Innate and ozone-induced airway hyperresponsiveness in obese mice: role of TNF-α

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Williams AS, Mathews JA, Kasahara DI, Wurmbrand AP, Chen L, Shore SA. Inactive and ozone-induced airway hyperresponsiveness in obese mice; role of TNF-α. Am J Physiol Lung Cell Mol Physiol 308: L1168–L1177, 2015. First published April 3, 2015; doi:10.1152/ajplung.00393.2014.—Invasive airway hyperresponsiveness (AHR) and augmented responses to ozone, an asthma trigger, are characteristics of obese mice. Systemic inflammation, a condition of increased circulating concentrations of inflammatory moieties, occurs in obesity. We hypothesized that TNF-α, via its effects as a master effector of this systemic inflammation, regulates innate AHR and augmented responses to ozone in obese mice. Therefore, we examined pulmonary inflammation and airway responsiveness in unexposed or ozone-exposed (2 ppm for 3 h) lean wild-type and obese Cpefat mice that were TNF-α sufficient or deficient. Cpefat mice lack carboxypeptidase E, which regulates satiety. Compared with wild type, Cpefat mice had elevated serum IL-17A, G-CSF, KC, MCP-1, IL-9, MIG, and leptin, indicating systemic inflammation. Despite reductions in most of these moieties in TNF-α-deficient vs. -sufficient Cpefat mice, we observed no substantial difference in airway responsiveness in these two groups of mice. Ozone-induced increases in bronchoalveolar lavage (BAL) neutrophils and macrophages were lower, but ozone-induced AHR and increases in BAL hyaluronan, osteopontin, IL-13, and protein carbonyls, a marker of oxidative stress, were augmented in TNF-α-deficient vs. -sufficient Cpefat mice. Our data indicate that TNF-α has an important role in promoting the systemic inflammation but not the innate AHR of obesity, suggesting that the systemic inflammation of obesity is not the major driver of this AHR. TNF-α is required for the augmented effects of acute ozone exposure on pulmonary inflammatory cell recruitment in obese mice, whereas TNF-α protects against ozone-induced AHR in obese mice, possibly by suppressing ozone-induced oxidative stress.

bronchoalveolar lavage; systemic inflammation; hyaluronan; osteopontin; IL-13

OBESITY IS A RISK FACTOR for asthma (6, 12, 39, 49). Obese asthmatic patients are more symptomatic, use more healthcare resources, and have worse quality of life than lean asthmatic patients (53). Importantly, weight loss improves asthma outcomes including airway hyperresponsiveness (AHR) (7, 11), a defining feature of asthma. Especially in subjects with AHR, obesity exacerbates reductions in lung function induced by acute exposure to ozone (O3), an asthma trigger (1, 4). These data suggest that exposure to this common air pollutant may be a particular problem for the obese person with asthma. Obese mice also exhibit innate AHR and have greater airway responsiveness after acute exposure to O3 than lean mice (32, 50, 58, 59, 62).

The mechanistic basis for obesity-related asthma is poorly understood. The chronic, low-grade, systemic inflammation that characterizes obesity promotes other obesity-related conditions (18, 23, 42, 54) and may also play a role in the etiology of obesity-related asthma (26, 49, 53). Circulating TNF-α is increased both in obese humans and obese mice (22, 36, 45, 58) and may be of relevance for asthma, since exogenous administration of TNF-α has the capacity to induce AHR (55). TNF-α also has the capacity to induce many of the other cytokines and chemokines whose circulating concentrations are elevated in obesity, including MCP-1, G-CSF, IL-9, IP-10, IL-17A, and KC (2, 15, 33, 41, 47, 58).

Studies in obese mice suggest a complex role for TNF-α in obesity-related AHR (58, 59, 62). TNF-α can bind to either of two receptors, TNFR1 and TNFR2. We have reported that innate AHR is not observed in obese mice lacking TNFR2 (58), whereas it is augmented in obese mice lacking TNFR1 (62). These opposing effects of TNFR1 and TNFR2 deficiency likely reflect differences in downstream signaling events, since these receptors differ in their inflammatory potential, their ability to promote apoptosis, and their angiogenic effects (17, 27, 35, 40). Furthermore, TNFR2 interacts only with membrane-bound TNF-α, whereas TNFR1 can bind both soluble and membrane-bound TNF-α (35). Whether signaling through one TNF receptor counterbalances the opposing effects on AHR of signaling through the other, or whether one receptor dominates the pulmonary effects of TNF-α in obese mice, is unknown but important, since anti-TNF-α-based therapies block TNF-α signaling through both receptors.

The role of TNFR2 in pulmonary responses to acute O3 exposure also differs in lean and obese mice (10, 51, 59). In lean mice, TNFR2 deficiency reduces O3-induced AHR but does not affect O3-induced inflammation (10, 51, 59), whereas in obese mice TNFR2 deficiency augments O3-induced AHR but reduces O3-induced changes in baseline pulmonary mechanics and O3-induced recruitment of neutrophils and macrophages to lungs (59). The impact of overall TNF-α deficiency on obesity-related changes in the response to O3 has not yet been assessed but could differ from the impact of TNFR2 deficiency because of differences in TNFR1 and TNFR2 signaling described above.

We examined the hypothesis that TNF-α regulates the innate AHR of obesity via its effects as a regulator of the systemic inflammation of obesity. To do so, we generated obese Cpefat mice that were also genetically deficient in TNF-α (Cpefat TNF-α−/− mice). Cpefat mice are obese because they lack carboxypeptidase E (Cpe), an enzyme involved in processing neuropeptides involved in appetite regulation and energy expenditure (31). Serum was obtained from these mice to deter-
mine whether TNF-α regulates aspects of the systemic inflammation of obesity. We also measured airway responsiveness in otherwise naive Cpe<sup>fat</sup>/TNF-α<sup>−/−</sup> mice as well as in wild-type (WT), Cpe<sup>fat</sup>, TNF-α<sup>−/−</sup> mice. Finally, we examined the impact of overall TNF-α deficiency on obesity-related increases in pulmonary responses to O<sub>3</sub> exposure. To do so, we compared responses to acute O<sub>3</sub> exposure (2 ppm for 3 h) in WT, Cpe<sup>fat</sup>, TNF-α<sup>−/−</sup>, and Cpe<sup>fat</sup>/TNF-α<sup>−/−</sup> mice.

**METHODS**

**Animals.** The Harvard Medical Area Standing Committee on Animals approved this study. Heterozygous Cpe<sup>+/−</sup> mice and TNF-α<sup>−/−</sup> mice were purchased from Jackson Laboratory (Bar Harbor, ME) and mated. Cpe<sup>+/−</sup>/TNF-α<sup>−/−</sup> offspring were bred back to TNF-α<sup>−/−</sup> mice. Cpe<sup>+/−</sup>/TNF-α<sup>−/−</sup> mice from this mating were then bred together to obtain Cpe<sup>fat</sup>/TNF-α<sup>−/−</sup> mice and TNF-α<sup>−/−</sup> controls. Cpe<sup>+/−</sup> mice were mated together to yield WT and Cpe<sup>fat</sup> mice. Mice were on a C57BL/6 background, were fed standard mouse chow diets, and were 10–12 wk of age at the time of study.

**Protocol.** Female mice were either unexposed or exposed to O<sub>3</sub> (2 ppm for 3 h) as previously described (50). At 24 h after exposure, mice were anesthetized and instrumented for the measurement of pulmonary mechanics and airway responsiveness to inhaled aerosolized methacholine. Bronchoalveolar lavage (BAL) was then performed, followed by lung tissue harvest. Pulmonary mechanics and some BAL data from the WT and Cpe<sup>fat</sup> mice described below were previously reported (58). The TNF-α<sup>−/−</sup> and Cpe<sup>fat</sup>/TNF-α<sup>−/−</sup> mice whose data are reported below were studied concurrently with those WT and Cpe<sup>fat</sup> mice. In another cohort, WT, Cpe<sup>fat</sup>, TNF-α<sup>−/−</sup>, Cpe<sup>fat</sup>/TNF-α<sup>−/−</sup> mice were euthanized with an overdose of anesthetic without assessment of pulmonary function. Blood was then collected by cardiac puncture and serum was prepared to assess systemic inflammation. In this second cohort, mice were not exposed

![Fig. 1. TNF-α deficiency reduces the systemic inflammation associated with obesity. Serum IL-17A (A), G-CSF (B), KC (C), MCP-1 (D), IL-9 (E), MIG (F), IL-1α (G), IP-10 (H), and leptin (I) in wild-type (WT), Cpe<sup>fat</sup>, TNF-α<sup>−/−</sup>, and Cpe<sup>fat</sup>/TNF-α<sup>−/−</sup> mice not exposed to ozone. Results are means ± SE of data from 7–8 mice/group. *P < 0.05 vs. TNF-α genotype-matched Cpe-sufficient mice. #P < 0.05 vs. Cpe genotype-matched TNF-α-sufficient mice.](http://ajplung.physiology.org/)
to O₃. Results from serum from some of the WT and Cpefat mice from this cohort were previously described (58).

Pulmonary mechanics and airway responsiveness. Mice were anesthetized and instrumented for the measurement of pulmonary mechanics, as previously described (58). Quasi-static lung pressure-volume loops were obtained as previously described (58). From these loops, we computed A, the difference between total lung capacity and end-expiratory volume. Baseline total lung impedance (ZL) was then obtained by the forced oscillation technique. A parameter-estimation model (19) was used to partition ZL into components representing Newtonian resistance (Rn), which mainly reflects changes in the mechanical properties of the airways, and the coefficients of lung tissue damping (G) and lung tissue elastance (H), measures of changes in the lung periphery, including airway closure. Measurements of Rn, G, H were then obtained after inhalation of aerosols containing PBS and increasing concentrations of methacholine from 1 to 100 mg/ml, as previously described (58, 59).

Bronchoalveolar lavage. The lungs were lavaged and total cell numbers and differentials assessed as previously described (58, 59). Lavage supernatants were frozen at −80°C until analyzed for MCP-1, G-CSF, hyaluronic, osteopontin, and protein carbonyls by ELISA [all R&D Systems, except for protein carbonyls (Cell Biolabs, San Diego, CA) and hyaluronan (Echelon Biosciences, Salt Lake City, UT)]. Serum was prepared from blood obtained by cardiac puncture and frozen at −80°C until analyzed by multiplex assay for 35 different cytokines, chemokines, and growth factors (Eve Technologies, Calgary, Alberta, Canada) (58, 62).

RNA extraction and real-time PCR. RNA was extracted from lung tissue and cDNA prepared as previously described (58). Real-time PCR with SYBR-green detection was used to assess changes in M1l, M12, and Hmox1 mRNA. Primers were previously described (52). The ΔΔCt method was used to assess changes in the gene of interest relative to a housekeeping gene, 36B4 (Rplp0), a ribosomal protein.

Statistics. Differences in outcome indicators were assessed by factorial ANOVA using Cpe genotype and TNF-α type or TNF-α deficiency on the systemic inflammation of obesity, we performed a multiplex assay on serum from WT, Cpefat, TNF-α−/−, and Cpefat/TNF-α−/− mice not exposed to O₃ (Fig. 1). Note that we have previously reported an approximate twofold increase in serum TNF-α in Cpefat vs. WT mice (58). Factorial ANOVA indicated a significant effect of Cpe genotype or TNF-α genotype or a significant interaction between Cpe genotype and TNF-α genotype for serum IL-17A, G-CSF, KC, MCP-1, IL-9, MIG, IL-1α, and IP-10 (Fig. 1). Other cytokines and chemokines were either unaffected or were below the limit of detection of the multiplex assay in most mice. Serum IL-17A, G-CSF, KC, MCP-1, IL-9, MIG were elevated in Cpefat vs. WT mice and lower in Cpefat/TNF-α−/− than Cpefat mice (Fig. 1). Serum IL-1α and IP-10 were unaffected by Cpe genotype but were lower in TNF-α-deficient vs. TNF-α-sufficient mice (Fig. 1, G and H). We also examined serum concentrations of the satiety hormone leptin by ELISA (Fig. 1I). Serum leptin was markedly increased in the obese groups of mice, consistent with previous reports (58, 62), but was not significantly altered by TNF-α genotype in either lean or obese mice.

Pulmonary mechanics and airway responsiveness in unexposed mice. Baseline pulmonary mechanics (Rn, G, H) were elevated and lung volume (A) was reduced in obese vs. lean mice (Table 1, air values), as previously described (59). However, obesity-related alterations in TNF-α signaling did not contribute to these changes, since baseline Rn, G, H, and A were not different in Cpefat vs. Cpefat/TNF-α−/− mice.

As previously reported (58), airway responsiveness was greater in unexposed Cpefat than WT mice, whether G (Fig. 2A) or Rn (data not shown) was used as the index of response. Airway responsiveness was not different in TNF-α−/− vs. WT mice (Fig. 2A). There was no substantial difference in airway responsiveness in Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 2A).

Pulmonary mechanics and airway responsiveness in ozone-exposed mice. O₃ exposure had no effect on Rn in mice of any genotype, but increased baseline G and H in Cpefat mice (Table 1). In Cpefat/TNF-α−/− mice, O₃ also significantly increased G, whereas H was unaffected (Table 1).

### RESULTS

**Body mass.** Cpefat and Cpefat/TNF-α−/− mice both weighed more than twice as much as WT or TNF-α−/− mice, but body mass was not different in Cpefat vs. Cpefat/TNF-α−/− (data not shown). Therefore, we refer to both Cpefat and Cpefat/TNF-α−/− mice as obese and both WT and TNF-α−/− mice as lean.

**Systemic inflammation.** To determine the effect of overall TNF-α deficiency on the systemic inflammation of obesity, we performed a multiplex assay on serum from WT, Cpefat, TNF-α−/−, and Cpefat/TNF-α−/− mice not exposed to O₃ (Fig. 1). Note that we have previously reported an approximate twofold increase in serum TNF-α in Cpefat vs. WT mice (58). Factorial ANOVA indicated a significant effect of Cpe genotype or TNF-α genotype or a significant interaction between Cpe genotype and TNF-α genotype for serum IL-17A, G-CSF, KC, MCP-1, IL-9, MIG, IL-1α, and IP-10 (Fig. 1). Other cytokines and chemokines were either unaffected or were below the limit of detection of the multiplex assay in most mice. Serum IL-17A, G-CSF, KC, MCP-1, IL-9, MIG were elevated in Cpefat vs. WT mice and lower in Cpefat/TNF-α−/− than Cpefat mice (Fig. 1). Serum IL-1α and IP-10 were unaffected by Cpe genotype but were lower in TNF-α-deficient vs. TNF-α-sufficient mice (Fig. 1, G and H). We also examined serum concentrations of the satiety hormone leptin by ELISA (Fig. 1I). Serum leptin was markedly increased in the obese groups of mice, consistent with previous reports (58, 62), but was not significantly altered by TNF-α genotype in either lean or obese mice.

**Pulmonary mechanics and airway responsiveness in unexposed mice.** Baseline pulmonary mechanics (Rn, G, H) were elevated and lung volume (A) was reduced in obese vs. lean mice (Table 1, air values), as previously described (59). However, obesity-related alterations in TNF-α signaling did not contribute to these changes, since baseline Rn, G, H, and A were not different in Cpefat vs. Cpefat/TNF-α−/− mice.

As previously reported (58), airway responsiveness was greater in unexposed Cpefat than WT mice, whether G (Fig. 2A) or Rn (data not shown) was used as the index of response. Airway responsiveness was not different in TNF-α−/− vs. WT mice (Fig. 2A). There was no substantial difference in airway responsiveness in Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 2A).

**Pulmonary mechanics and airway responsiveness in ozone-exposed mice.** O₃ exposure had no effect on Rn in mice of any genotype, but increased baseline G and H in Cpefat mice (Table 1). In Cpefat/TNF-α−/− mice, O₃ also significantly increased G, whereas H was unaffected (Table 1).

### Table 1. Baseline pulmonary mechanics in unexposed and O₃-exposed mice

<table>
<thead>
<tr>
<th></th>
<th>A, ml</th>
<th>Rn, cmH₂O·ml⁻¹·s⁻</th>
<th>G, cmH₂O/ml</th>
<th>H, cmH₂O/ml</th>
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<tr>
<td>WT</td>
<td>0.74±0.04</td>
<td>0.20±0.01</td>
<td>5.2±0.3</td>
<td>29.9±2.6</td>
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<tr>
<td>Air</td>
<td>0.80±0.02</td>
<td>0.21±0.01</td>
<td>5.9±0.3</td>
<td>30.2±1.8</td>
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<tr>
<td>O₃</td>
<td>0.74±0.03</td>
<td>0.26±0.03</td>
<td>4.9±0.3</td>
<td>26.8±1.6</td>
</tr>
<tr>
<td>TFN-α−/−</td>
<td>0.97±0.04†</td>
<td>0.22±0.02</td>
<td>5.0±0.4</td>
<td>24.5±2.2</td>
</tr>
<tr>
<td>Cpefat</td>
<td>0.52±0.03†</td>
<td>0.43±0.05†</td>
<td>7.5±0.6†</td>
<td>47.2±3.0†</td>
</tr>
<tr>
<td>Air</td>
<td>0.49±0.03†</td>
<td>0.40±0.02†</td>
<td>9.0±1.0†</td>
<td>56.2±4.6†</td>
</tr>
<tr>
<td>O₃</td>
<td>0.54±0.03†</td>
<td>0.40±0.03†</td>
<td>7.0±0.5†</td>
<td>44.9±2.8†</td>
</tr>
<tr>
<td>Cpefat/TNF-α−/−</td>
<td>0.52±0.03†</td>
<td>0.37±0.06†</td>
<td>9.1±1.0†</td>
<td>44.2±5.4†</td>
</tr>
</tbody>
</table>

Results are means ± SE of data from 5–9 mice per group. A, difference between total lung capacity and end-expiratory volume; Rn, Newtonian resistance; G, coefficient of lung tissue damping; H, coefficient of lung tissue elastance. *P < 0.05 vs. genotype-matched air; †P < 0.05 vs. lean mice with same TNF-α genotype; ‡P < 0.05 vs. TNF-α-sufficient mice with same Cpe genotype.
The magnitude of O₃-induced AHR was significantly greater in Cpefat than WT mice (Fig. 2), as previously described (59). AHR was similar in O₃-exposed TNF-α⁻/⁻ mice (Fig. 2B). In contrast, AHR was significantly increased in O₃-exposed Cpefat/TNF-α⁻/⁻ mice vs. Cpefat mice (Fig. 2B), similar to previously reported results obtained in Cpefat/TNFR2⁻/⁻ mice (59). Similar results were obtained by using H rather than G as the index of responsiveness (data not shown).

Pulmonary inflammation in ozone-exposed mice. O₃ increased BAL neutrophils and macrophages (Fig. 3, A and B) and BAL protein (Fig. 3C), a marker of O₃-induced injury, to a greater extent in Cpefat vs. WT mice, as previously described (59). There was no difference in BAL neutrophils, macrophages, or protein in TNF-α⁻/⁻ vs. WT mice (Fig. 3). In contrast, BAL neutrophils and macrophages were significantly reduced and BAL protein was significantly increased in O₃-exposed Cpefat/TNF-α⁻/⁻ vs. Cpefat mice (Fig. 3).

The reductions in neutrophil and macrophage recruitment in the Cpefat/TNF-α⁻/⁻ vs. Cpefat mice exposed to O₃ were not the result of reductions in the expression of the neutrophil survival factor, G-CSF (Fig. 4A), or the macrophage chemotactic factor, MCP-1 (Fig. 4B). In fact, both BAL G-CSF and MCP-1 were actually significantly increased in Cpefat/TNF-α⁻/⁻ vs. Cpefat mice (Fig. 4, A and B).
Blocking IL-13 attenuates O3-induced increases in BAL neutrophils and macrophages in obese but not lean mice (59), suggesting that changes in IL-13 might account for TNF-α-dependent changes in BAL neutrophils and macrophages in these mice (Fig. 3, A and B). To test this hypothesis, we measured BAL IL-13. Compared with air, exposure to O3 increased BAL IL-13 in Cpefat but not WT mice (Fig. 4C), as previously described (59). However, BAL IL-13 was significantly greater in O3-exposed Cpefat/TNFR2−/− than in Cpefat mice (Fig. 4C).

IL-17A has the capacity to induce both AHR and neutrophil recruitment (28, 29, 38). We and others have reported that IL-17 contributes to the AHR caused by repeated O3 exposure in lean mice (43, 44). Consequently, we assessed Il17a mRNA abundance in lung tissue from these mice. Compared with air, O3 exposure caused a significant increase in lung Il17a mRNA abundance in TNF-α-sufficient but not in TNF-α-deficient mice (Fig. 4C). Although there was a trend toward increased Il17a mRNA in lungs of Cpefat vs. WT mice exposed to O3, the effect did not reach statistical significance.

Fragmentation of the matrix glycoprotein hyaluronan contributes to O3-induced AHR in lean mice (14). There is also evidence that the matrix protein osteopontin contributes to O3-induced AHR in lean mice (3). O3 increased both BAL hyaluronan (Fig. 5A) and BAL osteopontin (Fig. 5B) to a greater extent in obese than lean mice. In addition, BAL hyaluronan and BAL osteopontin were significantly greater in Cpefat/TNFR2−/− than Cpefat mice exposed to O3. Because fragmentation of hyaluronan by O3 exposure is likely the result of oxidative stress (14), and because oxidative stress also induces osteopontin (34, 56), we measured BAL protein carbonyls, a marker of oxidative stress (Fig. 5C). O3 significantly increased BAL protein carbonyls in obese (P < 0.01) but not lean mice (Fig. 5C). Moreover, BAL carbonyls were significantly greater in Cpefat/TNFR2−/− vs. Cpefat mice exposed to O3 (Fig. 5C). Importantly, there was a strong and significant correlation between BAL hyaluronic acid and BAL protein carbonyls (r² = 0.433, P < 0.01) (Fig. 6A) and between BAL osteopontin and BAL carbonyls in O3-exposed mice (r² = 0.644, P < 0.01) (Fig. 6B). Obesity is itself a condition of increased oxidative stress (18, 23); there was also a significant increase in BAL carbonyls in obese vs. lean air-exposed mice, when these mice were considered separately from those exposed to O3 (Fig. 5C). These data provide evidence of increased oxidative stress in lungs of obese mice even in the absence of a stressor such as O3.

O3 induces expression of several antioxidant enzymes including Hmox1, Mt1, and Mt2 (24), and deficiency in these enzymes augments the response to O3 (20, 21). Moreover, TNF-α can induce expression of these antioxidants (8, 9). Consequently, we examined the hypothesis that augmented oxidative stress in O3-exposed Cpefat/TNFR2−/− mice (Fig. 5C) might result from loss of induction of Hmox1, Mt1, and Mt2 by TNF-α. O3 increased pulmonary mRNA abundance of Hmox1, Mt1, and Mt2 to a greater extent in obese than in lean mice (Fig. 7). Importantly, O3-induced expression of Mt1 was increased not reduced in Cpefat/TNFR2−/− vs. Cpefat mice (Fig. 7A). A similar trend was observed for Mt2 but not Hmox1 expression (Fig. 7, B and C). Thus lack of induction of antioxidant enzymes does not account for augmented O3-induced AHR observed in TNF-α-deficient mice (Fig. 2).
DISCUSSION

Chronic low-grade systemic inflammation is a common feature of obesity (18, 23). Serum TNF-α is elevated in obesity (22, 36, 45, 58), including in Cpefat vs. WT mice (58), and TNF-α can induce many of the other serum cytokines and chemokines that are also elevated in the serum in obesity (2, 15, 30, 33, 41, 47, 58). Our data indicate increases in serum concentrations of many cytokines and chemokines in Cpefat vs. WT mice (Fig. 1), consistent with previous reports in both obese mice and humans (18, 23). Importantly, there was a substantial reduction in many of these inflammatory moieties in Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 1), suggesting an important role for TNF-α as a master regulator of the systemic inflammation of obesity. Similarly, Salles et al. (48) reported reductions in fasting serum IL-6 and MCP-1 in TNF-α−/− vs. WT mice that had been rendered obese with high-fat diets. TNF-α antagonists have also been shown to reduce some but not all aspects of the systemic inflammation of obese human subjects (5).

In contrast to the substantial reductions in systemic inflammation observed in Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 1), we have previously reported that few serum cytokines or
chemokines were reduced in either in Cpefat/TNFFR1−/− or Cpefat/TNFFR2−/− vs. Cpefat mice (58, 62), suggesting that signaling through either TNF-α is sufficient to induce the effects of TNF-α that promote systemic inflammation. Nevertheless, despite marked reductions in serum cytokines and chemokines in Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 1), we observed no amelioration of innate AHR in Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 2). The results suggest that factors other than the systemic inflammation of obesity are required for this innate AHR, although unmeasured aspects of obesity-related inflammation could be contributing.

Our data indicate no substantive differences in airway responsiveness in Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 2), suggesting little or no role for TNF-α in the innate AHR of obese mice. These data extend our previous observations on the role of TNF-α in the innate AHR of obesity as follows. We previously reported reduced airway responsiveness in otherwise naive Cpefat/TNFFR2−/− vs. Cpefat mice (58), but increased airway responsiveness in Cpefat/TNFFR1−/− vs. Cpefat mice (62). These opposing effects of TNFR1 and TNFR2 deficiency coupled with the lack of effect of overall TNF-α deficiency on obesity-related AHR are consistent with the hypothesis that signaling through one TNF receptor counter-balances the opposing effects on AHR of signaling through the other, so that when TNF-α signaling through both receptors was abolished, as in the TNF-α-deficient mice, no substantive effect on AHR is observed (Fig. 2).

Our results indicate greater increases in baseline pulmonary mechanics (Table 1), in airway responsiveness (Fig. 2), and in BAL neutrophils and macrophages (Fig. 3, A and B) after acute O3 exposure in Cpefat vs. WT mice, as previously described (59). There was little difference in the response to O3 in TNF-α−/− vs. WT mice. In contrast, baseline H (Table 1), a measure of airway closure, and BAL neutrophils and macrophages (Fig. 3, A and B) were reduced and airway responsiveness was increased (Fig. 2) in O3-exposed Cpefat/TNF-α−/− vs. Cpefat mice. Taken together, the results indicate important roles for TNF-α during O3 exposure in obese mice and indicate differences in the role of TNF-α in regulating pulmonary responses to O3 in lean and obese mice.

In Cpefat O3-exposed mice, both TNFR2 (59) and TNF-α deficiency (Fig. 3) reduced BAL neutrophils and macrophages to levels observed in WT mice. Hence, TNF-α plays a key role in the augmented O3-induced recruitment of these cells to the lungs observed in obesity. Circulating leukocytes were not different in O3-exposed Cpefat/TNF-α−/− vs. Cpefat mice (data not shown), indicating that this reduction in cellular recruitment to the lungs was not the result of changes in the number of leukocytes available for recruitment. Since BAL concentrations of both the neutrophil survival factor G-CSF and the macrophage chemotactic factor MCP-1 were augmented rather than reduced in Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 4), it is unlikely that changes in these moieties contribute to reductions in BAL neutrophils and macrophages. Similarly, osteopontin has been shown to contribute to O3-induced neutrophil recruitment in lean mice (3), and IL-13 has been shown to contribute to O3-induced neutrophil and macrophage recruitment in obese mice (59). These inflammatory moieties cannot account for reductions in BAL neutrophils in Cpefat/TNF-α−/− vs. Cpefat mice, since both BAL osteopontin and BAL IL-13 were actually greater in Cpefat/TNF-α−/− than Cpefat mice exposed to O3 (Fig. 4C, 5B). In contrast, we have previously reported that O3-induced increases in MIP3α (CCL20) are reduced in TNFR2−/− deficient vs. −/− obese mice (59). Furthermore, TNF-α has the capacity to increase MIP-3α expression in airway epithelial cells (46). Hence it is conceivable that MIP-3α or moieties released from cells recruited by this chemokine contribute to the reductions in BAL inflammatory cells observed in Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 3). The observation that O3-induced increases in IL17a mRNA abundance were observed in TNF-α-deficient but not TNF-α-deficient mice (Fig. 4D), coupled with the important role for IL-17A in neutrophil recruitment (37), suggests that the reduction in BAL neutrophils in the Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 3A) may be the result reduced IL-17A in these mice.

We considered several factors that might have accounted for the augmented airway responsiveness observed in Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 2B). Others have reported greater increases in body weight in TNF-α-deficient vs. TNF-α-sufficient db/db mice (16). However, we observed no such difference in TNF-α-deficient vs. TNF-α-sufficient Cpefat mice, nor does TNF-α deficiency impact weight gain in mice on high-fat diets (57). Taken together, the results suggest that the impact of TNF-α deficiency on weight gain (16) may be restricted to the setting of leptin signaling deficiency. The results also indicate that augmented O3-induced AHR in Cpefat/TNF-α−/− vs. Cpefat

Fig. 7. Impact of ozone on pulmonary mRNA abundance of M1l (A), M12 (B), and Hmox1 (C) in WT, Cpefat, TNF-α−/−, and Cpefat/TNF-α−/− mice. Results are means ± SE of data from 7–9 mice/group. *P < 0.05 vs. genotype-matched unexposed mice; #P < 0.05 vs. TNF-α genotype-matched Cpefat-sufficient mice; %P < 0.05 vs. Cpe genotype-matched TNF-α-sufficient mice.
mice was not the result of greater weight gain in the Cpefat/TNF-α−/− mice. Similarly, TNF-α-dependent differences in lung IL-17A cannot account for the augmented O3-induced AHR in Cpefat/TNF-α−/− vs. Cpefat mice. Although IL-17A can induce AHR, especially in combination with IL-13 (29), lung IL17a mRNA abundance was, if anything, lower not higher in Cpefat/TNF-α−/− compared with Cpefat mice (Fig. 4D). IL-13 can cause AHR (60); BAL IL-13 was greater in Cpefat/TNF-α−/− than in Cpefat mice (Fig. 4C). However, we have previously reported that anti-IL-13 does not attenuate O3-induced AHR in either Cpefat or Cpefat/TNF2−/− mice (59), suggesting that changes in IL-13 are likely not responsible for the augmented O3-induced AHR observed in Cpefat/TNF-α−/− vs. Cpefat mice. Changes in hyaluronan and osteopontin may account for the augmented O3-induced AHR observed in Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 2). Hyaluronan fragmentation and osteopontin are both required for O3-induced AHR in lean mice (3, 14). O3 increased both BAL hyaluronan and BAL osteopontin to a greater extent in Cpefat than WT mice, and both BAL hyaluronan and BAL osteopontin were further augmented in Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 5, A and B), consistent with the observed changes in AHR (Fig. 2). Our data also suggest that the greater increases in BAL hyaluronan and osteopontin in Cpefat/TNF-α−/− vs. Cpefat mice are the result of greater oxidative stress in the Cpefat/TNF-α−/− mice. O3 fragments hyaluronan via oxidative stress (14), oxidative stress is also a stimulus for osteopontin release (34, 56), and we observed greater BAL protein carbonyls, a marker of oxidative stress, in Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 5C). Importantly, linear regression indicated a significant correlation between either BAL hyaluronan (Fig. 6A) or BAL osteopontin (Fig. 6B) and BAL protein carbonyls.

Oxidative stress represents an imbalance between oxidant production and antioxidant levels. Our data indicate increases in markers of oxidative stress in Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 5C). This increased oxidative stress is not the result of reduced levels of the antioxidants Hmox1, Mt1, or Mt2 in the Cpefat/TNF-α−/− mice (Fig. 7), although we cannot rule out the possibility that TNF-α-related changes in other unmeasured antioxidants are involved. Inflammatory cells can also contribute to oxidative stress, but our data indicate reduced, not elevated, numbers of neutrophils and macrophages in O3-exposed Cpefat/TNF-α−/− vs. Cpefat mice (Fig. 3). Both obesity (13, 25) and O3 exposure (61) are conditions of increased oxidative stress and it is conceivable that TNF-α deficiency also impacts the production of oxidants in these mice.

The impact of TNF-α deficiency on responses to O3 in obese mice was qualitatively similar to the impact of TNFR2 deficiency previously reported (59): in both O3-exposed Cpefat/TNF-α−/− and Cpefat/TNF2−/− mice, BAL neutrophils and baseline pulmonary mechanics were lower and airway responsiveness was higher than in O3-exposed Cpefat mice. The similarity in the response to either TNF-α or TNFR2 deficiency suggests either that signaling through TNFR2 dominates the effects of TNF-α in the obese O3-exposed lung or that signaling through either TNF-α receptor is sufficient to mediate these effects. Although studies in mice may not necessarily be translatable to human subjects, these data also suggest that anti-TNF-α strategies would have little therapeutic benefit in obese asthmatic patients and may even lead to worsening responses to asthma triggers that act via changes in oxidative stress.

In summary, our data indicate no net role for TNF-α in the innate AHR of obese mice, despite substantial reductions in many aspects of the systemic inflammation characteristic of obesity in TNF-α-deficient obese mice. These data suggest that the systemic inflammation of obesity is not the major driver of this AHR or that inflammatory moieties not impacted by TNF-α deficiency are involved. Our results also indicate an important role for TNF-α in promoting the augmented effects of acute O3 exposure on baseline pulmonary mechanics and inflammatory cell recruitment observed in obese mice. In contrast, TNF-α protects against augmented O3-induced AHR observed in obese mice, likely by suppressing O3-induced oxidative stress and the resulting changes in hyaluronan and osteopontin, although the mechanism by which this change in oxidative stress occurs remains to be established.

REFERENCES


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