Less Airway Inflammation and Goblet Cell Metaplasia in an IL-33-Induced Asthma Model of Leptin-Deficient Obese Mice

Atsushi Kurokawa
Tokyo Women's Medical University: Tokyo Joshi Ika Daigaku
Mitsuko Kondo (kondo.mitsuko@twmu.ac.jp)
Tokyo Women's Medical University: Tokyo Joshi Ika Daigaku https://orcid.org/0000-0003-3673-5431
Ken Arimura
Tokyo Women's Medical University: Tokyo Joshi Ika Daigaku
Shigeru Ashino
Tokyo Women's Medical University: Tokyo Joshi Ika Daigaku
Etsuko Tagaya
Tokyo Women's Medical University: Tokyo Joshi Ika Daigaku

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Less airway inflammation and goblet cell metaplasia in an IL-33-induced asthma model of leptin-deficient obese mice

Atsushi Kurokawa[1)], Mitsuko Kondo[1)], Ken Arimura[1)], Shigeru Ashino[2)], Etsuko Tagaya[1)]

1) Department of Respiratory Medicine, Tokyo Women's Medical University, Tokyo 162-8666, Japan.
2) Department of Microbiology and Immunology, Tokyo Women's Medical University, Tokyo 162-8666, Japan.

Corresponding author
Mitsuko Kondo, M.D.
Department of Respiratory Medicine, Tokyo Women's Medical University, Tokyo 162-8666, Japan.
E-mail: kondo.mitsuko@twmu.ac.jp
Tel: 81-3-3353-8111
Fax: 81-3-5269-7616

Abstract

Background: Asthma with obesity is a phenotype of severe asthma. Leptin exerts an immunomodulatory effect and its level is increased in obesity. IL-33 is associated with innate immunity and induces type 2 inflammation, and is present in adipose tissue. However, the role of IL-33 and leptin in obesity-associated asthma is not fully understood. We examined the effect of IL-33 on eosinophilic inflammation, goblet cell metaplasia, and airway responsiveness in leptin-deficient obese (ob/ob) and wild-type mice, and examined the effect of exogenous leptin pretreatment.

Methods: In ob/ob and wild-type mice, IL-33 was instilled intranasally on three consecutive days. In part of the animals, leptin was injected intraperitoneally prior to IL-33 treatment. The mice were challenged with methacholine and resistance of the respiratory system (Rrs) was measured using the forced oscillation technique. Cell differentiation, IL-5, IL-13, eotaxin, KC in bronchoalveolar lavage fluid (BALF), and histology of the lung were analyzed. For *the in vitro* study, NCI-H292 cells were stimulated with IL-33 in the presence or absence of leptin, and MUC5AC levels were measured by ELISA.

Results: Ob/ob mice showed greater baseline Rrs than wild-type mice. IL-33 and IL-33 with leptin did not enhance Rrs challenged with methacholine compared to non-treatment in ob/ob mice, whereas IL-33 with leptin enhanced Rrs in wild-type mice. Ob/ob mice showed less IL-33-induced eosinophil numbers, IL-5, IL-13, eotaxin, and KC levels in BALF and eosinophilic infiltration around bronchi and goblet cell metaplasia than wild-type mice, but leptin pretreatment attenuated these changes in ob/ob mice. MUC5AC levels were increased by co-stimulation with IL-33 and leptin *in vitro*.

Conclusions: Leptin plays an important role in IL-33-induced inflammation and goblet cell metaplasia in the airway, but obesity *per se* increases airway hyperresponsiveness independent of inflammation. These results explain some aspects of the pathogenesis of obesity-related asthma.

Keywords: asthma, obesity, eosinophils, goblet cell metaplasia, innate immunity

Background

The incidence of obesity is on the rise worldwide and is currently an important public health problem. Obesity is a risk factor for the development of asthma and is associated with poor control and frequent exacerbation [1]. Halder et al. previously reported that obesity-related asthma shows the characteristic phenotype with female-dominant, late-onset, non-eosinophilic, and highly symptomatic [2]. However, to date, there is no specific treatment except for weight reduction in obesity-related asthma [3].

Leptin is a hormone secreted by adipocytes and acts on the hypothalamus to inhibit hunger and stimulate satiety. In obesity, serum leptin levels are generally elevated because leptin resistance occurs and a feeling of hunger continues despite high energy stores [4]. However, the developmental mechanism of leptin resistance remains unclear [5]. As leptin is known to exert an immunomodulatory effect, it may be involved in the pathogenesis of obesity-related asthma. In obese women with asthma, airway reactivity is significantly related to leptin expression in visceral fat [6]. Moreover, leptin and its receptor are expressed in the airway epithelium [7].

Some studies have investigated the role of leptin in allergic animal models; leptin enhanced airway responsiveness in ovalbumin (OVA)-sensitized mice [8]. Spontaneously generated leptin-deficient obese (Ob/ob) mice, show innate airway hyperresponsiveness (AHR). Moreover, OVA-sensitized ob/ob mice show enhanced AHR without an increase in type-2 inflammation [9]. However, these reports were focused on adaptive immunity in the asthmatic response. On the other hand, the influence of leptin on innate immunity has not yet been fully investigated.

IL-33 is associated with innate immunity and induces type-2 inflammation in the airway. IL-33 is released from injured airway epithelial cells and stimulates type-2 innate lymphocytes (ILC2), which release IL-5 and IL-13. These cytokines lead to eosinophilic inflammation, goblet cell metaplasia, and hyperresponsiveness in the airway [10]. IL-33 and its receptor ST2 have also been shown to be present in human adipose tissue [11]. However, the effect of obesity on IL-33-induced asthma, especially the interaction between leptin and IL-33, is not fully understood. Herein we examined the effect of IL-33 on eosinophilic inflammation, goblet cell metaplasia, and airway responsiveness in ob/ob mice and leptin-treated wild-type C57BL/6J mice.

Methods

Animal models

This animal protocol was approved by the Animal Care and Use Committee of Tokyo Women's Medical University (license numbers: AE20-065-B). Ob/ob mice (genetically leptin-deficient obese mice, female, 7-9 weeks old) and C57BL/6J wild-type mice (age and sex-matched with ob/ob mice) were divided into three groups (non-treated, IL-33-treated, and Leptin + IL-33-treated). Recombinant mouse IL-33 (SRP3210, Sigma-Aldrich, St Louis, MO, USA: 1µg dissolved in 50 µL phosphate-buffered saline [PBS]) was instilled intranasally on days 9-11, and recombinant murine leptin (450-31, PeproTech, Cedarbrook Drive Cranbury, NJ, USA: 25 µg/125 µL PBS for wild-type, 50 µg/250 µL PBS for ob/ob) was injected intraperitoneally on days 1, 3, 5, and 8-11, as the experimental protocol shown in Fig. 1a.

Measurement of airway responsiveness

Mice were anesthetized, ventilated (flexiVent; SCIREQ, Montreal, Canada), challenged with increasing doses of inhaled methacholine (3.125, 6.25, 12.5, 25, and 50 mg/mL, purchased from Sigma-Aldrich, St Louis, MO, USA), and the resistance of the respiratory system (Rrs) was measured by the forced oscillation technique as previously described [12]. Airway responsiveness was assessed by a fold change from baseline Rrs.

Bronchoalveolar lavage fluid (BALF) analysis

After measuring airway responsiveness, the BALF was collected by lavaging the lungs with 1.8 mL PBS. The BALF was centrifuged at 500 × *g* for 3 min, and the supernatant was collected for subsequent analysis. Total cell number was counted using a hemocytometer, and the cell differentials were counted by staining with May-Giemsa. Cytokine or chemokine levels (IL-5, IL-13, eotaxin, KC) were analyzed using a mouse ELISA kit (R&D system, Minneapolis, MN, USA).

Lung histology

The lungs were fixed with 10% formalin and embedded in paraffin. Sections were cut 5 μm thick and stained with periodic acid-Schiff/Alcian-blue. To assess goblet cell metaplasia in the bronchi, mucus scores were obtained as previously described [13]. In brief, bronchi with internal diameter measuring > 200 μm in cross section is assessed. Scores were obtained based on the ratio of goblet cell area to whole cross-sectional epithelial area in each round bronchus: a score of 0 indicates none, a score of 1 indicates occupation of < 1/3 of the epithelial area, a score of 2 indicates occupation of ≥ 1/3 to < 2/3 of the epithelial area; and a score of 3 indicates occupation of ≥ 2/3 of the epithelial area. The mucus score was obtained by averaging the scores of the measured bronchi.

In vitro study using NCI-H292 cells

For *the in vitro* study of mucin synthesis, the human pulmonary mucoepidermoid carcinoma cell line NCI-H292 cells were cultured in RPMI 1640 medium (GIBCO; Invitrogen Co. Grand Island, NY, USA) with 10% fetal calf serum, penicillin (100 U/mL), streptomycin (100 µg/mL), and fungizone (2.5 µg/mL) at 37 °C in a humidified 5% CO2 incubator. Cells were stimulated with IL-33 (0.5 ng/mL) in the presence or absence of leptin (1 ng/mL). 24 h after stimulation, mucin-5AC (MUC5AC) protein levels in cell lysates were measured with an ELISA kit (Cloud-Clone Corp, TX, USA) as previously described [14]. The data are shown as percentages in non-stimulated control cells.

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using the Prism 8 software package (GraphPad Software, San Diego, CA, USA). Airway responsiveness was evaluated using two-way repeated ANOVA with Turkey's post hoc test. All other data were evaluated using one-way ANOVA with Turkey's post hoc test. A p-value of less than 0.05 was considered statistically significant. In preliminary experiments, as we confirmed that leptin *i.p.* alone did not affect airway hyperresponsiveness, BALF, or histology, we did not include the data analysis.

149 Results

150 **Leptin-deficiency was associated with less IL-33-induced eosinophilia and goblet**
151 **cell metaplasia in ob/ob mice**

152 Body weight did not significantly change during the experiment in all mice, and ob/ob
153 mice were significantly heavier than wild-type mice (ob/ob 38.73 ± 2.55 g, wild-type
154 18.67 ± 1.02 g; $p < 0.001$, on day 12) (Fig. 1b). Ob/ob mice showed significantly
155 greater baseline Rrs than wild-type mice (1.264 ± 0.107 vs. 0.721 ± 0.023
156 $cmH_2O.s/mL$; $p < 0.01$) (Fig. 2a) and showed greater response to methacholine than
157 wild-type mice (Fig. 2b). However, in ob/ob mice, IL-33 with or without leptin induced
158 no significant change in airway responsiveness (Fig. 2d). The total cell counts in BALF
159 were lower in IL-33-treated ob/ob mice than in IL-33-treated wild-type mice (1.63 ±
160 0.33 vs. 4.36 ± 1.49 × 10^4 per mL) (Fig. 3a). In wild-type mice, IL-33 induced marked
161 eosinophilia in BALF (non-treated vs. IL-33-treated: 0.0 vs. 34.4 ± 10.8%; $p < 0.05$).
162 However, in ob/ob mice, IL-33 did not induce significant eosinophilia (non-treated vs.
163 IL-33-treated: 0.0 vs. 2.1 ± 1.4%). In ob/ob mice, leptin treatment prior to IL-33
164 instillation induced a significant increase in eosinophils (IL-33-treated vs. Leptin + IL-
165 33-treated: 2.1 ± 1.4 vs. 11.8 ± 4.1%; $p < 0.05$) (Fig. 3c), and also induced a significant
166 increase in neutrophils (0.8 ± 0.4 vs. 6.8 ± 2.8%; $p < 0.05$) (Fig. 3d). IL-5 and IL-13
167 levels in BALF were significantly lower in IL-33-treated ob/ob mice than in IL-33-
168 treated wild-type mice (IL-5: 4.5 ± 2.4 vs. 46.6 ± 8.6 pg/mL; $p < 0.001$, IL-13: 1.1 ±
169 0.9 vs. 13.9 ± 5.5 pg/mL; $p < 0.05$) (Fig. 4a, 4b). Eotaxin levels tended to be lower in
170 IL-33-treated ob/ob mice than in IL-33-treated wild-type mice (10.3 ± 0.5 vs. 61.5 ±
171 38.2 pg/mL) (Fig. 4c). KC level was significantly lower in IL-33-treated ob/ob mice

than in IL-33-treated wild-type mice (8.9 ± 2.3 vs. 44.1 ± 2.1 pg/mL; $p < 0.05$) (Fig. 4d). In ob/ob mice, leptin treatment increased IL-5, IL-13, eotaxin, and KC levels (IL-33-treated vs. Leptin + IL-33-treated; IL-5: 4.5 ± 2.4 vs. 6.9 ± 4.2 pg/mL, IL-13: 1.1 ± 0.9 vs. 4.6 ± 0.6 pg/mL, eotaxin: 10.3 ± 0.5 vs. 69.7 ± 57.7 pg/mL, KC: 8.9 ± 2.3 vs. 31.7 ± 11.1 pg/mL), but these changes were not significant (Fig. 4a–4d). In non-treated wild-type and ob/ob mice, neither airway inflammation nor goblet cell metaplasia were observed (Fig. 5a, 5d). In IL-33-treated wild-type mice, airway inflammation and goblet cell metaplasia were observed (Fig. 5b). On the other hand, in IL-33-treated ob/ob mice, the changes were attenuated (Fig. 5e). However, addition of exogenous leptin induced inflammation and goblet cell metaplasia (Fig. 5f). The mucus score was lower in IL-33-treated ob/ob mice than in IL-33-treated wild-type mice (0.33 ± 0.07 vs. 1.00 ± 0.12, $p < 0.01$), but the addition of exogenous leptin significantly increased the mucus score in ob/ob mice (IL-33-treated vs. Leptin + IL-33-treated; 0.33 ± 0.07 vs. 1.02 ± 0.29; $p < 0.05$) (Fig. 5g).

Co-stimulation with leptin and IL-33 induced AHR in wild-type mice

In wild-type mice, airway hyperresponsiveness was induced by leptin + IL-33, but not by IL-33 alone (Fig. 2c). In wild-type mice, leptin + IL-33 tended to increase eosinophils and neutrophils in BALF compared to IL-33 alone, but these changes were not significant (IL-33-treated vs. Leptin + IL-33-treated; eosinophil: 34.4 ± 10.8% vs. 43.9 ± 3.3%, neutrophil: 3.7 ± 1.4% vs. 14.4 ± 6.1%) (Fig. 3c, 3d). Leptin + IL-33 treatment significantly increased eotaxin and KC levels compared to IL-33 alone (IL-33-treated vs. Leptin + IL-33-treated; eotaxin: 61.5 ± 38.2 vs. 209.9 ± 13.9 pg/mL; $p <$

0.05; KC: 44.1 ± 2.1 vs. 85.9 ± 9.8 pg/mL; $p < 0.01$) (Fig. 4c, 4d). In Leptin + IL-33-treated wild-type mice, marked inflammation and goblet cell metaplasia were observed (Fig. 5c).

***In vitro* study using NCI-H292 cells**

MUC5AC levels did not change with leptin and IL-33 alone, but were increased by co-stimulation with leptin and IL-33 *in vitro* (leptin vs. leptin + IL-33; 100.6 ± 2.6 vs. 118.9 ± 3.6 %; $p < 0.01$, IL-33 vs. leptin + IL-33; 105.6 ± 3.0 vs. 118.9 ± 3.6 %; $p < 0.05$) (Fig. 6).

Discussion

In this study, ob/ob mice showed less eosinophilic inflammation and goblet cell metaplasia induced by IL-33 compared to wild-type mice, but these changes were attenuated by the exogenous administration of leptin. Furthermore, AHR was spontaneously elevated in ob/ob mice regardless of IL-33 treatment. These findings suggest that leptin enhances IL-33-induced eosinophilic inflammation and goblet cell metaplasia in the airway, and that obesity *per se* is associated with AHR, independent of inflammation.

We demonstrated that IL-33-induced airway eosinophilic inflammation was attenuated in ob/ob mice (Fig. 3c). In addition, IL-5, IL-13, eotaxin, and KC levels in BALF were lower in IL-33-treated ob/ob mice than in IL-33-treated wild-type mice (Fig. 4a–4d). Furthermore, the exogenous administration of leptin in ob/ob mice attenuated these changes. Classically, it is known that OVA-sensitized ob/ob mice do not show an

218 increase in type-2 inflammation [9]. Given that IL-33 induces type-2 cytokines from
219 ILC2, the attenuated eosinophilia in ob/ob mice may have been associated with the
220 decrease in IL-5, IL-13, and eotaxin. Zheng et al. demonstrated that leptin promotes the
221 proliferation of Th2 cells and ILC2s. They also demonstrated that leptin-deficiency leads
222 to reduced ILC2s and attenuated type-2 cytokine production [15]. Ding et al. reported that
223 ILC2 decreases in the adipose tissue of ob/ob mice [16]. Therefore, our results may have
224 been caused by a decrease in ILC2 in ob/ob mice.

225 In wild-type mice, IL-33 induced goblet cell metaplasia (Fig. 5b) and increased
226 mucus score (Fig. 5g). Conversely, in ob/ob mice, IL-33-induced goblet cell metaplasia
227 was attenuated, and exogenous leptin administration reversed this change (Fig. 5e, 5f).
228 This may have been caused by the decrease in IL-13 in IL-33-treated ob/ob mice because
229 IL-13 plays an important role in the induction of goblet cell metaplasia [17]. Furthermore,
230 in our *in vitro* study, MUC5AC levels were increased by co-stimulation with IL-33 and
231 leptin (Fig. 6). Leptin and its receptor are expressed in the airway epithelium [7]. The
232 asthmatic airway epithelium shows an increase in IL-33 expression [18]. Leptin is
233 reported to have the potency to induce mucin protein expression in human airway
234 epithelial cells [19]. Therefore, our *in vitro* study supports the hypothesis that leptin
235 deficiency disturbs mucin production in the IL-33-stimulated airway epithelium in ob/ob
236 mice.

237 Subsequently, ob/ob mice spontaneously showed increased AHR compared to
238 wild-type mice in the absence of IL-33 (Fig. 2b). Ob/ob mice have innate AHR, which
239 might be induced by mechanical factors (low functional residual capacity or tidal
240 volume) and systemic inflammation caused by obesity or weight gain [20]. In our study,
241 exogenous leptin induced no significant change in AHR in IL-33-treated ob/ob mice

(Fig. 2d), although airway inflammation and mucus secretion were augmented. One possible explanation may be that the dose of exogenous leptin was insufficient to enhance AHR. Conversely, leptin administration prior to IL-33 enhanced AHR in wild-type mice (Fig. 2c). The addition of leptin to IL-33 induced a significant increase in BALF eotaxin and KC in wild-type mice (Fig. 4c, 4d). It is suggested that augmented airway inflammation plays a role in increased AHR in wild-type mice. Others also reported that the addition of the OVA challenge [21] or IL-17A [22] to IL-33 enhanced AHR compared to IL-33 alone.

We used female mice in this study. Obesity-related asthma is known to be more prevalent in females than in males. Sood et al. reported that the association between leptin and asthma appeared stronger in women than in men [23]. Visceral fat leptin expression is significantly related to AHR in women with asthma [6]. It is well known that obese women show severe airflow limitation, little eosinophilic inflammation, and steroid unresponsiveness [24, 25]. Uddén et al. showed that corticosteroids induce elevated serum leptin levels in women [26]. Increased body weight and leptin levels induce much more AHR and worsening symptoms, especially in women.

Based on the results of this experiment, we hypothesized that the mechanism of obesity-related severe asthma is as follows. Increased body weight induces AHR due to its mechanical factor, and elevated leptin combined with IL-33 induces airway inflammation, goblet cell metaplasia, and more enhanced AHR. These two factors (airway inflammation and AHR) could be associated with severe asthma. However, human obesity shows increased leptin levels that differ from ob/ob mice. Even short-

term high-fat-diet treatment is reported to induce leptin and AHR [27]. Therefore, further studies are needed to clarify leptin- and obesity-related asthma in high-fat-diet-induced animal models.

Conclusions

In summary, we showed that leptin combined with IL-33 plays an important role in airway inflammation and goblet cell metaplasia, and that obesity *per se* increases AHR independent of inflammation.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

List of abbreviations

AHR: airway hyperresponsiveness

BALF: bronchoalveolar lavage fluid

ILC2: type-2 innate lymphocytes

Rrs: resistance of the respiratory system

Declarations

Acknowledgements

The authors have no conflicts of interest to declare. The authors thank Masayuki Shino and Yoshimi Sugimura (Tokyo Women's Medical University) for their technical support.

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Authors' Contributions

AK, MK, and KA designed the study and wrote the manuscript. SA and ET interpreted the results. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This animal protocol was approved by the Animal Care and Use Committee of Tokyo Women's Medical University (license numbers: AE20-065-B).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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391

392 Figure legends

393 Figure 1. (a) Experimental protocol. In leptin-treated groups, leptin (25 μg/125 μL
394 phosphate-buffered saline (PBS) for wild-type, 50 μg/250 μL PBS for ob/ob) were
395 injected intraperitoneally on days 1, 3, 5, and 8-11. In IL-33-treated groups, IL-33 (1
396 μg/50 μL PBS) was instilled intranasally on days 9-11. On day 12, airway
397 responsiveness, BALF, and lung sections were assessed. (b) Body weight. Data are
398 expressed as mean ± standard error of the mean (SEM). n = 4-6 for each group. ### $p <$
399 0.001 vs. mice with an identical treatment.
400

401 Figure 2. Airway responsiveness to methacholine. (a) Baseline resistance of the
402 respiratory system (Rrs) in wild-type and ob/ob mice. Airway responsiveness in (b)
403 non-treated wild-type and ob/ob mice, (c) non-treated, IL-33-treated, Leptin + IL-33-
404 treated wild-type mice, and (d) non-treated, IL-33-treated, Leptin + IL-33-treated ob/ob
405 mice. Data are expressed as mean ± standard error of the mean (SEM). n = 4-5 for each
406 group. (a) ** $p < 0.01$, (b-d) Rrs is shown as fold change from baseline. *Closed circle*:
407 non-treated wild-type mice. *Open circle*: non-treated ob/ob mice. *Closed square*: IL-33-
408 treated wild-type mice. *Open square*: IL-33-treated ob/ob mice. *Closed triangle*: Leptin
409 + IL-33-treated wild-type mice. *Open triangle*: Leptin + IL-33-treated ob/ob mice. * $p <$
410 0.05, ** $p < 0.01$ vs. non-treated wild-type mice.
411

412 Figure 3. The cell differentials of bronchoalveolar lavage fluid. (a) Total cells, (b) %
413 macrophages, (c) % eosinophils, (d) % neutrophils, and (e) % lymphocytes. Data are

414 expressed as mean ± standard error of the mean (SEM). n = 6-9 for each group. * p <
415 0.05, ** p < 0.01, *** p < 0.001 vs. genotype-matched mice. # p < 0.05, ## p < 0.01, ###
416 vs. p < 0.001 vs. mice with an identical treatment.
417
418 Figure 4. The cytokine and chemokine analysis in bronchoalveolar lavage fluid. (a) IL-
419 5, (b) IL-13, (c) Eotaxin, (d) KC. Data are expressed as mean ± standard error of the
420 mean (SEM). n = 6-9 for each group. * p < 0.05, ** p < 0.01, *** p < 0.001 vs.
421 genotype-matched mice. # p < 0.05, ## p < 0.01, ### vs. p < 0.001 vs. mice with an
422 identical treatment.
423
424 Figure 5. The light microscopic photographs. (a) non-treated wild-type, (b) IL-33-
425 treated wild-type, (c) Leptin + IL-33-treated wild-type, (d) non-treated ob/ob, (e) IL-33-
426 treated ob/ob, (f) Leptin + IL-33-treated ob/ob mice. PAS/Alcian blue stain. Scale bar =
427 200 μm. (g) Mucus score. Data are expressed as mean ± standard error of the mean
428 (SEM). n = 3-4 for each group. * p < 0.05, *** p < 0.001 vs. genotype-matched mice.
429 ## p < 0.01 vs. mice with an identical treatment.
430
431 Figure 6. MUC5AC level induced by leptin and IL-33 *in vitro*. Data are expressed as
432 mean ± standard error of the mean (SEM). n = 5-6 for each group. ** p < 0.01, Leptin +
433 IL-33 vs. Leptin. * p < 0.05, Leptin + IL-33 vs. IL-33.
434
435

Figure. 1

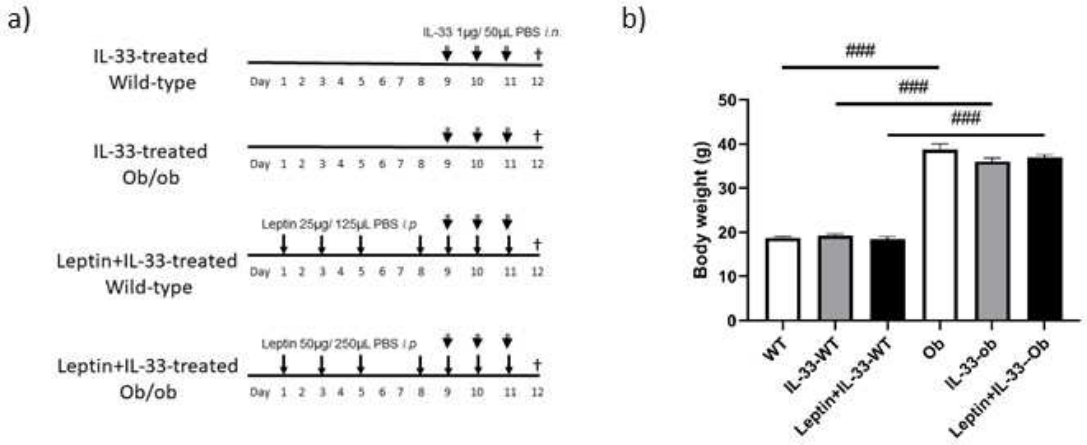

a)
IL-33 1μg/ 50μL PBS i.n.
IL-33-treated Wild-type
Day 1 2 3 4 5 6 7 8 9 10 11 12
IL-33-treated Ob/ob
Leptin 25μg/ 125μL PBS i.p
Leptin+IL-33-treated Wild-type
Leptin 50μg/ 250μL PBS i.p
Leptin+IL-33-treated Ob/ob
b)
###
###
###
Body weight (g)
0
10
20
30
40
50
WT
IL-33-WT
Leptin+IL-33-WT
Ob
IL-33-ob
Leptin+IL-33-Ob

Figure. 2

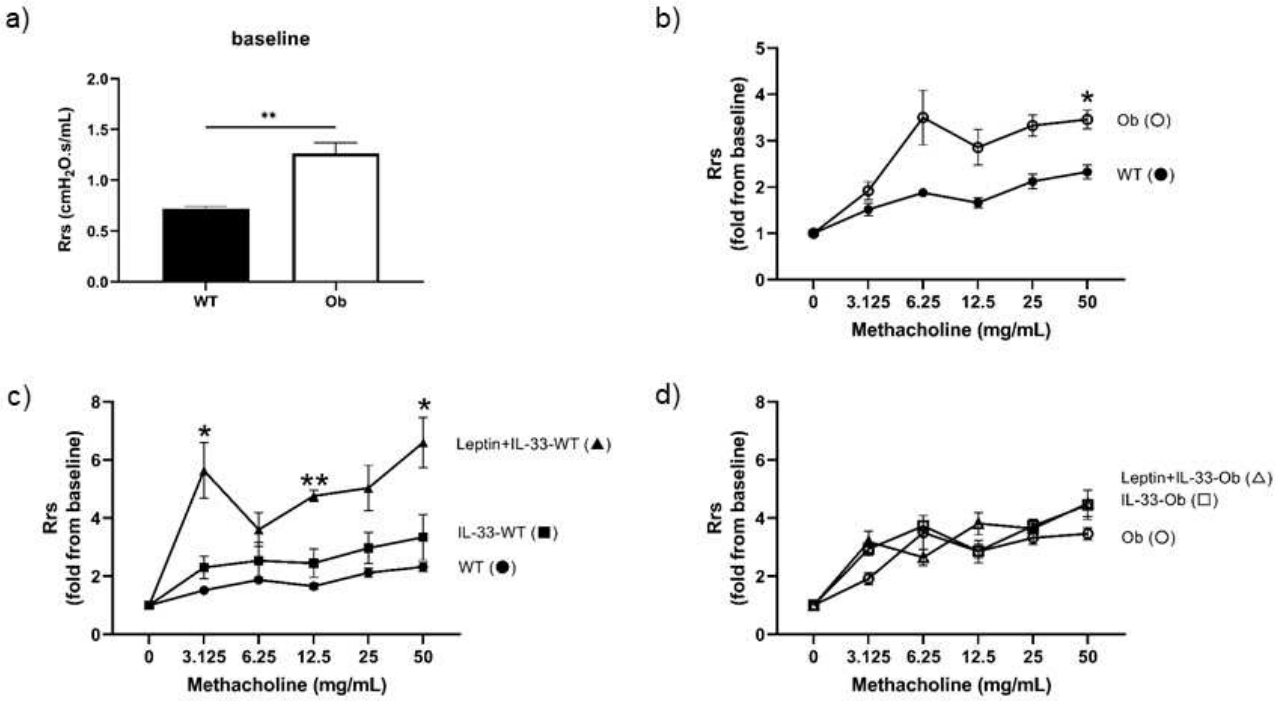

a)
baseline
Rrs (cmH2O.s/mL)
**
WT
Ob
b)
Rrs (fold from baseline)
*
Ob (O)
WT (●)
0
3.125
6.25
12.5
25
50
Methacholine (mg/mL)
c)
Rrs (fold from baseline)
*
**
*
Leptin+IL-33-WT (▲)
IL-33-WT (■)
WT (●)
Methacholine (mg/mL)
d)
Rrs (fold from baseline)
Leptin+IL-33-Ob (△)
IL-33-Ob (□)
Ob (O)
Methacholine (mg/mL)

Figure. 3

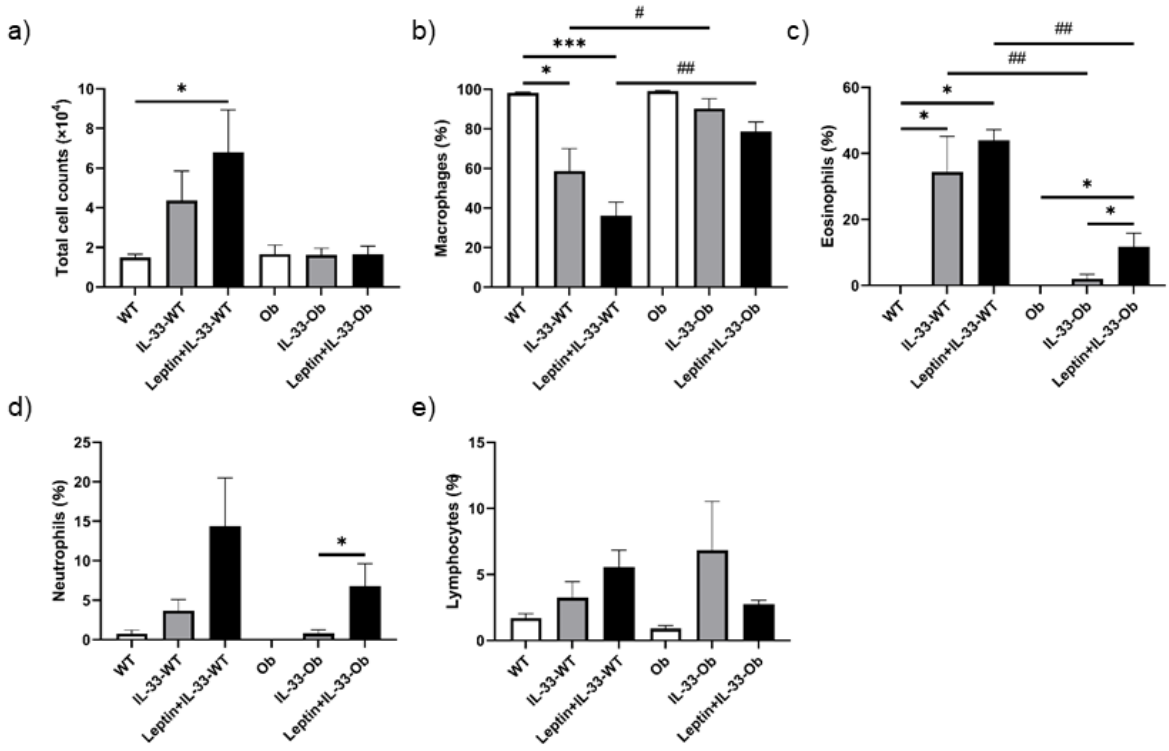

a)
Total cell counts (×10⁴)
b)
Macrophages (%)
c)
Eosinophils (%)
d)
Neutrophils (%)
e)
Lymphocytes (%)
WT
IL-33-WT
Leptin+IL-33-WT
Ob
IL-33-Ob
Leptin+IL-33-Ob

Figure. 4

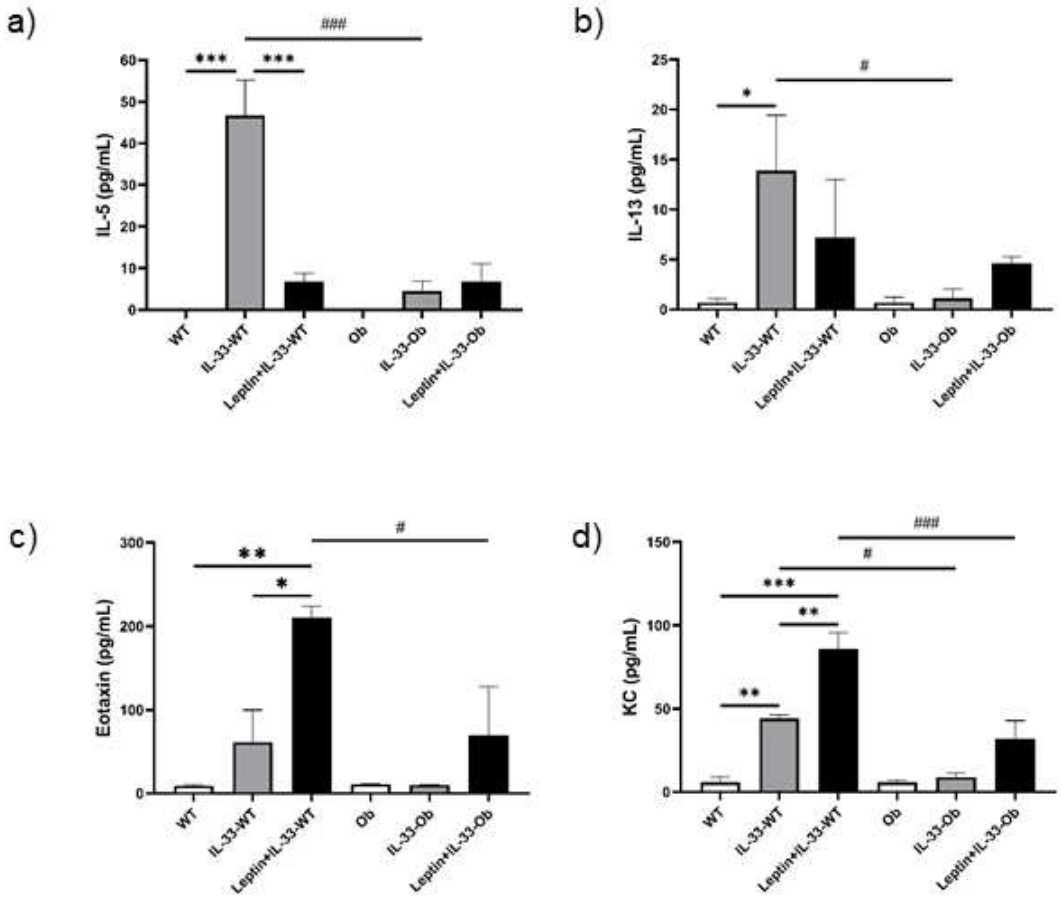
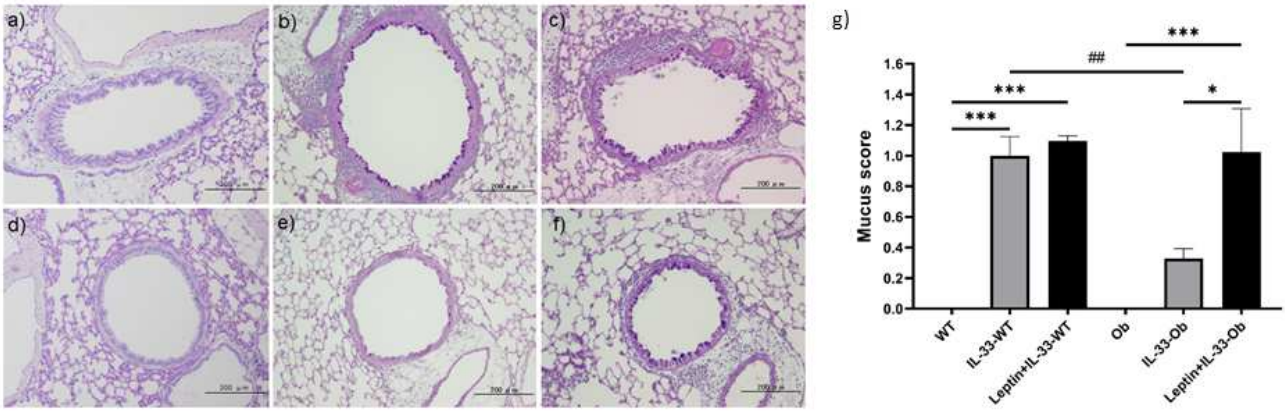

a)
IL-5 (pg/mL)
***
***
###
WT
IL-33-WT
Leptin+IL-33-WT
Ob
IL-33-Ob
Leptin+IL-33-Ob
b)
IL-13 (pg/mL)
*
#
c)
Eotaxin (pg/mL)
**
*
#
d)
KC (pg/mL)
**
***
**
#
###

Figure. 5

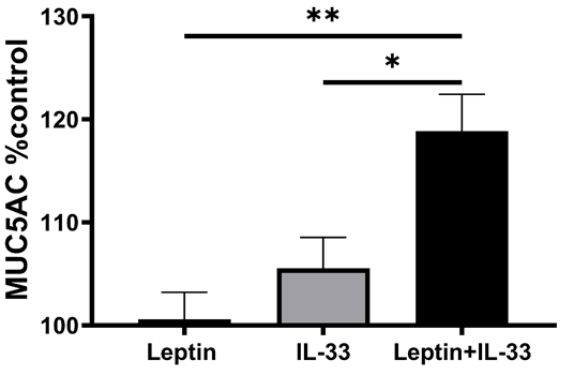

a)
b)
c)
d)
e)
f)
g)
Mucus score
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
***
##
***
*
WT
IL-33-WT
Leptin+IL-33-WT
Ob
IL-33-Ob
Leptin+IL-33-Ob

507

508

509

510 Figure. 6



511

Figures

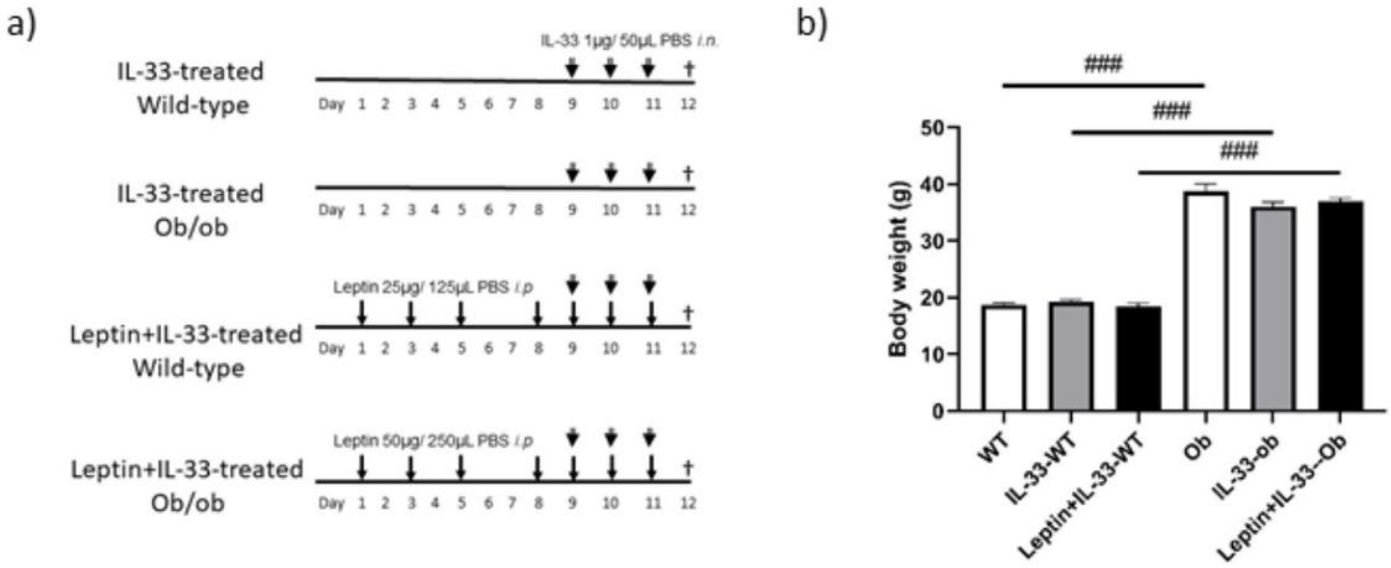


Figure 1

(a) Experimental protocol. In leptin-treated groups, leptin (25 μg/125 μL phosphate-buffered saline (PBS) for wild-type, 50 μg/250 μL PBS for ob/ob) were injected intraperitoneally on days 1, 3, 5, and 8-11. In IL-33-treated groups, IL-33 (1 μg/50 μL PBS) was instilled intranasally on days 9-11. On day 12, airway responsiveness, BALF, and lung sections were assessed. (b) Body weight. Data are expressed as mean ± standard error of the mean (SEM). n = 4-6 for each group. ### p <0.001 vs. mice with an identical treatment.

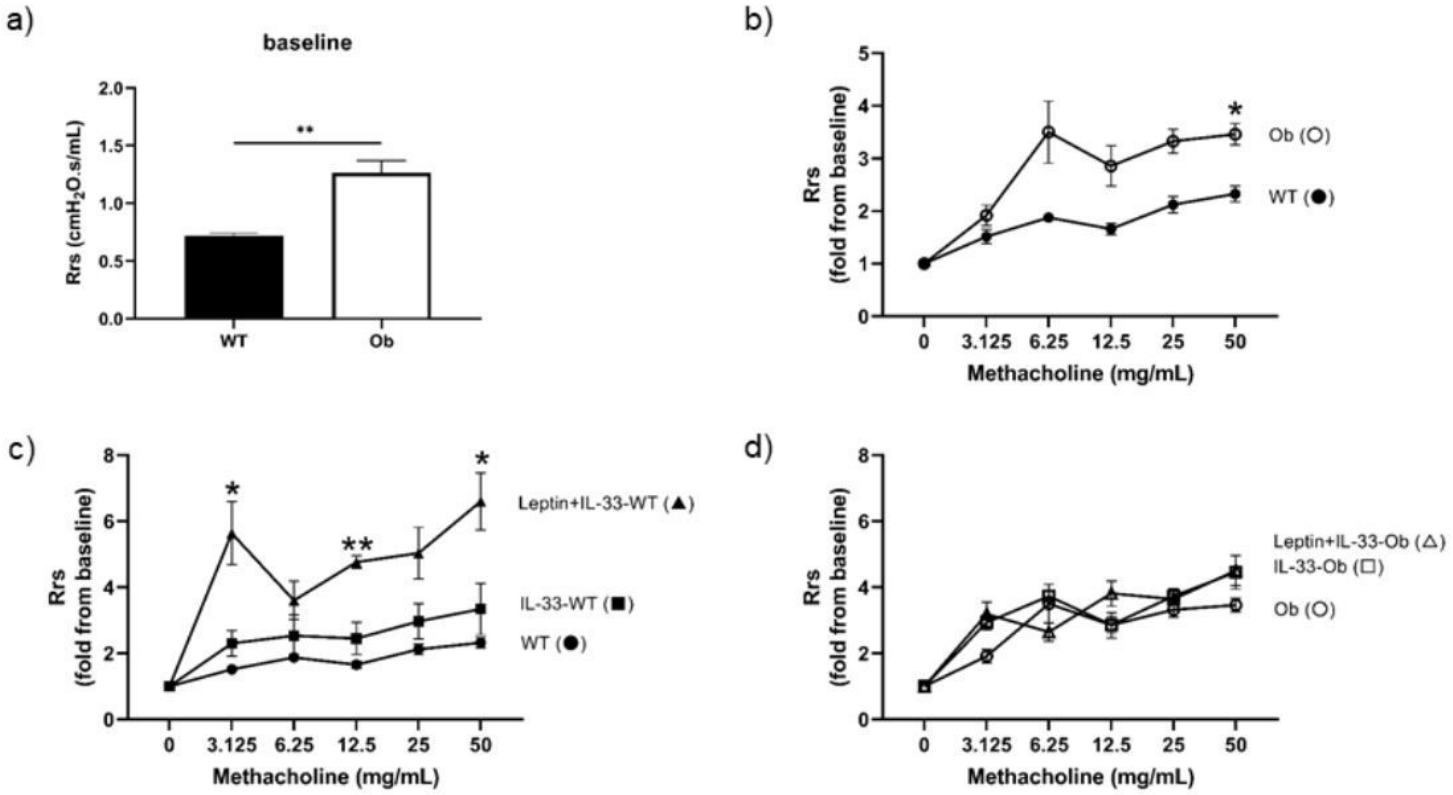


Figure 2

Airway responsiveness to methacholine. (a) Baseline resistance of the respiratory system (Rrs) in wild-type and ob/ob mice. Airway responsiveness in (b) non-treated wild-type and ob/ob mice, (c) non-treated, IL-33-treated, Leptin + IL-33- treated wild-type mice, and (d) non-treated, IL-33-treated, Leptin + IL-33-treated ob/ob mice. Data are expressed as mean ± standard error of the mean (SEM). n = 4-5 for each group. (a) ** $p < 0.01$, (b-d) Rrs is shown as fold change from baseline. Closed circle: non-treated wild-type mice. Open circle: non-treated ob/ob mice. Closed square: IL-33- treated wild-type mice. Open square: IL-33-treated ob/ob mice. Closed triangle: Leptin + IL-33-treated wild-type mice. Open triangle: Leptin + IL-33-treated ob/ob mice. * $p < 0.05$, ** $p < 0.01$ vs. non-treated wild-type mice.

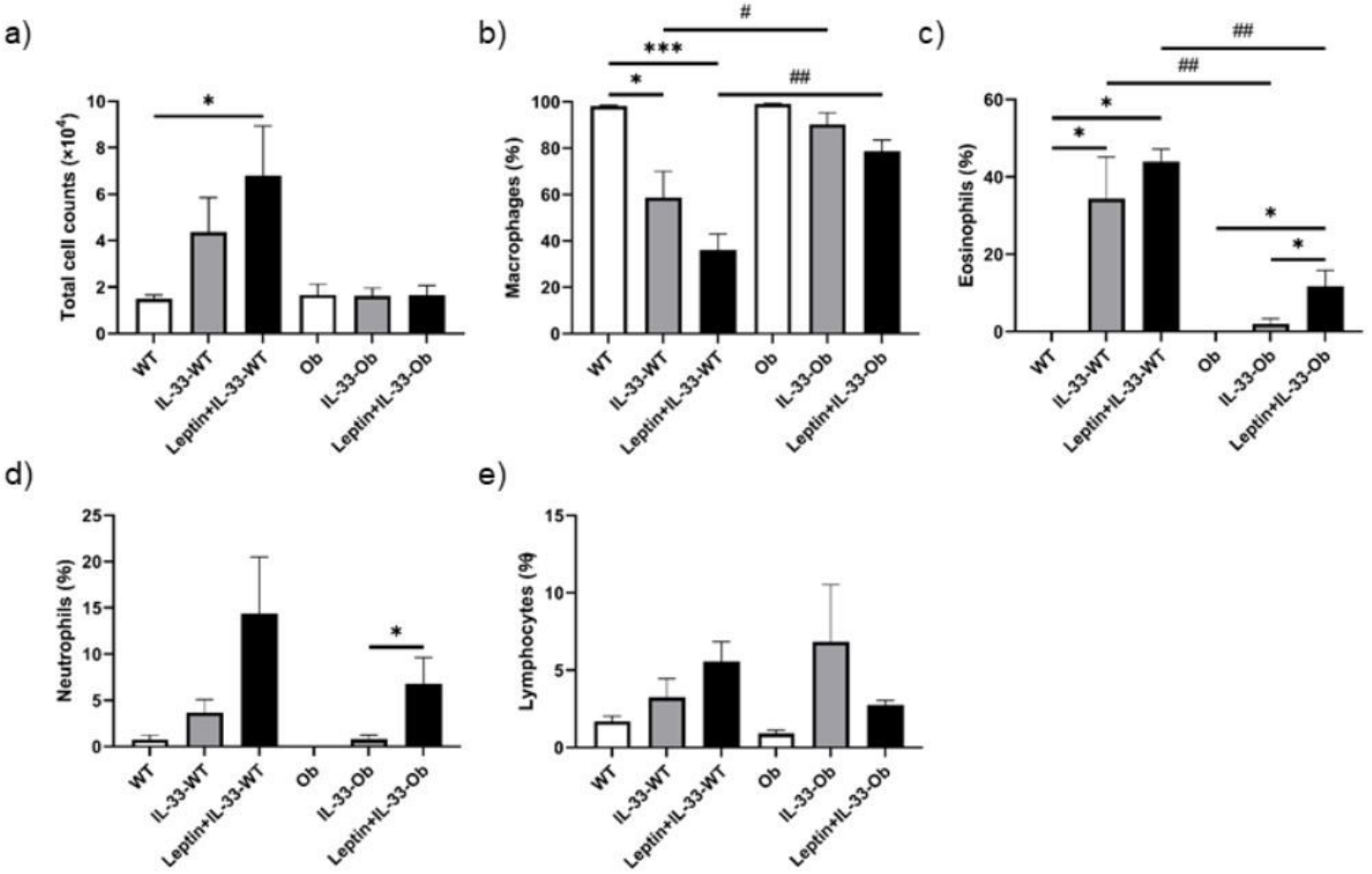


Figure 3

The cell differentials of bronchoalveolar lavage fluid. (a) Total cells, (b) % macrophages, (c) % eosinophils, (d) % neutrophils, and (e) % lymphocytes. Data are expressed as mean ± standard error of the mean (SEM). n 414 = 6-9 for each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. genotype-matched mice. □ $p < 0.05$, □□ $p < 0.01$, □□□ vs. $p < 0.001$ vs. mice with an identical treatment.

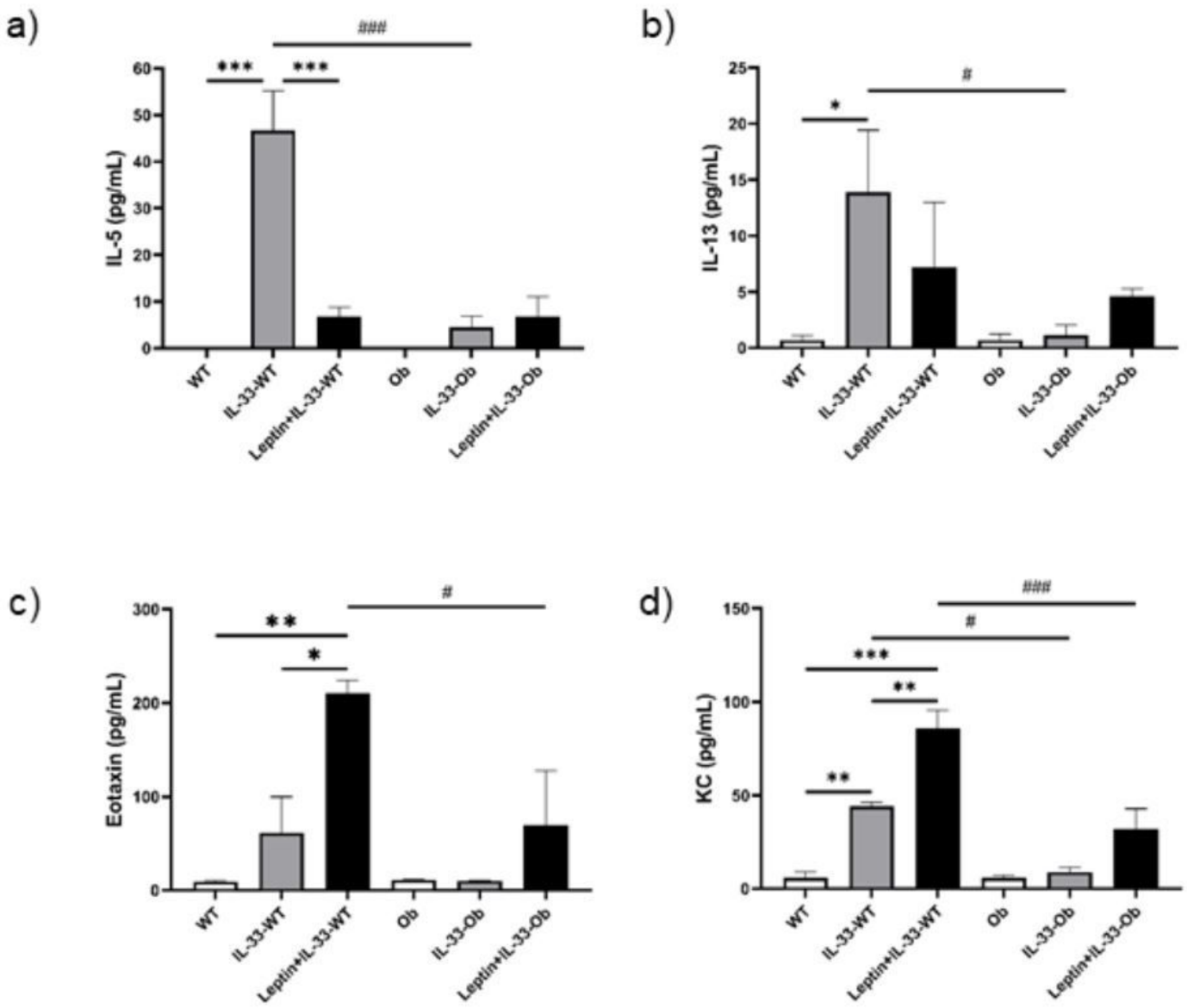


Figure 4

The cytokine and chemokine analysis in bronchoalveolar lavage fluid. (a) IL- 5, (b) IL-13, (c) Eotaxin, (d) KC. Data are expressed as mean ± standard error of the mean (SEM). n = 6-9 for each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. genotype-matched mice. □ $p < 0.05$, □□ $p < 0.01$, □□□ vs. $p < 0.001$ vs. mice with an identical treatment.

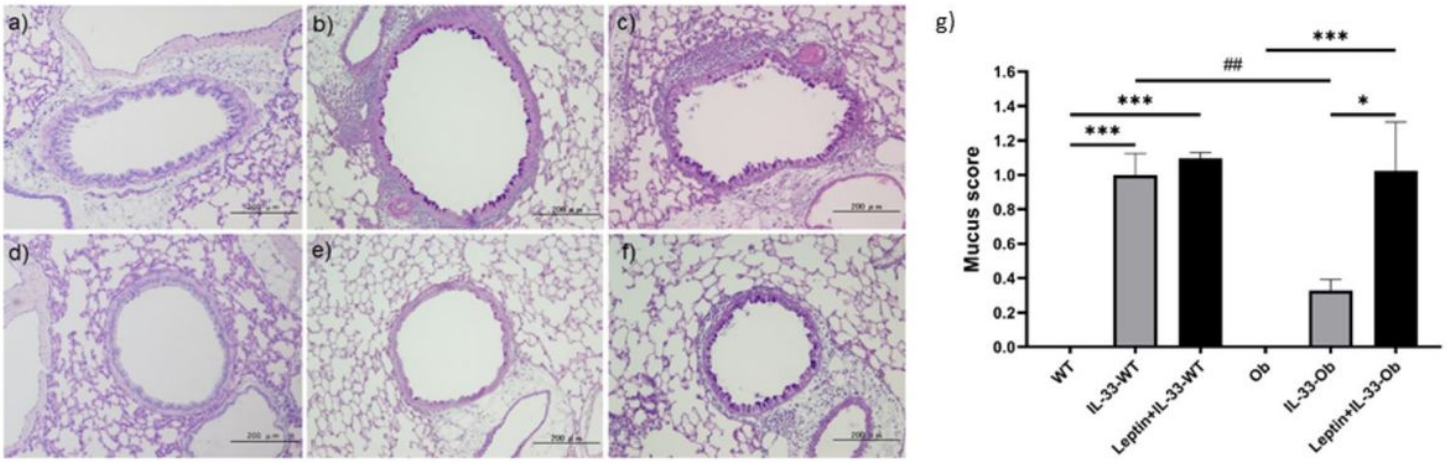


Figure 5

The light microscopic photographs. (a) non-treated wild-type, (b) IL-33- treated wild-type, (c) Leptin + IL-33-treated wild-type, (d) non-treated ob/ob, (e) IL-33- treated ob/ob, (f) Leptin + IL-33-treated ob/ob mice. PAS/Alcian blue stain. Scale bar = 200 μm. (g) Mucus score. Data are expressed as mean ± standard error of the mean (SEM). n = 3-4 for each group. * $p < 0.05$, *** $p < 0.001$ vs. genotype-matched mice. ## $p < 0.01$ vs. mice with an identical treatment.

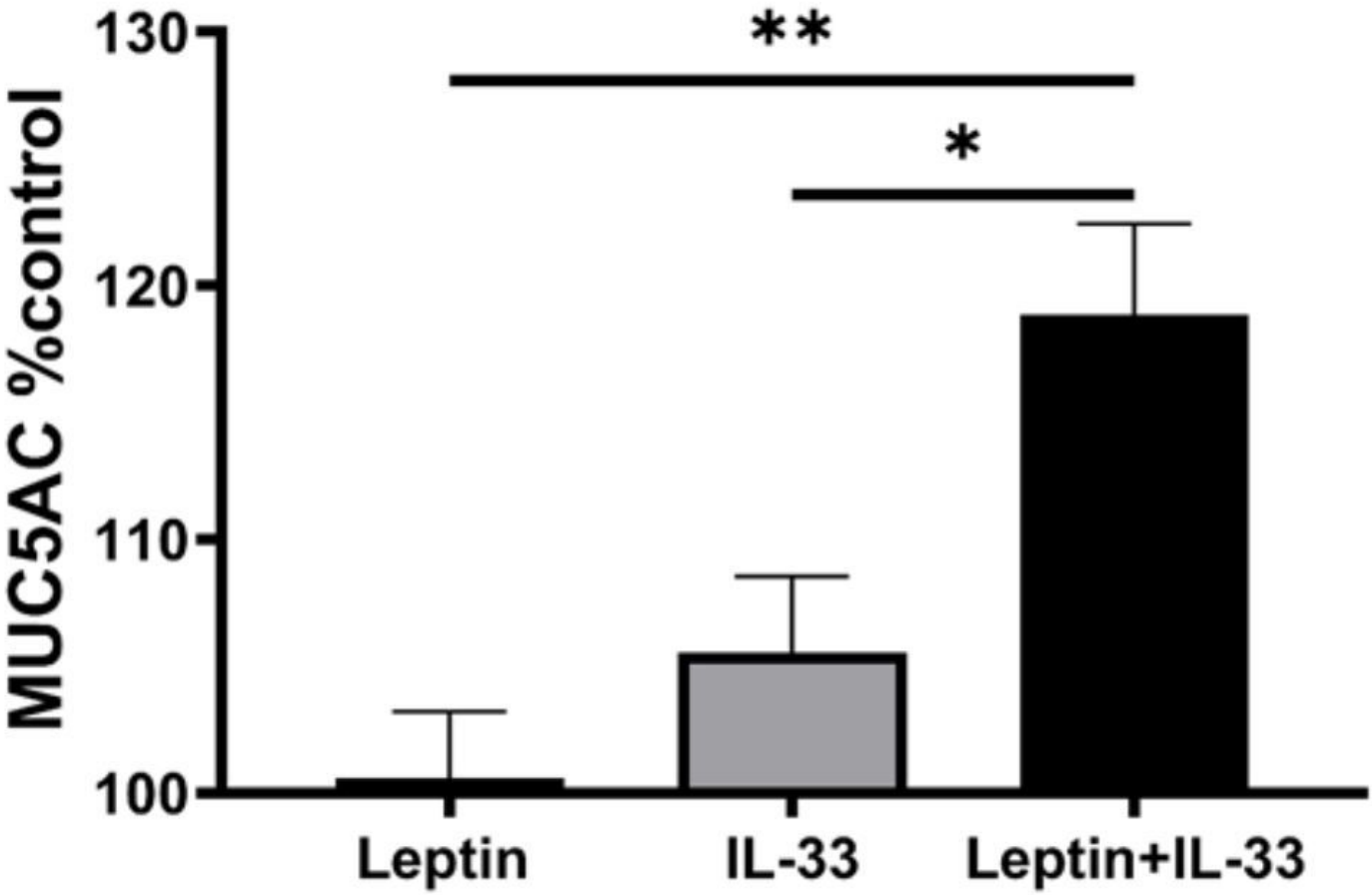


Figure 6

MUC5AC level induced by leptin and IL-33 in vitro. Data are expressed as mean ± standard error of the mean (SEM). n = 5-6 for each group. ** $p < 0.01$, Leptin + IL-33 vs. Leptin. * $p < 0.05$, Leptin + IL-33 vs. IL-33.