ exposure protocol (25).

Increases in BAL neutrophils, BAL protein, a marker of lung injury, BAL sTNFR1, and in some chemokines such as IP-10, are usually just detectable 4 h after cessation of acute O₃ exposure (2 ppm for 3 h) but continue to increase through 24 h. In contrast, IL-6 itself and many other chemokines that are induced by O₃ are observed in BAL fluid 4 h after cessation of acute O₃ exposure, but then decline towards basal values by 24 h. Consequently, to assess inflammation and injury, mice were studied 4 or 24 h after exposure. BAL was performed, blood was obtained by cardiac puncture, and the lungs were collected and snap frozen at ~80°C for subsequent protein extraction.

BAL. Four or 24 h after cessation of O₃ exposure, mice were euthanized with an overdose of pentobarbital sodium. The trachea was cannulated with an 18-gauge tubing adaptor, and the lungs were lavaged twice with 1 ml of PBS/0.6 mM EDTA, which was instilled and then slowly withdrawn over 30 s. Approximately 80–90% of the BAL fluid was recovered (the % did not differ between strains) and was placed on ice until it was centrifuged at 2,000 rpm at 4°C for 10 min. Cell pellets were resuspended in Hanks’ balanced salt solution, and the total number of cells was counted with a hemocytometer. Aliquots of cells were also centrifuged onto glass slides at 800 rpm for

10 min (Cytospin 3; Shandon, Sewickley, PA), air-dried, and stained with Wright-Giemsa (LeukoStat; Fisher Scientific, Pittsburgh, PA). Cell differentials were determined by counting 300 cells under ×400 magnification. The supernatant was frozen at ~80°C and subsequently analyzed for total protein concentration by using the Bio-Rad dye reagent (Bio-Rad, Richmond, CA) according to the manufacturer’s instructions. This assay has a sensitivity of ~1.25 μg/ml. The concentrations of BAL IL-6, KC, MIP-2, sTNFR1, IP-10, lipopolysaccharide-induced CXC chemokine (LIX, CXCL5), MCP-1, leukemia inhibitor factor (LIF), eotaxin, and serum albumin in the BAL fluid were determined with ELISA kits (Endogen, Woburn, MA) for IL-6; Immunology Consultants Laboratory, Newberg, OR for serum albumin, and R&D Systems, Minneapolis, MN for all others). Different markers of inflammation were assessed 4 and 24 h after O₃ exposure, based on previously reported differences in the time course of induction of these moieties by O₃ (12, 26, 34, 46, 49). Serum was isolated and stored at ~20°C until assayed for IL-6 by ELISA (Endogen).

Western blotting. The excised right lung was homogenized in lysis buffer (buffered saline solution containing Nonidet P-40 (Calbiochem), sodium deoxycholate, SDS, sodium orthovanadate, and protease inhibitors) using a Kinematica Polytron. Total protein was separated by SDS-PAGE and transferred to Immobilon-P polyvinylidene fluoride membranes. Membranes were probed with rabbit anti-phosphoTy705-STAT-3, anti-STAT-3, anti-phosphoSTAT-1, or anti-STAT-1 (Cell Signaling, Woburn, MA). After washing, membrane-bound primary antibodies were detected on autoradiographic film by horseradish peroxidase-conjugated secondary antibodies and the ECL Plus chemiluminescent system (Amersham Pharmacia Biotech). Protein bands were quantified by laser densitometer using Gel Pro Analyzer (Media Cybernetics, Silver Spring, MD), and band density values were expressed in arbitrary OD units. For STAT-3 and pTyr-STAT-3, two bands were typically observed, as previously reported (27). Since densitometric analysis indicated no effect of O₃ exposure, genotype, or antibody on the relative expression of these two bands, they were summed together for analysis.

Statistics. Differences in BAL cells, protein, serum albumin, cyto- kines, and chemokines, and in STAT expression, were assessed by factorial ANOVA using exposure, genotype, and antibody treatment as main effects. In each case, the Fisher least significant differences test was used for post hoc comparisons. Statistical analyses were carried out by using SAS software (SAS Institute, Cary, NC). All results are presented as means ± SE unless otherwise indicated. P < 0.05 was considered statistically significant.

RESULTS

Body mass. Wild-type and ob/ob mice weighed 20.7 ± 0.5 g and 58.4 ± 0.9 g, respectively (P < 0.05). Both male and female ob/ob mice weighed more than wild-type controls; females and males averaged, respectively, 190% and 170% more than wild-type controls. No substantial gender differences in response were observed, and data from both genders are summarized.

Intravenous anti-IL-6 antibody blocks IL-6 in the lung. We have previously reported that BAL IL-6 is increased 4 h after acute exposure to O₃ (2 ppm) and then declines to near basal levels by 24 h (25, 26, 34, 46). Therefore, to verify the efficacy of the anti-IL-6 antibody treatment, we measured IL-6 in BAL fluid of mice treated with anti-IL-6 or control antibody 4 h after acute exposure to O₃ (Fig. 1). Following O₃ exposure, BAL IL-6 levels increased markedly compared with air exposure in mice treated with control antibody, as previously described (25, 26, 34, 46). However, in mice treated with anti-IL-6 antibody, there was a marked reduction in O₃-induced BAL.

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Mice were injected by tail vein 24 h before exposure with either anti-IL-6 Ab or control Ab. Results are means ± SE. *P < 0.05 vs. mice of same genotype and Ab treatment exposed to air; #P < 0.05 vs. control Ab-injected mice of the same genotype. N = 6 per group.

IL-6 (Fig. 1), indicating that the antibody effectively neutralized IL-6 in the lung. By 24 h, BAL IL-6 was not significantly different from air-exposed mice in any group (data not shown). Serum IL-6 for all groups was below the limit of detection of our ELISA assay.

Effect of IL-6 antibody on pulmonary inflammation measured 4 h post-O3 exposure. In mice treated with control antibody, exposure to O3 caused a significantly greater inflammatory response in obese than in lean mice, as previously described (26, 34, 46). BAL neutrophils were significantly greater in O3-exposed ob/ob mice than in lean controls (Fig. 2A). Similar results were obtained for MIP-2, eotaxin, MCP-1, and LIX, and the same trend was observed for IP-10 and KC (Fig. 2). Treatment with IL-6 antibody had no effect on any of these inflammatory moieties in wild-type mice. However, in O3-exposed ob/ob mice, anti-IL-6 antibody significantly reduced BAL neutrophils (Fig. 2A) and BAL eotaxin (Fig. 2B) to levels not significantly different from lean controls. Anti-IL-6 antibody also reduced BAL MCP-1 (Fig. 2C) in ob/ob mice, although the effect was not nearly as profound as it was for neutrophils and eotaxin. BAL epithelial cells, BAL protein, and BAL serum albumin were not increased 4 h following acute O3 exposure in any group.

To determine whether other IL-6 family members might be induced by O3, we also assayed LIF in BAL fluid 4 h after O3 exposure (Fig. 2H). Compared with air, O3 caused a significantly greater increase in LIF in ob/ob vs. wild-type mice. There was no effect of antibody treatment on BAL LIF.

**Effect of IL-6 antibody on pulmonary inflammation and injury measured 24 h post-O3 exposure.** Different outcome indicators were assessed 4 and 24 h after cessation of O3 exposure based on differences in their time course of response following O3 exposure (26, 34, 46). Figure 3 shows BAL serum albumin and total BAL protein, measures of epithelial barrier injury, 24 h after exposure to O3 or room air. In mice treated with control antibody, exposure to O3 resulted in significant increases in BAL levels of serum albumin in ob/ob but not wild-type mice, and treatment with anti-IL-6 antibody completely ablated this difference (Fig. 3A). Similar results were obtained using BAL protein (Fig. 3B): O3 exposure resulted in significantly greater BAL protein in ob/ob vs. wild-type mice treated with control antibody, but this difference was abolished in mice treated with anti-IL-6 antibody.

In contrast to measures of pulmonary epithelial barrier integrity, O3-induced increases in BAL neutrophils were not significantly different in lean and obese mice 24 h postexposure (Fig. 4A), even though the early neutrophilic response was significantly greater in the obese vs. lean mice (Fig. 2A). Together, the results suggest that the neutrophilic response is accelerated in the obese mice. Anti-IL-6 antibody caused a significant decrease in neutrophil influx at 24 h post-O3 exposure in both obese and lean mice (Fig. 4A). In contrast, for other aspects of the O3 response, the effect of anti-IL-6 antibody differed between lean and obese mice. For example, in control antibody-treated mice, O3 exposure increased the number of BAL macrophages (Fig. 4B) and sloughed airway epithelial cells (Fig. 4C), but the effect was not substantially different in lean and obese mice. Compared with control antibody, anti-IL-6 antibody significantly reduced macrophage influx and airway epithelial sloughing in lean but not obese mice.
mice. O\textsubscript{3} caused significant increases in BAL IP-10 and sTNFR1 in obese, but not in lean, mice (Fig. 4, \textit{D} and \textit{E}). For IP-10, anti-IL-6 antibody significantly reduced the O\textsubscript{3}-induced increases observed in obese mice and ablated the difference between lean and obese mice. A similar trend was observed for sTNFR1 but did not reach statistical significance.

O\textsubscript{3} exposure causes STAT-3 and STAT-1 activation. Since many of the effects of IL-6 are mediated by STAT-3 and STAT-1, we examined activation of these transcription factors by O\textsubscript{3} exposure. Measurements were made 4 h after cessation of O\textsubscript{3} exposure, because induction of IL-6 by acute O\textsubscript{3} exposure peaks at that time and then declines to levels close to those observed in air-exposed mice by 24 h (26). There was no effect of O\textsubscript{3}, genotype, or antibody treatment on total STAT-3 (Fig. 5).

Compared with air, exposure to O\textsubscript{3} (2 ppm for 3 h) resulted in a robust increase in pTyr-STAT-3 in lung homogenates of mice euthanized 4 h after exposure (Fig. 5). Densitometric analysis indicated that O\textsubscript{3} exposure caused an \textasciitilde10-fold increase in pTyr-STAT-3 ($P < 0.01$) (Fig. 5). However, there was no effect of either genotype or antibody treatment on O\textsubscript{3}-induced tyrosine phosphorylation of STAT-3 (Fig. 5).

In wild-type mice treated with control antibodies, exposure to O\textsubscript{3} also increased pSTAT-1 (Fig. 6), although the magnitude of this effect was only about a 50% increase ($P < 0.05$, Fig. 6) vs. \textasciitilde10-fold for pSTAT-3 (Fig. 6). Remarkably, there was less total STAT-1 in lung homogenates from \textit{ob/ob} vs. wild-type mice regardless of exposure or antibody treatment. Densitometric analysis indicated that \textit{ob/ob} mice had approximately half the total STAT-1 of wild-type mice ($P < 0.01$) (Fig. 6). Neither O\textsubscript{3} exposure nor Ab treatment affected total STAT-1 expression. Phosphorylated STAT-1 was also reduced in \textit{ob/ob} vs. wild-type mice regardless of exposure or antibody treatment ($P < 0.01$) (Fig. 6), and in contrast to wild-type mice, O\textsubscript{3} exposure failed to increase pSTAT-1 in \textit{ob/ob} mice. Anti-IL-6 antibody did not affect either pSTAT-1 or total STAT-1 in either wild-type or \textit{ob/ob} mice (Fig. 6).

**DISCUSSION**

The results of these experiments (summarized in Table 1) indicate that IL-6 contributes to the pulmonary inflammation and injury that occurs with acute O\textsubscript{3} exposure in a manner that differs between lean and obese animals. For example, in obese mice, neutrophil influx into the lungs at 4 h after exposure was accelerated compared with lean mice, and this difference was markedly attenuated following IL-6 antibody treatment (Fig. 2A). In addition, O\textsubscript{3}-induced changes in BAL serum albumin...
and total BAL protein, indices of O₃-induced lung injury, were greater in obese vs. lean mice, and treatment with IL-6 antibody abolished the obesity-related differences in these outcomes (Fig. 3).

O₃ exposure leads to neutrophil influx into the lungs (10, 13, 25, 28, 35, 42, 56). Our results using IL-6 blocking antibodies (Figs. 2A and 4A) indicate that IL-6 is required for this response, confirming our previous results obtained using IL-6-
deficient mice (25), and are consistent with the results of Savov et al. (42) who reported an association between BAL IL-6 and BAL neutrophils across multiple strains of mice after acute O₃ exposure. IL-6 is also required for neutrophil recruitment to the lungs during Escherichia coli pneumonia (27). We observed a greater number of neutrophils in BAL fluid of obese vs. lean mice 4 h but not 24 h after O₃ exposure (Fig. 2A), suggesting that neutrophil influx was accelerated in the obese mice. Mice with other types of obesity also display greater lung neutrophilia following acute O₃ exposure than do lean controls (26). As previously discussed, this difference is unlikely to be related to obesity-related differences in the inhaled dose of O₃ (44). Importantly, treatment with IL-6 antibody markedly attenuated the earlier (4 h) increase in BAL neutrophils observed in the obese mice (Fig. 2A), rendering them similar to lean neutrophils at that time point. These data suggest that IL-6 contributes to obesity-related differences in the effects of O₃ on neutrophil influx.

We explored the possibility that differential effects of IL-6 on chemokine expression in lean and obese mice might be the cause of the accelerated neutrophil recruitment. We observed greater O₃-induced pulmonary chemokine expression in obese vs. lean mice treated with control antibody (Figs. 2 and 4) consistent with previous reports (26, 34, 46). Treatment with anti-IL-6 antibody had no effect on O₃-induced changes in BAL chemokines in lean mice (Fig. 2), consistent with previous reports in IL-6-deficient mice (25), and suggesting that in lean mice, the role of IL-6 in the O₃-induced influx of neutrophils is not mediated through changes in these chemotactic factors. In contrast, IL-6 antibody did attenuate O₃-induced expression of some chemokines in ob/ob mice (Figs. 2, B and C, and 4D). However, the chemokines affected by IL-6 antibody (eotaxin, MCP-1, and IP-10) are not ligands for the major neutrophil chemokine receptors CXCR1 and CXCR2. There is precedent for a role for IP-10 in the neutrophil recruitment elicited by O₃ exposure (35), but IL-6 and obesity-dependent changes in this chemokine were only observed 24 h post-O₃ (Fig. 4D), not at the 4-h time point where the accelerated neutrophil influx was observed (Fig. 2D). Consequently, it is likely that the apparent role of IL-6 in accelerating neutrophil influx into the lungs of obese mice is related to effects of IL-6 on the induction of other unmeasured neutrophil chemotactic agents or to effects of IL-6 on adhesion molecules or chemokine receptors.

Studies tracking movement of labeled tracers from the air spaces to the blood or vice versa indicate that O₃ can cause disruption of the epithelial barrier leading to increased permeability, thus permitting accumulation of serum proteins, such as albumin, in the air spaces (3). Thus our data indicate that O₃ caused greater epithelial barrier injury in obese vs. lean mice: O₃ exposure caused a marked increase in BAL serum albumin in ob/ob but not wild-type mice (Fig. 3A). Significantly greater increases in BAL protein, another marker of O₃-induced pulmonary edema (suggestive of pulmonary edema) in ob/ob but not wild-type mice (41). If such differences in the integrity of the epithelial barrier also occur in obese humans exposed to O₃, they could lead to increased uptake of and hence risk from other particulate copollutants (3). We did not perform a histological assessment of the lungs of these mice, so we cannot be certain of the anatomical correlates of the changes in BAL serum albumin and total protein that we observed.

The obesity-related differences in BAL albumin and protein were abolished in mice treated with IL-6 antibody (Fig. 3). The lack of effect of anti-IL-6 antibody on BAL protein in lean mice (Fig. 3B) is consistent with previous results from IL-6-deficient lean mice (25, 56). Together, the data indicate an important role for IL-6 in epithelial permeability in obese but not lean mice.

In contrast to pulmonary epithelial barrier injury, our data indicate that IL-6 does play a role in O₃-induced airway epithelial cell sloughing in lean mice (Fig. 4C), consistent with the data of Yu et al. (56), whereas no such effect of IL-6 was observed in obese mice (Fig. 4C). This discordance between epithelial barrier integrity as indexed by BAL protein and destruction of airway epithelial cells has been observed by others (54, 56) and suggests that different factors control these processes.

Obesity-related differences in O₃-induced IL-6 release do not account for the observed differences in the effect of anti-IL-6 antibody on responses to O₃ in ob/ob vs. wild-type mice: although there was a marked increase in BAL IL-6 with O₃, the magnitude of this change was the same in lean vs. obese mice (Fig. 1). Consequently, we considered the possibility that differences exist in the expression or activation of components of the IL-6 signal transduction pathway in the lungs of obese and lean mice. Indeed, we found that STAT-1 but not STAT-3 expression was substantially reduced in the lungs of ob/ob vs. wild-type mice, leading to reduced pSTAT-1.

### Table 1. Summary of obesity- and IL-6-dependent changes in the effects of O₃

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Effect of Obesity</th>
<th>Effect of IL-6 Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL neutrophils</td>
<td>Accelerates O₃-induced influx at 4 h</td>
<td>Ablates obesity-induced accelerated influx and markedly attenuates later influx in both obese and lean mice</td>
</tr>
<tr>
<td>BAL protein and BAL serum albumin</td>
<td>Augments O₃-induced increases at 24 h</td>
<td>Attenuates O₃-induced increase in obese but not lean mice, ablatating the obesity-related difference</td>
</tr>
<tr>
<td>BAL chemokines</td>
<td>Augments O₃-induced increases in most chemokines at 4 and 24 h</td>
<td>Attenuates O₃-induced increases in eotaxin, MCP-1, and IP-10 in obese but not lean mice</td>
</tr>
<tr>
<td>BAL LIF</td>
<td>Augments O₃-induced increases at 4 h</td>
<td>No effect</td>
</tr>
<tr>
<td>STAT-3</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>STAT-1</td>
<td>Decreases total STAT-1 and decreases O₃-induced pSTAT-1 at 4 h</td>
<td>No effect</td>
</tr>
</tbody>
</table>

O₃, ozone; BAL, bronchoalveolar lavage; LIF, leukemia inhibitory factor; STAT, signal transducer and activator of transcription; MCP-1, monocyte chemoattractant protein-1; IP-10, interferon-inducible protein 10.
upon O₃ exposure in ob/ob vs. wild-type mice (Fig. 6), whereas activation of STAT-3 was not affected by obesity (Fig. 5). STAT-3 and STAT-1 activation can have opposing effects on some cellular processes (9, 48), so it is conceivable that the obesity-related increase in the net ratio of STAT-3 to STAT-1 activation could be responsible for the increased effects of O₃ observed in obese vs. lean mice. The observation that STAT-1 is reduced in the lungs of ob/ob mice suggests that in addition to responses to O₃, other STAT-1-dependent processes in the lung may be impacted by obesity.

To our knowledge, we are the first to report that O₃ causes activation of STAT-3 in the lungs, although others have reported increased STAT-1 activation in alveolar macrophages from O₃-exposed mice (31). Since our measurements were made in lung homogenates, we cannot determine whether airway/air space epithelial cells, mesenchymal cells, or vascular cells are the source of these activated STAT proteins, and it is conceivable that there are more profound changes in some cell types that represent only a small fraction of the total lung protein. We expected activation of STAT-3 based on the robust increase in IL-6 release that occurs with O₃ exposure (Fig. 1). Hence, we were extremely surprised to find that treatment with anti-IL-6-Ab did not inhibit O₃-induced tyrosine phosphorylation of either STAT-3 or STAT-1 (Figs. 5 and 6), even though it markedly attenuated BAL IL-6 (Fig. 1). The data suggest that there are other moieties induced by O₃ exposure that cause STAT-3 and STAT-1 activation. Since other IL-6 family members are capable of activating these transcription factors, and since one of these, LIF, can be produced by many lung cell types (53), we assayed LIF in BAL fluid 4 h after O₃ exposure (Fig. 2H). Our results indicated that LIF was induced by O₃, especially in ob/ob mice. To our knowledge, this is the first report of LIF induction by O₃ in any species.

In summary, our results indicate that neutralizing IL-6 attenuates the accelerated neutrophil influx and the increased epithelial barrier injury that are observed in obese vs. lean mice following acute O₃ exposure, whereas other obesity-related differences in the response to O₃ are not dependent on IL-6. Prior reports have indicated that IL-6 can play differing roles (pro- or anti-inflammatory) depending on the nature of the inciting stimulus. Our results suggest that obesity may be another factor that influences the response to IL-6.

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