EFFECT OF ANTIGEN SENSITIZATION AND CHALLENGE ON OSCILLATORY MECHANICS OF THE LUNG AND PULMONARY INFLAMMATION IN OBESE CARBOXYPEPTIDASE E-DEFICIENT MICE

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Running Head: Antigen-induced lung pathophysiology in obese Cpefat mice
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ABSTRACT

Atopic, obese asthmatics exhibit airway obstruction with variable degrees of eosinophilic, airway inflammation. We previously reported that mice obese due to a genetic deficiency in either leptin (ob/ob mice) or the long isoform of the leptin receptor (db/db mice) exhibit enhanced airway obstruction in the presence of decreased numbers of bronchoalveolar lavage fluid (BALF) eosinophils as compared to lean, wild-type mice following antigen (ovalbumin; OVA) sensitization and challenge. To determine if the genetic modality of obesity induction influences the development of OVA-induced airway obstruction and OVA-induced pulmonary inflammation, we examined indices of these sequelae in mice obese due to a genetic deficiency in carboxypeptidase E, an enzyme that processes pro-hormones and pro-neuropeptides involved in satiety and energy expenditure (Cpefat mice). Accordingly, Cpefat and lean, wild-type (C57BL/6) mice were sensitized to OVA and then challenged with either aerosolized phosphate-buffered saline (PBS) or OVA. Compared to genotype-matched, OVA-sensitized and PBS-challenged mice, OVA sensitization and challenge elicited airway obstruction and increased BALF eosinophils, macrophages, neutrophils, interleukin (IL)-4, IL-13, IL-18, and chemerin. However, OVA challenge enhanced airway obstruction and pulmonary inflammation in Cpefat as compared to wild-type mice. These results demonstrate that OVA sensitization and challenge enhances airway obstruction in obese mice regardless of the genetic basis of obesity whereas the degree of OVA-induced pulmonary inflammation is dependent on the genetic modality of obesity induction. These results have important implications for animal models of asthma, as modeling the pulmonary phenotypes for subpopulations of atopic, obese asthmatics critically depends on selecting the appropriate mouse model.
Keywords: asthma, atopic, eosinophil, interleukin-13, and ovalbumin
INTRODUCTION

Obesity is an epidemic among children, adolescents, and adults and is a clearly recognized determinant of breast and colon cancer, cardiovascular disease, nonalcoholic steatohepatitis, and type 2 diabetes (24, 53, 58, 62). Furthermore, a number of investigators have reported an increased prevalence and incidence of asthma in obese children, adolescents, and adults (8, 50, 68). Obesity also increases asthma severity, decreases asthma control, and decreases the efficacy of standard asthma medications (40). As the prevalence of obesity is high in both developed and developing countries (80), a significant number of individuals are at risk for developing asthma. To understand and ultimately prevent the negative health effects of asthma caused by obesity, elucidating the mechanistic relationships between obesity and asthma is urgently needed.

Obesity is common in individuals with severe or refractory asthma (3, 33, 34, 46, 57). Data from Holguin and colleagues (33) demonstrate at least two distinct phenotypes of severe asthma in obesity: early- and late-onset asthma, which are categorized by the age of onset of asthma, airway responsiveness to methacholine, and atopic status. Specifically, obese subjects with early-onset asthma have significantly greater airway responsiveness to methacholine and a significantly higher prevalence of atopy as compared to obese subjects with late-onset asthma (33). Dixon et al. (20) also reported that atopic status can be used to differentiate obese, human asthmatic subjects into subpopulations. Specifically, surgically-induced weight loss significantly decreased airway responsiveness to methacholine in obese, non-atopic, but not obese, atopic asthmatic subjects (20). Based on these results, Dixon et al. (20) suggested that there are at least two distinct phenotypes of asthma in obesity: late-onset, non-atopic asthma due to obesity and
early-onset, atopic asthma that is complicated by the development of obesity. However, the mechanisms underlying the development of these distinct phenotypes in obese asthmatics are not well understood.

We have been using obese mice to characterize the mechanisms underlying the relationship between obesity and asthma, and we previously reported that obese mice exhibit innate airway hyperresponsiveness (AHR) to non-specific bronchoconstrictors such as methacholine and serotonin (36-39, 52, 64, 70). We have used mice obese due to a genetic deficiency in leptin, a satiety hormone, \(ob/ob\) mice, as a model for determining the effects of obesity on atopic asthma. Specifically, we have previously reported that \(ob/ob\) mice develop airway obstruction in the presence of decreased numbers of bronchoalveolar lavage fluid (BALF) eosinophils, lymphocytes, and macrophages as compared to lean, wild-type (C57BL/6) mice following antigen (ovalbumin; OVA) sensitization and challenge (39). OVA sensitization and challenge leads to a pulmonary phenotype in mice that mimics many of the characteristics features of atopic asthma in humans (44). We also obtained similar results with mice that are obese due to a genetic deficiency in the long isoform of the leptin receptor \(db/db\) mice (39).

Consistent with our observations in mice, data from human asthmatic subjects demonstrate that indices of atopic pulmonary inflammation, and in particular sputum eosinophils, decrease with increasing body mass index (20, 42, 73, 76). In contrast, recent data from Desai and colleagues (19) demonstrate that select indices of atopic, pulmonary inflammation, including interleukin (IL)-5 and submucosal eosinophils, increase with increasing body mass index. Taken together, these data suggest that even among atopic asthmatics, obesity has different effects on the development of pulmonary inflammation. Similarly, the genetic
101 modality of obesity induction in mice may result in different phenotypic responses to OVA
102 sensitization and challenge.

103 In this context, the major objective of this study was to determine the effect of OVA
104 sensitization and challenge on the oscillatory mechanics of the lung and pulmonary inflammation
105 in mice obese due to a genetic deficiency in carboxypeptidase E (Cpefat mice). Carboxypeptidase
106 E, a zinc-dependent exopeptidase, is expressed in the central nervous system and in endocrine
107 cells and processes pro-peptides, such as pro-insulin, pro-cholecystokinin, and pro-
108 opiomelanocortin, into biologically active peptides (14). Many of these biologically active
109 peptides generated from carboxypeptidase E-induced proteolytic processing of pro-peptides are
110 intimately involved in satiety and energy expenditure (14). Because of a missense mutation in
111 the gene encoding carboxypeptidase E in Cpefat mice, carboxypeptidase E enzymatic activity is
112 severely reduced in these animals (60), which prevents the processing of pro-peptides into their
113 biologically active peptide configuration (48). Consequently, due to disrupted satiety and energy
114 expenditure signaling pathways, Cpefat mice exhibit increased body mass by seven weeks of age
115 and extreme obesity by fourteen to sixteen weeks of age (37, 38).

116 In humans, a single nucleotide polymorphism in the gene encoding carboxypeptidase E is
117 positively associated with obesity (51). Furthermore, Cpefat mice, similar to db/db and ob/ob
118 mice, exhibit a number of obesity-related sequelae, including hypercholesterolemia (54),
119 hyperglycemia (26, 49, 66), insulin resistance (5, 35, 75), and tachypnea (52, 68, 70).
120 Collectively, these data demonstrate that Cpefat mice are a relevant, pre-clinical model of human
121 obesity that can be used to enhance our understanding of the mechanisms by which obesity
122 influences the development of atopic, pulmonary inflammation in asthmatics.
In this current study, we report that $Cpe^{fat}$ mice exhibit enhanced airway obstruction as compared to lean, wild-type (C57BL/6) mice following OVA sensitization and challenge, which is similar to our previous observations in $db/db$ and $ob/ob$ mice (39). However, in contrast to $db/db$ and $ob/ob$ mice, OVA-induced pulmonary inflammation is significantly greater in $Cpe^{fat}$ as compared to wild-type mice. These results demonstrate that OVA sensitization and challenge enhances airway obstruction in obese mice regardless of the genetic basis of obesity whereas the degree of OVA-induced pulmonary inflammation is dependent upon the genetic modality of obesity induction. Thus, when modeling the pulmonary phenotypes that exist in subpopulations of atopic, obese asthmatics, it is critically important to select the appropriate mouse model.
MATERIALS AND METHODS

Animals. Female $Cpe^{fat}$ mice were purchased from The Jackson Laboratory (Bar Harbor, ME) at 3–7 weeks of age. Because $Cpe^{fat}$ mice were backcrossed onto a C57BL/6J genetic background for at least ten generations, age-matched, female C57BL/6J mice were used as wild-type controls and purchased from The Jackson Laboratory at the same time as the $Cpe^{fat}$ mice.

All mice were housed in individually-ventilated, microisolator cages (Tecniplast S.p.a., Buguggiate, Varese, Italy), containing no more than five animals per cage, within a multi-species, modified barrier animal care facility, where they were given irradiated food (PicoLab® Rodent Diet 20, LabDiet®, Brentwood, MO) and autoclaved water ad libitum, exposed to a 12-hour:12-hour light-dark cycle, and acclimated to their new environment for at least 3 days prior to entering the experimental protocol at 4–9 weeks of age. All of the experimental protocols used in this study were approved by The University of Texas Health Science Center at Houston Animal Welfare Committee.

Antigen Sensitization and Challenge. On day 0 of the experimental protocol, when the mice were 4–9 weeks of age, each animal was initially sensitized to albumin from chicken egg white (ovalbumin, OVA, Grade V, Sigma-Aldrich Co., St. Louis, MO) via an intraperitoneal (i.p.) injection of 20 µg of OVA and 2 mg of adjuvant, aluminum hydroxide powder [Mallinckrodt Baker, Inc., Phillipsburg, NJ], dispersed in 0.2 mL of phosphate-buffered saline (PBS). On days 14 and 56 of the experimental protocol, each animal was given an i.p. injection of the same reagents that were administered on day 0. A 1 mL insulin syringe with a 28-gauge hypodermic needle (Becton, Dickinson and Company, Franklin Lakes, NJ) was used for all i.p. injections.
On days 70 through 76 of the experimental protocol, the animals were challenged for twenty-five minutes once per day with either an aerosol of PBS containing 4% OVA (weight/volume) or an aerosol of PBS alone. The aerosol was generated by driving air from a PRONEB® Ultra II air compressor (PARI Respiratory Equipment, Inc., Midlothian, VA) into a PARI LC® Sprint nebulizer (PARI Respiratory Equipment, Inc.), which contained 8 mL of either PBS containing 4% OVA or PBS alone. To challenge the mice with an aerosol, the animals were individually placed into one of twelve ventilated chambers of a circular pie cage (Braintree Scientific, Inc., Braintree, MA). On day 77 of the experimental protocol, which was twenty-four hours following the cessation of the final aerosol challenge, the animals, which were 15–20 weeks of age at this time, were subjected to one or more of the subsequently described experimental procedures.

**Protocol.** Three separate cohorts of wild-type and Cpefat mice were used in this study. In the first cohort, airway and lung parenchymal oscillation mechanics were assessed in anesthetized mice twenty-four hours following the cessation of the final PBS or OVA aerosol challenge. In the second cohort, blood was collected from and a bronchoalveolar lavage (BAL) was performed on mice that were euthanized twenty-four hours following the cessation of the final PBS or OVA aerosol challenge. In the third cohort, blood was collected from and the lungs were fixed in situ in mice that were euthanized twenty-four hours following the cessation of the final PBS or OVA aerosol challenge.

**Measurement of Airway and Lung Parenchymal Oscillation Mechanics.** Mice in the first cohort were anesthetized with pentobarbital sodium (50 mg/kg, i.p., Hospira, Lake Forest, IL)
and xylazine hydrochloride (7 mg/kg, i.p., Akorn, Decatur, IL) twenty-four hours following the cessation of the final PBS or OVA aerosol challenge. Once the mouse was acceptably anesthetized, as determined by unresponsiveness to a hind paw pinch, a tracheostomy was performed, and an 18-gauge tubing adaptor (Becton, Dickinson and Company) was inserted into the trachea. Subsequently, wild-type and \( Cpe^{fat} \) mice were artificially ventilated at 150 or 180 breaths per minute, respectively, with a tidal volume \((V_T)\) of 0.3 mL and a positive end-expiratory pressure of 3 cm H\(_2\)O using a specialized ventilator (flexiVent, SCIREQ Scientific Respiratory Equipment, Montréal, Québec, Canada) as we have previously described (2, 64, 70). The higher breathing frequency used for the \( Cpe^{fat} \) mice was chosen to conform to the spontaneous breathing frequency of these animals, yet \( V_T \) was slightly, but not significantly, reduced in \( Cpe^{fat} \) as compared to wild-type mice during spontaneous breathing (68). Consequently, spontaneous minute ventilation was not different between \( Cpe^{fat} \) and wild-type mice (68). Once ventilation was established, a wide incision in the chest wall was made bilaterally and any adipose tissue overlying the lungs was carefully removed in order to expose the lungs to atmospheric pressure.

Total lung impedance \((Z_L)\) at baseline was determined by the forced oscillation technique as described in detail by others (30, 32, 63). Specifically, \( Z_L \) was measured by perturbing ventilation for three seconds and then simultaneously delivering thirteen sinusoidal forcing functions, which ranged in frequency from 1 to 20.5 Hz, to the animal. The flexiVent was used to both ventilate the lungs and to deliver the sinusoidal forcing functions. The constant-phase model, as described by Hantos and colleagues (30), was used to partition \( Z_L \) into components representing airway resistance \((R_{aw})\), the coefficient of lung tissue damping \((G)\), the coefficient of lung tissue elastance \((H)\), and lung tissue hysteresivity \((\eta = G/H)\). Measurements of the real
and imaginary components of $Z_L$, which were obtained from OVA-sensitized and PBS-challenged, wild-type and $Cpe^{fat}$ mice, as a function of breathing frequency, conformed to the constant phase model (data not shown).

The following protocol was executed to facilitate measurements of $Z_L$ at baseline in our mice. First, ventilation was paused for six seconds, and a pressure of 30 cm H$_2$O was applied to the system to inflate the lungs to capacity to standardize lung volume history. Next, ventilation was allowed to resume for at least six seconds, and then a 2.5 Hz sinusoidal forcing function was applied, while ventilation was perturbed for 1.25 seconds, to measure total lung resistance ($R_L$) as described by Bates et al. (4). $R_L$ was then measured at least five more times using this method to ensure that $R_L$ was stable. Once $R_L$ was stable, a sinusoidal forcing function, as described above, perturbed ventilation for 1.25 seconds to measure $R_L$. Subsequently, $Z_L$ was determined by simultaneously delivering thirteen sinusoidal forcing functions to the animal, ranging in frequency from 1 to 20.5 Hz. At this time, $Z_L$ was also partitioned into $R_{aw}$, G, H, and $\eta$. The measurement of $R_L$ and $Z_L$ was repeated two more times for a total of three measurements. Once all measurements were complete, the perturbations ceased and ventilation resumed uninterrupted. The three measurements of $R_{aw}$, G, and H, which were partitioned from the three distinct measurements of $Z_L$, were averaged to determine a mean value of $R_{aw}$, G, and H for each animal.

In order to establish the goodness of fit of the constant phase model to our data, the *flexiVent* software (Version 5.3) calculates a coefficient of determination (COD). Any measurement with a COD less than 0.9 was excluded from our study.

**Blood Collection and Isolation of Serum.** Twenty-four hours following the cessation of the final PBS or OVA aerosol challenge, each mouse in the second cohort was given an i.p. injection
of pentobarbital sodium (200 mg/kg, i.p., Vortech Pharmaceuticals, Dearborn, MI). Once each mouse was deeply anesthetized and unresponsive to any stimuli, it was placed in the supine position, and a median sternotomy was performed to expose the heart and lungs in situ. To collect blood from the animal, the heart was punctured with a 25-gauge hypodermic needle attached to a 1 mL syringe (Becton, Dickinson and Company). After the blood was collected, it was placed into Microtainer® serum separator tubes (Becton, Dickinson and Company). The blood was then allowed to clot at room temperature for at least thirty minutes prior to being centrifuged at 15,000×g for two minutes at 4°C so as to isolate serum. The serum was then stored at −20°C until needed.

BAL. After blood was collected from each animal, the animal was prepared for and then subjected to a BAL. First, the trachea and larynx were exposed in situ by removing any overlying fur, skin, and fascia covering these tissues. Next, a small incision was made on the ventral surface of the trachea directly distal to the larynx with micro scissors. A 20-gauge fluorinated ethylene propylene polymer catheter (Becton, Dickinson and Company) attached to a 1 mL syringe containing 0.6 mL of lavage buffer [PBS containing 0.6 mM of ethylenediaminetetraacetic acid] was then inserted into the tracheal incision. Subsequently, the lungs were gently lavaged four times. The first lavage was with 0.6 mL of lavage buffer, and the second lavage was with the resulting lavagate instilled into the lungs again, subsequently retrieved a second time, and then stored on ice. This process was repeated again with a separate aliquot (0.6 mL) of lavage buffer, and the resulting lavagate from the second retrieval in this instance was pooled with the first lavagate, which was already stored on ice. The pooled lavagate was centrifuged at 600×g for ten minutes at 4°C. Once centrifugation was complete, the
supernatant was isolated and stored at −80°C until needed. The remaining cell pellet was resuspended in 1 mL of Hanks’ Balanced Salt Solution (HyClone Laboratories, Logan, UT) and the total number of cells within this cell suspension was enumerated with a hemacytometer. In order to perform a differential cell analysis on the cells recovered in the BALF, an aliquot of the cell suspension containing 25,000 cells was spun at 800 rpm for 10 minutes at room temperature using a Cytospin® 3 cytocentrifuge (Thermo Shandon Limited, Runcorn, United Kingdom). The slides were then air dried and stained with a Hema 3 stain set (Fisher Diagnostics, Middletown, VA). Cells were classified as eosinophils, lymphocytes, macrophages, neutrophils, or respiratory epithelial cells according to standard morphological characteristics (25, 27). Specifically, respiratory epithelial cells were identified by the presence of cilia. Cells which could not be clearly identified as one of the aforementioned cell types were categorized as indeterminates. At least 300 cells per slide were counted during the differential cell analysis.

**Enzyme-Linked Immunosorbent Assays.** The concentrations of adiponectin, chemerin, eotaxin, IL-4, IL-5, IL-13, IL-18, leptin, OVA-specific IgE and IgG1, and total IgE in the BALF and/or serum were measured using enzyme-linked immunosorbent assays. The OVA-specific and total IgE assays were purchased from BioLegend, Inc. (San Diego, CA) while the OVA-specific IgG1 assay was purchased from Cayman Chemical Company (Ann Arbor, MI). All other assays were purchased from R&D Systems, Inc. (Minneapolis, MN). The assays were performed according to the manufacturer’s instructions.

**Lung Histology.** Twenty-four hours following the cessation of the final PBS or OVA aerosol challenge, wild-type and *Cpe*<sup>fut</sup> mice in the third cohort, were administered an overdose of
pentobarbital sodium (200 mg/kg, i.p.), and once unresponsive to a hind paw pinch, a median sternotomy was performed to expose the heart and lungs in situ. Next, as described above, blood was collected from the heart, serum isolated from the blood, and the isolated serum stored at −20°C until needed. Afterwards, the abdominal aorta was severed and the right ventricle of the heart was punctured with a 25-gauge hypodermic needle (Smiths Medical ASD, Inc., Keene, NH) that was attached to a syringe containing 10 mL of ice-cold PBS. Subsequently, the heart and the circulatory system of the animal were perfused with 10 mL of ice-cold PBS. We then performed a tracheostomy on the animal and inserted a 19-gauge blunt needle (BRICO Medical Supplies, Inc., Dayton, NJ) into the trachea, which we secured by tying it in place. The lungs were then fixed in situ with 10% phosphate-buffered formalin (Fisher Scientific, Fair Lawn, NJ) at a pressure of 25 cm H2O and removed en bloc. The lungs were then placed in 10% phosphate-buffered formalin at 4°C where they remained for at least twenty-four hours. Afterwards, the lungs were dehydrated in ethanol gradients, cleared with xylene, and infiltrated with paraffin. The lungs were then embedded in paraffin blocks and four micrometer thick coronal sections of the right and left lung were cut with a microtome. The resulting sections were mounted onto a microscope slide. Sections were stained with hematoxylin and eosin or subjected to the Periodic Acid Schiff (PAS) reaction, dried overnight, covered with mounting medium, and overlaid with a cover slip.

Inflated portions of hematoxylin- and eosin-stained lung sections were blindly examined under light microscopy to assess the inflammation score of each section. We determined the inflammation score, which is the product of the severity and prevalence of inflammation, in a manner similar to Hamada et al. (28). Severity was assigned a numerical value based on the thickness of the inflammatory cell infiltrates surrounding the airways and blood vessels in the
lungs (0 = no cells; 1 = 1–3 cells thick; 2 = 4–6 cells thick; 3 = 7–9 cells thick; 4 = greater than or
equal to 10 cells thick). Prevalence was assigned a numerical value according to the percentage
of airways and blood vessels in each section encompassed by inflammatory cells (0 = no airways
or blood vessels; 1 = < 25%; 2 = 25–50%; 3 = 51–75%; 4 = > 75%).

Using light microscopy, mucin-containing goblet cells, which are those cells that
responded positively to the PAS reaction, were enumerated on a blinded basis in lung sections
obtained from our animals. Specifically, the number of PAS-positive goblet cells along an
inflated, non-oblique 100 µm section of the respiratory mucosa from three different secondary or
tertiary airways was counted. The three different enumerations of PAS-positive goblet cells
were averaged to create a mean value for each mouse.

Statistical Analysis of Data. The effect of genotype and aerosol challenge on indices of airway
and lung parenchymal oscillation mechanics, BALF and histological indices of inflammation, the
number of mucin-containing goblet cells, and serum immunoglobulins were assessed by a two-
way analysis of variance (ANOVA) for normally distributed data or by a Kruskal-Wallis one-
way ANOVA for non-normally distributed data. In all of these analyses, genotype (wild-type
and Cpefat) and aerosol challenge (PBS and OVA) were the main effects. Depending on whether
the data were normally or non-normally distributed, the Fisher’s least significant difference test
or the Wilcoxon signed-rank test, respectively, were used as a follow-up to determine the
significance of differences between the various experimental groups. Body masses and serum
levels of adiponectin, chemerin, eotaxin, IL-18, and leptin were analyzed using an unpaired
Student’s *t*-test or the Wilcoxon signed-rank test as appropriate. Stata® 12 (StataCorp LP,
College Station, TX) was used for all statistical analyses. The results are expressed as the
mean±standard error of the mean. A $p$ value equal to or less than 0.05 was considered significant.
RESULTS

Effect of carboxypeptidase E deficiency on body mass. On the final day of the experimental protocol (day 77) when the mice were 15–20 weeks of age, the body mass of each animal in this study was measured prior to initiating the experimental procedures described in the Materials and Methods. Consistent with our previous observations (37, 38, 69), Cpe<sup>fat</sup> mice weighed significantly more than wild-type, C57BL/6 mice (46.0±0.7 and 21.5±0.2 g, respectively).

Effect of obesity and OVA sensitization and challenge on airway and lung parenchymal oscillation mechanics. Figure 1 illustrates the baseline values of airway (Raw) and lung parenchymal (G and H) oscillation mechanics in OVA-sensitized, wild-type and Cpe<sup>fat</sup> mice twenty-four hours following the cessation of the final PBS or OVA aerosol challenge. Raw, G, and H were greater in PBS-challenged, Cpe<sup>fat</sup> as compared to PBS-challenged, wild-type mice. However, statistical significance was only achieved for Raw (p = 0.002). OVA challenge significantly increased Raw, G, and H in wild-type and Cpe<sup>fat</sup> mice, and there were genotype-related differences in these indices following OVA challenge. Specifically, Raw, G, and H were significantly greater in Cpe<sup>fat</sup> as compared to wild-type mice following OVA challenge. Finally, because indices of airway and lung parenchymal oscillation mechanics were extremely elevated in Cpe<sup>fat</sup> mice following OVA challenge, airway responsiveness to methacholine was not assessed in any of our mice since any further increases in these indices induced by methacholine could not be accurately measured.
Effect of obesity and OVA sensitization and challenge on BALF cell differentials. For each of the cell types enumerated in the BALF twenty-four hours following the cessation of the final PBS or OVA aerosol challenge, a Kruskal-Wallis one-way ANOVA by ranks revealed a significant difference between at least two of the four experimental groups \((p = 0.0001\) in each instance; Figure 2). The number of total cells retrieved from the BALF of PBS-challenged, \(Cpe^{fat}\) mice was greater than those of PBS-challenged, wild-type mice. However, this difference was not statistically significant \((p = 0.07);\) Figure 2A). The greater number of total cells in the BALF of PBS-challenged, \(Cpe^{fat}\) mice was driven by a significant increase in the number of BALF macrophages \((p = 0.04);\) Figure 2B). There were no genotype-related differences in the number of BALF eosinophils, lymphocytes, or neutrophils in PBS-challenged mice (Figure 2C, Figure 2D, and Figure 2E). In fact, these cell types were rarely observed in the BALF of PBS-challenged, wild-type and \(Cpe^{fat}\) mice. OVA challenge caused a very robust and significant increase in the number of total cells, macrophages, eosinophils, lymphocytes, and neutrophils in the BALF of wild-type and \(Cpe^{fat}\) mice (Figure 2). However, the number of BALF total cells, macrophages, eosinophils, and neutrophils were significantly greater in OVA-challenged, \(Cpe^{fat}\) mice as compared to OVA-challenged, wild-type mice.

Effect of obesity and OVA sensitization and challenge on lung histopathology. Because the number of inflammatory cells in the air spaces of \(Cpe^{fat}\) mice was significantly greater than those of wild-type mice following OVA sensitization and challenge (Figure 2), we semi-quantitatively determined the degree of peribronchiolar and perivascular inflammation in hematoxylin- and eosin-stained sections that were prepared from formalin-fixed lungs. The lungs were obtained from OVA-sensitized, wild-type and \(Cpe^{fat}\) mice twenty-four hours following the cessation of the
final PBS or OVA aerosol challenge. No significant inflammatory lesions were observed in PBS-challenged, wild-type and Cpe<sup>fat</sup> mice (Figure 3A and Figure 3B). OVA-challenged, wild-type and Cpe<sup>fat</sup> mice exhibited significant peribronchiolar and perivascular inflammation, which was primarily characterized by mononuclear inflammatory cells (macrophages and lymphocytes) with moderate to heavy infiltrates of eosinophils (Figure 3C and Figure 3D). The inflammation was primarily directed toward blood vessels with collateral involvement of the airways (Figure 3E and Figure 3F). We observed no genotype-related differences in peribronchiolar and perivascular inflammation scores following OVA challenge (Figure 3E and Figure 3F).

There were no PAS-positive, mucin-containing goblet cells present in the lung sections of OVA-sensitized and PBS-challenged, wild-type and Cpe<sup>fat</sup> mice (Figure 4A and Figure 4B). Although OVA challenge significantly increased the number of PAS-positive, mucin-containing goblet cells in the lungs of wild-type and Cpe<sup>fat</sup> mice, no genotype-related differences existed following OVA challenge (Figure 4C, Figure 4D, and Figure 4E).

**Effect of obesity and OVA sensitization and challenge on BALF cytokines.** Twenty-four hours following the cessation of the final PBS aerosol challenge, there were no differences in the levels of BALF IL-4, IL-13, IL-18, eotaxin, and chemerin between wild-type and Cpe<sup>fat</sup> mice (Figure 5). OVA challenge significantly increased the levels of BALF IL-4, IL-13, IL-18, and chemerin in both wild-type and Cpe<sup>fat</sup> mice (Figure 5). However, the levels of these cytokines were significantly greater in Cpe<sup>fat</sup> as compared to wild-type mice following OVA challenge. Eotaxin was significantly increased by OVA challenge in Cpe<sup>fat</sup> but not wild-type mice (Figure 5D). In addition, eotaxin levels were significantly higher in OVA-challenged, Cpe<sup>fat</sup> as compared to OVA-challenged, wild-type mice. Finally, we analyzed the BALF for IL-5.
However, in both wild-type and $Cpe^{fat}$ mice, IL-5 was below the detection limit of our assay regardless of the aerosol challenge (data not shown).

**Effect of obesity and OVA sensitization and challenge on total IgE and OVA-specific immunoglobulins in the serum.** There were no genotype-related differences in the serum IgE levels of OVA-sensitized, wild-type and $Cpe^{fat}$ mice challenged with PBS (Figure 6A). OVA challenge significantly increased IgE levels in the serum of both wild-type and $Cpe^{fat}$ mice. However, no genotype-related differences in IgE existed following OVA challenge. Similar results were obtained for OVA-specific IgE and IgG1 (Figure 6B and Figure 6C).

**Effect of obesity on indices of chronic systemic inflammation.** A number of obesity-related sequelae, including asthma, cardiovascular disease, and type 2 diabetes, have been mechanistically linked to obesity-induced, chronic systemic inflammation, which is characterized by elevated blood levels of cytokines, chemokines, hormones, and soluble cytokine receptors (7, 9, 10, 13, 37-39, 59, 73, 79, 84). The majority of these moieties, collectively termed adipokines, are pro-inflammatory in nature. Thus, to confirm previous observations of the existence of chronic systemic inflammation in $Cpe^{fat}$ mice (37, 38, 79, 84), and perhaps provide a mechanistic basis for our current observations in $Cpe^{fat}$ mice, we measured a number of indices of chronic systemic inflammation in the serum of OVA-sensitized and PBS-challenged, wild-type and $Cpe^{fat}$ mice (Figure 7). Twenty-four hours following the cessation of the final PBS aerosol challenge, the serum levels of chemerin, eotaxin, IL-18, and leptin were significantly greater in $Cpe^{fat}$ as compared to wild-type mice. There were no genotype-related differences in serum adiponectin levels following PBS challenge (Figure 7E).
DISCUSSION

In comparison to wild-type mice sensitized to and challenged with OVA, our data demonstrate that OVA-sensitized and challenged, \textit{Cpe}^{fat} mice exhibit greater airway obstruction and increased pulmonary inflammation. The greater airway obstruction observed in \textit{Cpe}^{fat} mice following OVA sensitization and challenge (Figure 1) is consistent with our previous observations in obese \textit{db/db} and obese \textit{ob/ob} mice (39). However, in contrast to \textit{db/db} and \textit{ob/ob} mice (39), \textit{Cpe}^{fat} mice exhibit increased pulmonary inflammation in response to OVA sensitization and challenge (Figures 2 and 5). Taken together, these results demonstrate that enhanced airway obstruction is consistently observed in genetically obese mice following OVA sensitization and challenge, whereas the development of pulmonary inflammation in obese mice in response to OVA sensitization and challenge is dependent upon the genetic modality of obesity induction.

\[ \text{Raw}, \] which is a measure of airflow obstruction within the conducting airways (74), was significantly greater in OVA-sensitized, \textit{Cpe}^{fat} as compared to OVA-sensitized, wild-type mice following PBS challenge (Figure 1A). A linear relationship exists between airway conductance, the inverse of \text{Raw}, and lung volume (83), which is reduced in obesity due to a decrease in lung and chest wall compliance from the mass loading effects of obesity on the chest wall and abdomen (61). However, it is doubtful that a reduction in lung volume in \textit{Cpe}^{fat} mice as a consequence of decreased chest wall compliance accounts for the observed increase in \text{Raw} since all of our mice were studied with an open chest and a fixed positive end-expiratory pressure. Following PBS challenge, we also observed no genotype-related differences in \text{H} (Figure 1C), the inverse of compliance. Accordingly, differences in lung compliance between \textit{Cpe}^{fat} and
wild-type mice can also be excluded as a potential mechanism by which $R_{aw}$ is elevated in OVA-sensitized and PBS-challenged, $Cpe^{fat}$ mice. However, our data and those of other investigators suggest that chronic systemic inflammation, which is exhibited by OVA-sensitized and PBS-challenged, $Cpe^{fat}$ mice [Figure 7 and (37, 38, 79, 84)] contributes to the increase in $R_{aw}$ that we observed in these animals (Figure 1A). For example, IL-17A and TNF-$\alpha$, which are two pro-inflammatory adipokines elevated in the serum of $Cpe^{fat}$ mice (79, 84), enhance airway smooth muscle contractility in response to bronchoconstrictors (1, 43). Thus, the increase in $R_{aw}$ that we observed in OVA-sensitized and PBS-challenged, $Cpe^{fat}$ mice may be a result of these adipokines enhancing the effects of endogenous acetylcholine on basal airway smooth muscle contraction. Our observation that pro-inflammatory adipokines are elevated in the serum of OVA-sensitized and PBS-challenged $Cpe^{fat}$ mice supports this hypothesis (Figure 7).

OVA sensitization and challenge caused significant airway obstruction in wild-type and $Cpe^{fat}$ mice (Figure 1A). Furthermore, airway obstruction induced by OVA sensitization and challenge was exacerbated in $Cpe^{fat}$ mice. OVA sensitization and challenge can enhance methacholine-induced airway obstruction through a number of diverse inflammatory moieties, including IL-4, IL-13, IL-18, major basic protein, and neutrophil elastase (23, 29, 41, 47, 81). Because the aforementioned cytokines, eosinophils (the source of major basic protein), and neutrophils (the source of neutrophil elastase), are increased in the lungs of OVA-sensitized and challenged wild-type and $Cpe^{fat}$ mice (Figures 2 and 5), it is probable that the increased airway obstruction observed in OVA-sensitized and challenged mice of both genotypes is mechanistically linked to the pulmonary inflammation seen in these animals. For example, the aforementioned inflammatory moieties may be acting directly on the airway smooth muscle or affecting neural innervation to potentiate the contractile effects of endogenous acetylcholine on
the airway smooth muscle. Because inflammatory moieties are increased in the BALF of Cpe\textsuperscript{fat} as compared to wild-type mice following OVA sensitization and challenge, any effect of these moieties on airway obstruction is likely enhanced in Cpe\textsuperscript{fat} mice. Thus, increased pulmonary inflammation is a potential mechanism by which airway obstruction is exacerbated in OVA-challenged, Cpe\textsuperscript{fat} mice. Taken together with our prior observations in db/db and ob/ob mice (39), our results demonstrate that OVA sensitization and challenge enhances airway obstruction in obese mice regardless of the modality of obesity induction.

As Figure 1B and Figure 1C illustrate, OVA sensitization and challenge significantly increased G and H, which are indices of lung parenchymal resistance and elastance, respectively (31). Increases in G and H could result from enhanced closure of the small airways due to greater constriction of the conducting airways and/or surfactant dysfunction (77, 78). Similar to Raw, G and H were increased to a greater extent in Cpe\textsuperscript{fat} as compared to wild-type mice following OVA sensitization and challenge. Such increases in G and H in Cpe\textsuperscript{fat} mice could be a result of the greater airway constriction exhibited by these animals. Alternatively, OVA sensitization and challenge leads to surfactant dysfunction (82), which may be exacerbated in Cpe\textsuperscript{fat} mice due to greater levels of BALF IL-13, which inhibits surfactant function (85). Thus, obesity has deleterious effects on the oscillation mechanics of both the conducting airways and lung parenchyma following OVA sensitization and challenge.

Because airway obstruction is exacerbated in genetically obese mice following OVA sensitization and challenge, this suggests that a common mechanism may exist among Cpe\textsuperscript{fat}, db/db, and ob/ob mice to account for this phenomenon. However, as discussed in detail below, potential shared mechanisms such as increased pulmonary inflammation, decreased serum adiponectin levels, and increased serum IgE levels are unlikely. If a common mechanism does
exist, our data and those from others suggest that it may be chronic systemic inflammation. First, increased pulmonary inflammation can be excluded as a common mechanism, as indices of pulmonary inflammation such as IL-4, IL-13, eosinophils, and neutrophils, were either not different or were reduced in \(db/db\) and/or \(ob/ob\) as compared to wild-type mice following OVA sensitization and challenge (39). Secondly, although we have previously reported that leptin can enhance methacholine-induced airway obstruction in OVA-challenged, wild-type mice (71), we can also exclude leptin as a common mechanism by which airway obstruction is enhanced in obese mice following OVA sensitization and challenge since leptin signaling is impaired in \(db/db\) and \(ob/ob\) mice (48). Thirdly, in comparison to age- and gender-matched, wild-type mice, \(db/db\) and \(ob/ob\) mice have significantly lower circulating levels of adiponectin, an anti-inflammatory adipokine (18, 52). Shore et al. (72) has previously demonstrated that elevating serum levels of adiponectin by administering exogenous adiponectin reduces OVA-induced AHR and inflammation. However, it is very doubtful that that the enhanced airway obstruction in obese mice following OVA sensitization and challenge is due to a decrease in serum adiponectin since there were no differences in serum adiponectin between wild-type and \(Cpe^{fat}\) mice (Figure 7E). Fourthly, the role of IgE in the development of OVA-induced airway obstruction and pulmonary inflammation in mice is controversial (17, 29, 45, 55). However, there are studies which demonstrate that IgE is important to the development of OVA-induced AHR (17, 45). We previously reported that the serum levels of IgE are elevated in OVA sensitized and challenged \(ob/ob\) mice (39). However, it is doubtful that IgE is the common mechanism that enhances airway obstruction in obese mice following OVA sensitization and challenge since we observed no differences in total or OVA-specific IgE between \(Cpe^{fat}\) and wild-type mice following OVA challenge (Figure 6). Finally, if a common mechanism does
account for the enhanced airway obstruction observed in genetically obese mice following OVA sensitization and challenge, then chronic systemic inflammation is a plausible consideration. *Cpe*fat, *db/db*, and *ob/ob* mice develop chronic systemic inflammation [Figure 7 and (37-39, 52, 79, 84)], and Dixon and colleagues (73) have previously reported that obesity-induced asthma is more strongly associated with systemic and adipose tissue inflammation as opposed to pulmonary inflammation. Dixon *et al.* (20) demonstrated that obese asthmatics who lose weight *via* bariatric surgery become less reactive to methacholine despite the fact that pulmonary inflammation actually increases. Taken together, these data demonstrate that increased pulmonary inflammation does not account for the increased airway responsiveness to methacholine observed in obese, human asthmatic subjects. Consequently, despite the fact that *Cpe*fat mice exhibit increased pulmonary inflammation, the primary mechanism for enhanced airway obstruction in these animals may be systemic in nature.

The marked differences in the development of pulmonary inflammation among *Cpe*fat, *db/db*, and *ob/ob* mice in response to OVA sensitization and challenge is intriguing given that we previously reported that pulmonary inflammation is enhanced in *Cpe*fat, *db/db*, and *ob/ob* mice following exposure to ozone, a common environmental pollutant (37, 38, 52, 70). The mechanism underlying the divergent inflammatory responses to OVA sensitization and challenge among these obese mice is not known. However, we have considered two potential possibilities for this observation. First, in contrast to *db/db* and *ob/ob* mice, leptin signaling is intact in *Cpe*fat mice (48). Furthermore, leptin is elevated in the serum of *Cpe*fat mice, and leptin is a pro-inflammatory cytokine [Figure 7 and (37, 38, 70, 84)]. We previously reported that exogenous leptin administration had no effect on OVA-induced pulmonary inflammation in wild-type mice following OVA sensitization and challenge (71). Furthermore, mice with diet-induced obesity,
which also have elevated leptin (36), have reduced BALF eosinophils following OVA sensitization and challenge (12). Taken together, it is doubtful that leptin enhances OVA-induced pulmonary inflammation in Cpefat mice. Secondly, because of a missense mutation in the gene encoding carboxypeptidase E, Cpefat mice cannot process a number of pro-hormones and pro-neuropeptides into biologically active peptides (14, 48, 60). For example, pro-opiomelanocortin and pro-cholecystokinin are processed by carboxypeptidase E into their biologically active forms: α-melanocyte-stimulating hormone (α-MSH) and cholecystokinin. Both α-MSH and cholecystokinin have anti-inflammatory effects and are reduced in mice with impaired carboxypeptidase E activity (6, 11, 15, 21, 56, 65). Of particular interest to this study, α-MSH has been shown to decrease antigen-induced skin inflammation (6, 21). Furthermore, pro-opiomelanocortin can be processed by carboxypeptidase E into adrenocorticotropin (14), which is also reduced in Cpefat mice (67). Adrenocorticotropin stimulates the release of corticosteroids, which have been previously shown to inhibit OVA-induced pulmonary inflammation (22). Interestingly, corticosterone levels are elevated in db/db and ob/ob mice (48), which exhibit decreased OVA-induced pulmonary inflammation, whereas corticosterone levels are not elevated in Cpefat mice (49), which exhibit enhanced OVA-induced pulmonary inflammation. Taken together, the inability of Cpefat mice to produce sufficient quantities of endogenous, anti-inflammatory mediators due to improper processing of their precursors would be expected to exacerbate systemic and pulmonary inflammation.

In humans, obesity appears to be a stronger risk factor for non-atopic as compared to atopic asthma (16), yet the development of obesity appears to worsen pre-existing atopic asthma (20). Given that the degree of pulmonary inflammation among obese individuals with atopic asthma varies substantially even though all exhibit airway obstruction (19, 20, 42, 73, 76)
suggests that different obese mice may be necessary to model the different phenotypes observed among the various human subpopulations of atopic, obese asthmatics. To that end, we propose that \( db/db \) and \( ob/ob \) mice are better suited to study mechanisms underlying airway obstruction in atopic, obese asthmatics with minimal, eosinophilic pulmonary inflammation whereas \( Cpe^{fat} \) mice are more suited to study the mechanisms of airway obstruction in atopic, obese asthmatics with substantial pulmonary inflammation, such as those described in the study by Desai et al. (19).

**Perspectives and Significance**

In conclusion, our results demonstrate that OVA sensitization and challenge enhances airway obstruction in obese mice regardless of the genetic basis of obesity whereas the development of OVA-induced pulmonary inflammation is dependent upon the genetic modality of obesity induction. Given that pulmonary inflammation in response to OVA sensitization and challenge develops according to the genetic modality of obesity induction, future studies warrant an investigation into the molecular basis of this phenomenon. However, the results of this study have important implications for animal models of asthma, as modeling the pulmonary phenotypes for subpopulations of atopic, obese asthmatics critically depends on selecting the appropriate mouse model. Consequently, obese mice will be useful tools to test the pre-clinical efficacy of medications aimed at preventing or alleviating airway obstruction in various subpopulations of atopic, obese asthmatics with variable degrees of pulmonary inflammation.
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**FIGURE CAPTIONS**

**Figure 1.** Measurements of (A) airway resistance ($R_{aw}$), (B) the coefficient of lung tissue damping ($G$), and (C) the coefficient of lung tissue elastance ($H$) obtained from ovalbumin (OVA)-sensitized, wild-type (C57BL/6) and carboxypeptidase E-deficient (Cpe$^{fat}$) mice challenged with aerosols of either phosphate-buffered saline (PBS) or PBS containing 4% OVA (weight/volume) once per day for seven consecutive days. Measurements were made twenty-four hours following the cessation of the final aerosol challenge. Each value is expressed as the mean±standard error of the mean. $n = 7–9$ mice for each group. *$p < 0.05$ compared to genotype-matched mice challenged with PBS. **$p < 0.05$ compared to wild-type (C57BL/6) mice with an identical series of aerosol challenges.

**Figure 2.** The number of (A) total cells, (B) macrophages, (C) eosinophils, (D) lymphocytes, and (E) neutrophils in the bronchoalveolar lavage fluid (BALF) obtained from ovalbumin (OVA)-sensitized, wild-type (C57BL/6) and carboxypeptidase E-deficient (Cpe$^{fat}$) mice challenged with aerosols of either phosphate-buffered saline (PBS) or PBS containing 4% OVA (weight/volume) once per day for seven consecutive days. BALF was obtained from the mice twenty-four hours following the cessation of the final aerosol challenge. Each value is expressed as the mean±standard error of the mean. $n = 9–13$ mice for each group. *$p < 0.05$ compared to genotype-matched mice challenged with PBS. **$p < 0.05$ compared to wild-type (C57BL/6) mice with an identical series of aerosol challenges.
Figure 3. (A–D) Representative light micrographs of hematoxylin- and eosin-stained histologic sections as well as (E) peribronchiolar and (F) perivascular inflammation scores from the lungs of ovalbumin (OVA)-sensitized, wild-type (C57BL/6) and carboxypeptidase E-deficient (Cpefat) mice challenged with aerosols of either phosphate-buffered saline (PBS) or PBS containing 4% OVA (weight/volume) once per day for seven consecutive days. A and B are lung sections from PBS challenged, wild-type and Cpefat mice, respectively. C and D are lung sections from OVA challenged, wild-type and Cpefat mice, respectively. The lungs were fixed in situ with 10% phosphate-buffered formalin twenty-four hours following the cessation of the final aerosol challenge. In A–D, the images have been magnified with a 20× objective lens while each of the scale bars in A–D represent 100 µm. In E and F, each value is expressed as the mean±standard error of the mean. n = 8–9 mice for each group. *p < 0.05 compared to genotype-matched mice challenged with PBS.

Figure 4. (A–D) Representative light micrographs of histologic sections demonstrating the prevalence of the periodic-acid Schiff (PAS) reaction as well as (E) the number of mucin-containing goblet cells in the lungs of ovalbumin (OVA)-sensitized, wild-type (C57BL/6) and carboxypeptidase E-deficient (Cpefat) mice challenged with aerosols of either phosphate-buffered saline (PBS) or PBS containing 4% OVA (weight/volume) once per day for seven consecutive days. A and B are lung sections from PBS challenged, wild-type and Cpefat mice, respectively. C and D are lung sections from OVA challenged, wild-type and Cpefat mice, respectively. The arrows in C and D are directed at PAS-positive, mucin-containing goblet cells, which were found in the respiratory mucosa of secondary or tertiary bronchi. The lungs were fixed in situ with 10% phosphate-buffered formalin twenty-four hours following the cessation of the final aerosol
challenge. In A–D, the images have been magnified with a 40× objective lens while each of the scale bars in A–D represent 50 µm. In E, each value is expressed as the mean±standard error of the mean. \( n = 8–9 \) mice for each group. \( ^* p < 0.05 \) compared to genotype-matched mice challenged with PBS.

**Figure 5.** The concentration of (A) interleukin (IL)-4, (B) IL-13, (C) IL-18, (D) eotaxin, and (E) chemerin in the bronchoalveolar lavage fluid (BALF) obtained from ovalbumin (OVA)-sensitized, wild-type (C57BL/6) and carboxypeptidase E-deficient (Cpe\(^{fat}\)) mice challenged with aerosols of either phosphate-buffered saline (PBS) or PBS containing 4% OVA (weight/volume) once per day for seven consecutive days. BALF was obtained from the mice twenty-four hours following the cessation of the final aerosol challenge. Each value is expressed as the mean±standard error of the mean. \( n = 4–11 \) mice for each group. \( ^* p < 0.05 \) compared to genotype-matched mice challenged with PBS. \( ^\# p < 0.05 \) compared to wild-type (C57BL/6) mice with an identical series of aerosol challenges.

**Figure 6.** The concentration of (A) immunoglobulin (Ig) E, (B) ovalbumin (OVA)-specific IgE, and (C) OVA-specific IgG\(_1\) in serum isolated from the blood of OVA-sensitized, wild-type (C57BL/6) and carboxypeptidase E-deficient (Cpe\(^{fat}\)) mice challenged with aerosols of either phosphate-buffered saline (PBS) or PBS containing 4% OVA (weight/volume) once per day for seven consecutive days. Blood was obtained from the mice and serum isolated from the blood twenty-four hours following the cessation of the final aerosol challenge. Each value is expressed as the mean±standard error of the mean. \( n = 8–13 \) mice for each group. \( ^* p < 0.05 \) compared to genotype-matched mice challenged with PBS.
Figure 7. The concentration of (A) chemerin, (B) eotaxin, (C) interleukin (IL)-18, (D) leptin, and (E) adiponectin in the serum of OVA-sensitized, wild-type (C57BL/6) and carboxypeptidase E-deficient (CpeΔat) mice challenged with an aerosol of phosphate-buffered saline (PBS) once per day for seven consecutive days. Serum was isolated from the blood that was obtained from the mice twenty-four hours following the cessation of the final PBS challenge. Each value is expressed as the mean±standard error of the mean. $n=5–9$ mice for each group. $^\# p<0.05$ compared to wild-type (C57BL/6) mice.
**Figure 1**

(A) Graph showing the relationship between $R_{aw}$ (cm H$_2$O/ml/s) and treatment with PBS or OVA. The bars for OVA are higher than those for PBS, indicating a significant difference. 

(B) Graph showing the relationship between $G$ (cm H$_2$O/ml) and treatment with PBS or OVA. The bars for OVA are significantly higher than those for PBS. 

(C) Graph showing the relationship between $H$ (cm H$_2$O/ml) and treatment with PBS or OVA. The bars for OVA are significantly higher than those for PBS.

Legend:
- **Wild-type**
- $Cpe^fat$
Figure 2

(A) Total Cells (×10^4): PBS vs OVA

(B) Macrophages (×10^4): PBS vs OVA

(C) Eosinophils (×10^4): PBS vs OVA

(D) Lymphocytes (×10^4): PBS vs OVA

(E) Neutrophils (×10^4): PBS vs OVA

- PBS
- OVA

Wild-type
\(Cpe^{fat}\)
Figure 3

Wild-type

Cpe\textsuperscript{fat}

PBS

OVA

Inflammation Score

Peribronchiolar

Perivascular

Inflammation Score

PBS OVA PBS OVA
Figure 4

Wild-type

Cpe\textsuperscript{fat}

A

B

C

D

PBS

OVA

E

Number of Mucin-Containing Goblet Cells per 100 μm of Basement Membrane

PBS

OVA

Wild-type

Cpe\textsuperscript{fat}

*
Figure 5

- **Panel A**: IL-4 (pg/ml) levels in PBS and OVA groups.
- **Panel B**: IL-13 (pg/ml) levels in PBS and OVA groups.
- **Panel C**: IL-18 (pg/ml) levels in PBS and OVA groups.
- **Panel D**: Eotaxin (pg/ml) levels in PBS and OVA groups.
- **Panel E**: Chemerin (pg/ml) levels in PBS and OVA groups.

**Legend**
- **Wild-type** (filled bars)
- **Cpe^fat** (open bars)

**Significance**
- *: p < 0.05 (compared to PBS)
- #: p < 0.05 (compared to OVA)

**Groups**
- PBS
- OVA
Figure 6

(A) IgE levels (ng/ml) in PBS and OVA-treated groups.

(B) OVA-Specific IgE levels (ng/ml) in PBS and OVA-treated groups.

(C) OVA-Specific IgG1 levels (mg/ml) in PBS and OVA-treated groups for Wild-type and Cpe<sup>fat</sup> mice.
Figure 7

A. Chemerin (ng/ml)
B. Eotaxin (pg/ml)
C. IL-18 (pg/ml)
D. Leptin (ng/ml)
E. Adiponectin (µg/ml)

Wild-type vs. Cpe\textsuperscript{fat}

# indicates significant difference.