

Obese mice are resistant to eosinophilic airway inflammation induced by diesel exhaust particles

Rie Yanagisawa^{a*}, Eiko Koike^a, Takamichi Ichinose^b and Hirohisa Takano^c

ABSTRACT: Particulate matter can exacerbate respiratory diseases such as asthma. Diesel exhaust particles are the substantial portion of ambient particulate matter with a $<2.5\ \mu\text{m}$ diameter in urban areas. Epidemiological data indicate increased respiratory health effects of particulate matter in obese individuals; however, the association between obesity and diesel exhaust particle-induced airway inflammation remains unclear. We aimed to investigate the differences in susceptibility to airway inflammation induced by exposure to diesel exhaust particles between obese mice (db/db) and lean mice (db/+m). Female db/db and db/+m mice were intratracheally administered diesel exhaust particles or vehicle every 2 weeks for a total of seven times. The cellular profile of bronchoalveolar lavage fluid and histological changes in the lungs were assessed and the lungs and serum were analyzed for the generation of cytokines, chemokines and soluble intercellular adhesion molecule 1. Diesel exhaust particle exposure-induced eosinophilic infiltration in db/+m mice accompanied by T-helper 2 cytokine, chemokine and soluble intercellular adhesion molecule 1 expression in the lungs. In contrast, it induced mild neutrophilic airway inflammation accompanied by elevated cytokines and chemokines in db/db mice. The lungs of db/db mice exhibited decreased expression of eosinophil activators/chemoattractants such as interleukin-5, interleukin-13 and eotaxin compared with those of db/+m mice. In addition, serum eotaxin and monocyte chemoattractant protein-1 levels were significantly higher in db/db mice than in db/+m mice. In conclusion, obesity can affect susceptibility to diesel exhaust particle-induced airway inflammation, which is possibly due to differences in local and systemic inflammatory responses between lean and obese individuals. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: diesel exhaust particles; neutrophil; eosinophil; cytokines; chemokines; airway inflammation

Introduction

Over the past two decades, substantial evidence has suggested that the effect of ambient particulate matter (PM) on the cardiopulmonary system is a significant public health concern in developed and developing countries (Pope *et al.*, 2004; Miller *et al.*, 2007; Brook *et al.*, 2010). Individuals with respiratory and cardiovascular diseases are usually more sensitive to PM exposure. Several recent studies have shown that individuals with diabetes may also be susceptible to the adverse effects of PM with a $<2.5\ \mu\text{m}$ diameter (PM_{2.5}) (Zanobetti and Schwartz, 2001; Zanobetti and Schwartz, 2002; Goldberg *et al.*, 2006; O'Neill *et al.*, 2007). Although biological mechanisms underlying these associations remain unclear, Brook *et al.* suggested that PM exposure affects cardiovascular disease through systemic inflammation and/or oxidative stress activation and may promote type 2 diabetes mellitus (Brook, 2008). Experimental studies have demonstrated that PM_{2.5} exposure induces insulin resistance and increases visceral inflammation and adiposity in rodents (Sun *et al.*, 2009; Xu *et al.*, 2010; Yan *et al.*, 2011). Our previous study suggested that pulmonary exposure to diesel exhaust particles (DEP), the substantial portion of ambient PM_{2.5} in urban areas, enhances hepatic steatosis in obese mice with type 2 diabetes through oxidative stress activation (Tomaru *et al.*, 2007). A recent epidemiologic study showed that PM concentrations are associated with respiratory symptoms and asthma in obese children (Dong *et al.*, 2013). Therefore, obese individuals are probably vulnerable to PM such as DEP; however, supportive evidence remains inconclusive.

Epidemiological data indicate that the prevalence of obesity in adults and children has dramatically increased worldwide (McClean *et al.*, 2008). Obesity is a known risk factor for type 2 diabetes mellitus, atherosclerosis, hypertension and some types of cancer (Field *et al.*, 2001). It also increases the prevalence and incidence of asthma and worsens asthma control (Akerman *et al.*, 2004). Worldwide cross-sectional studies have indicated that asthma prevalence is higher in obese than in lean individuals. In addition, obesity may be a particularly important factor influencing asthma severity because more than 75% of asthmatic individuals visiting the emergency room are obese or overweight (Thomson *et al.*, 2003). In animal studies, inflammatory responses in the lung have been shown to influence the production of the adipocytokines, leptin, cytokines, acute phase proteins and other mediators produced by adipose tissue resulting in involved immune responses of the lung (Mancuso,

*Correspondence to: Rie Yanagisawa, PhD, Center for Environmental Health Sciences, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba 305-8506, Japan. Email: yanagisawa.rie@nies.go.jp

^aCenter for Environmental Health Sciences, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba 305-8506, Japan

^bDepartment of Health Sciences, Oita University of Nursing and Health Sciences, Notsuharu, Oita 870-1201, Japan

^cGraduate School of Engineering, Kyoto University, Kyoto-Daigaku-Katsura, Nishikyo-ku, Kyoto 615-8530, Japan

2009). These findings suggest that obesity can play an important role in the exacerbation of respiratory disorders; however, it has not been established. Thus, it remains unclear whether obesity affects susceptibility to airway inflammation induced by PM, including DEP. This study aimed to investigate the differences in DEP-induced airway inflammation between obese (db/db) and lean mice (db/+m) and assess changes in systemic inflammation in both groups to identify pathological factors that may attribute to susceptibility to DEP.

Material and Methods

Animals

Five-week-old female mice (BKS.Cg- $\text{Lepr}^{\text{db}}/\text{Jcl}$ [db/db] and BKS.Cg-m/+ $\text{Lepr}^{\text{db}}/\text{Jcl}$ [db/+m]) purchased from Japan Clea Co. (Tokyo, Japan) and were used for the experiments. Animals were fed a commercial diet (CE-2; Japan Clea Co.) and water *ad libitum* and housed in an animal facility maintained at 22–26 °C and 40–69% humidity under a 12 h light/dark cycle. All mice were treated humanely and every effort to alleviate suffering was made in accordance with guidelines established for animal experiments by the National Institute for Environmental Studies. The institutional review board approved all protocols involving the animals.

Collection of Diesel Exhaust Particles

A 4JB1-type, light duty, four-cylinder, 2.74 l, Isuzu diesel engine (Isuzu Automobile Company, Tokyo, Japan) under computer control was connected to a dynamometer (Meiden-sha, Tokyo, Japan). The methods of engine operation and collection of DEP were identical to those previously reported (Sagai *et al.*, 1996). The mass median aerodynamic diameter of the DEP, as determined by an Anderson Air Sampler (Shibata Science Technology, Tokyo, Japan), was 0.4 μm . Most particles were globular in shape.

Study Protocol

db/db and db/+m mice were randomized into two experimental groups. The vehicle group received phosphate-buffered saline (pH, 7.4; Life Technologies Corp., Carlsbad, CA, USA) containing 0.025% Tween 80 (Nacalai Tesque, Kyoto, Japan). The DEP group received 100 μg of suspended DEP in the same vehicle. The DEP suspensions were sonicated for 2 min with an ultrasonic disruptor (UD-201; Tomy Seiko, Tokyo, Japan). Mice were anesthetized with 4% halothane (Takeda Chemical Industries, Ltd., Osaka, Japan), and intratracheally instilled with 100 μl aliquots of aqueous solution every 2 weeks for a total of seven times.

Blood Retrieval and Bronchoalveolar Lavage

All mice were killed under diethyl ether anesthesia 24 h after the final intratracheal instillation. The chest and abdominal walls were opened, and blood was retrieved by cardiac puncture. The lungs were lavaged with 1 ml of sterile saline at 37 °C three times by syringe. The lavaged fluid was harvested by gentle aspiration. The average volume was 90% of the amount instilled (3 ml). The lavage fluid was centrifuged at 300 g for 10 min at 4 °C to recover alveolar free cells. The total cell count was

determined on a fresh fluid specimen using a hemocytometer. Differential cell counts were prepared using the Autospin (Sakura Seiki Co., Tokyo, Japan) and stained with Diff-Quik (International Reagents Co., Kobe, Japan). A total of 500 cells were counted under a microscope (AX80; Olympus, Tokyo, Japan). The lungs were retrieved immediately after bronchoalveolar lavage (BAL). The serum and lungs were stored at – 80 °C until further use.

Histopathological Examination

The lungs ($n=5$ in each group) were fixed in 10% phosphate-buffered formalin (pH 7.4) 24 h after the final intratracheal instillation. After separation of the lobe, 3 mm thick blocks were taken for paraffin embedding and cut into 3 μm sections. Histological specimens were then stained with hematoxylin and eosin. Pathological analysis was performed using an Olympus AX80 microscope (Olympus Corp.). The degree of inflammation was quantified by the percentage of airways and blood vessels surrounded by inflammatory cells (0, not present; 1, < 10%; 2, 10–20%; 3, 20–40%; 4, 40–60%; 5, > 60%).

Enzyme-linked Immunosorbent Assay

The lungs ($n=13$ –17 in each group) removed after BAL were homogenized with 10 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM ethylenediaminetetraacetic acid (Sigma-Aldrich Co., St. Louis, MO, USA), 0.1 mM phenylmethanesulfonyl fluoride (Nacalai Tesque, Inc., Kyoto, Japan), 1 μM pepstatin (Peptide Institute, Inc., Osaka, Japan) and 2 μM leupeptin (Peptide Institute). The homogenates were then centrifuged at 105,000 g for 1 h at 4 °C. The supernatants were dispensed and stored at – 80 °C. Enzyme-linked immunosorbent assay for chemokine (C-C motif) ligand 2/monocyte chemoattractant protein 1 (CCL2/MCP-1; R&D Systems Inc., Minneapolis, MN), chemokine (C-C motif) ligand 3/macrophage inflammatory protein 1 α (CCL3/ MIP-1 α ; R&D Systems), chemokine (C-X-C motif) ligand 1/keratinocyte chemoattractant (CXCL1/KC; R&D Systems), chemokine (C-C motif) ligand 5/regulated and normal T-cell expressed and secreted (CCL5/RANTES; R&D Systems), chemokine (C-C motif) ligand 11 (CCL11)/eotaxin (R&D Systems), interleukin (IL)-13 (R&D Systems), IL-5 (Thermo Fisher Scientific Inc., Waltham, MA, USA), IL-6 (Life Technologies), and tumor necrosis factor- α (TNF- α ; Thermo Fisher Scientific) in the lung tissue supernatants and CCL11/eotaxin, CCL2/MCP-1 (R&D Systems) and soluble intercellular adhesion molecule 1 (ICAM-1; Thermo Fisher Scientific) in the serum ($n=18$ –22 in each group) was conducted according to the manufacturer's instructions.

Statistical Analysis

Data are expressed as mean $\pm$ SEM. The significance of variation among different groups was determined by one-way ANOVA or Kruskal–Wallis analysis. Differences between groups and was determined Tukey or Steel–Dwass multiple comparison tests using the Excel Statistics 2010 statistical software (Social Survey Research Information Co., Ltd., Tokyo, Japan). $P < 0.05$ was considered statistically significant.

Results

Cellular Profiles of Bronchoalveolar Lavage Fluid and Histological Findings in The Lungs after Diesel Exhaust Particles Exposure

Cellular infiltration into BALF and histological changes in the lungs were confirmed to assess the differences between db/+m mice and db/db mice following DEP exposure. Administration of DEP increased the number of leukocytes compared with vehicle administration in both db/+m and db/db mice (Fig. 1). Increases in the number of eosinophils and lymphocytes were more prominent in db/+m mice than in db/db mice (eosinophils; $P < 0.01$, and lymphocytes; $P < 0.01$, respectively; Fig. 1C,D). In contrast, an increase in the number of neutrophils was slightly greater in db/db mice than in db/+m mice following exposure to DEP (Fig. 1E).

Next, a histopathological analysis of hematoxylin and eosin-stained lung sections was performed 24h after the final intratracheal instillation. No pathological alterations were observed in the vehicle-exposed lungs of either db/+m or db/db mice (Fig. 2A,C,E). A significant accumulation of eosinophils, neutrophils and mononuclear cells was observed in the peribronchial and perivascular regions of db/+m mice exposed to DEP (Fig. 2B,D,E). On the other hand, DEP-exposed db/db mice showed a mild increase in inflammatory cells such as neutrophils and mononuclear cells in the lung (Fig. 2D).

Cytokine, Chemokine and Soluble Intercellular Adhesion Molecule-1 Expression in the Lung

We investigated the effect of DEP on protein levels of proinflammatory molecules in the lungs 24 h after final instillation. Compared with vehicle treatment, DEP treatment in db/+m mice caused a significant increase in the protein expression of MCP-1, KC, MIP-1 α , IL-6, IL-1 β , IL-5, IL-13 and eotaxin in the lungs. In db/db mice, DEP administration resulted in a significant increase in MCP-1, KC, MIP-1 α , IL-6 and IL-1 β compared with vehicle administration ($P < 0.05$ for MCP-1, $P < 0.01$ for KC,

$P < 0.01$ for MIP-1 α , $P < 0.05$ for IL-6, $P < 0.01$ for IL-1 β ; Fig. 3); however, these increases were significantly lesser in db/db mice than in db/+m mice. Lung TNF- α and RANTES expression was lower in db/db mice than in db/+m mice and showed no significant differences in the presence and absence of DEP. To assess the local expression of ICAM-1, which contributes to eosinophil recruitment in the lungs (Kokuludag *et al.*, 2002), soluble ICAM-1 was measured in the lung supernatant (Fig. 3K). DEP exposure elevated soluble ICAM-1 levels in the lungs of db/+m and db/db mice compared with vehicle exposure; however, soluble ICAM-1 expression was lower in db/db mice than in db/+m mice.

Systemic Expression of Chemokines

Obesity is associated with chronic low-grade systemic inflammation characterized by increases in serum cytokine, chemokine, acute-phase protein and inflammatory cell levels. To assess systemic inflammation in obese mice in the presence and absence of DEP exposure, the protein expression of eotaxin and MCP-1 in the serum was measured. Eotaxin and MCP-1 levels were much higher in db/db mice than in db/+m mice in the absence of DEP exposure (Fig. 4). DEP exposure tended to increase eotaxin and MCP-1 levels in both db/+m and db/db mice compared with vehicle exposure, whereas there were no significant alterations.

Discussion

This and a previous study (Sagai *et al.*, 1993) demonstrated that intratracheal exposure to DEP results in prominent eosinophilic airway inflammation in lean (db/+m) mice. These results were paralleled by the expression of proinflammatory molecules such as T-helper 2 (Th2) cytokines, chemokines and soluble ICAM-1 in the lungs. In contrast, DEP exposure attenuated eosinophilic inflammation in obese (db/db) mice but caused mild neutrophilic inflammation and elevation in cytokine and chemokine levels compared with vehicle exposure.

Numerous epidemiological studies have reported associations between PM exposure and adverse cardiopulmonary health

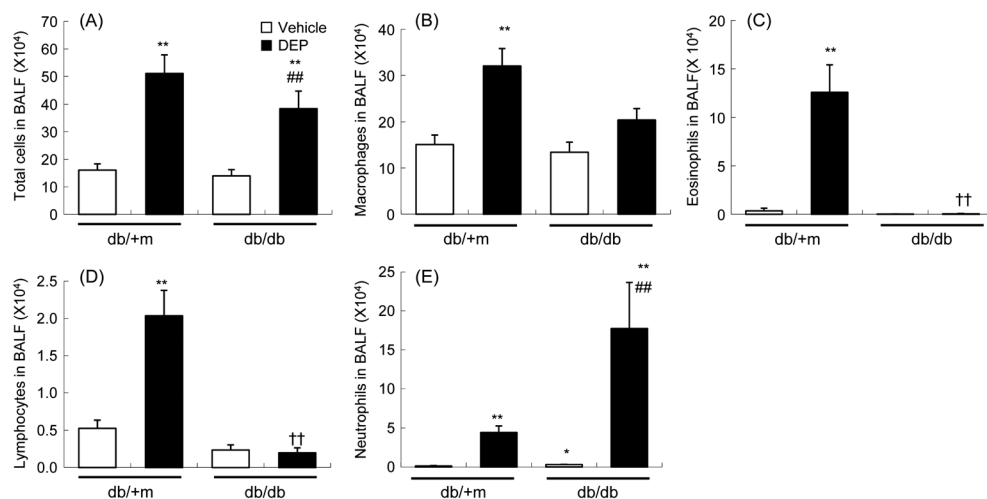


Figure 1. The number of inflammatory cells in BALF following exposure to DEP in db/+m and db/db mice. Twenty-four hours after the final intratracheal administration of vehicle or DEP, BALF was harvested and centrifuged to recover inflammatory cells. (A) Total cells; (B) macrophages; (C) eosinophils; (D) lymphocytes; and (E) neutrophils. White and black columns indicate vehicle- and DEP-exposed specimens, respectively. Data represent means $\pm$ SEM of 13–17 animals per group. * $P < 0.05$ versus vehicle-exposed db/+m mice, ** $P < 0.01$ versus vehicle-exposed db/+m mice, ## $P < 0.01$ versus vehicle-exposed db/db mice, †† $P < 0.01$ versus DEP-exposed db/+m mice. BALF, bronchoalveolar lavage fluid; DEP, diesel exhaust particles.

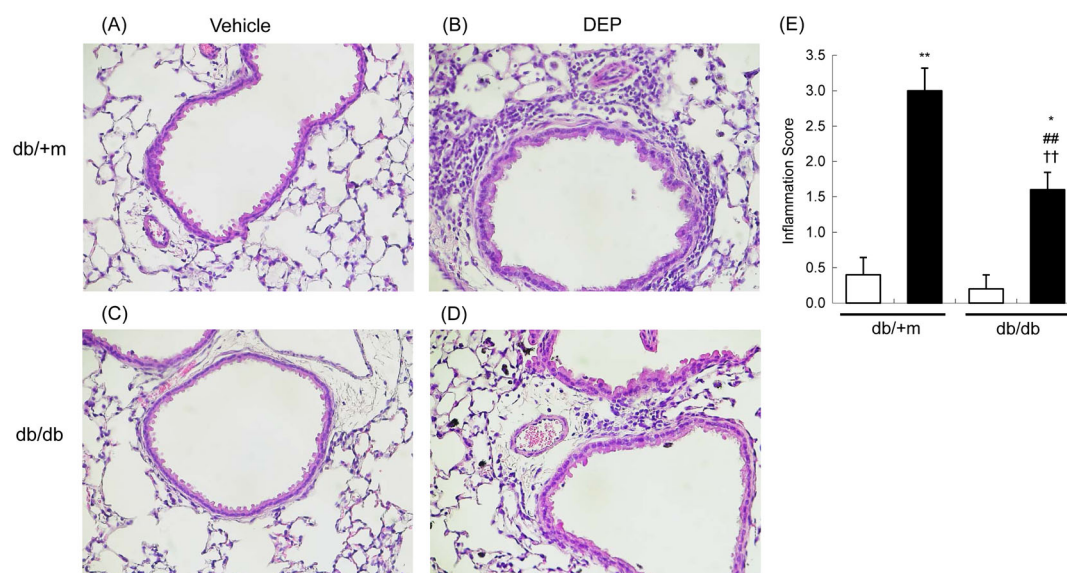


Figure 2. Histological findings in the lungs following exposure to DEP in db/+m and db/db mice. We evaluated the infiltration of inflammatory cells into the lungs using hematoxylin and eosin staining 24 h after the last intratracheal instillation. (A) Vehicle-exposed db/+m mice; (B) DEP-exposed db/+m mice; (C) vehicle-exposed db/db mice; (D) DEP-exposed db/db mice; and (E) inflammation score. Data represent means $\pm$ SEM of five animals per group. * $P < 0.05$ versus vehicle-exposed db/+m mice, ** $P < 0.01$ versus vehicle-exposed db/+m mice, ## $P < 0.01$ versus vehicle-exposed db/db mice, †† $P < 0.01$ versus DEP-exposed db/+m mice. DEP, diesel exhaust particles.

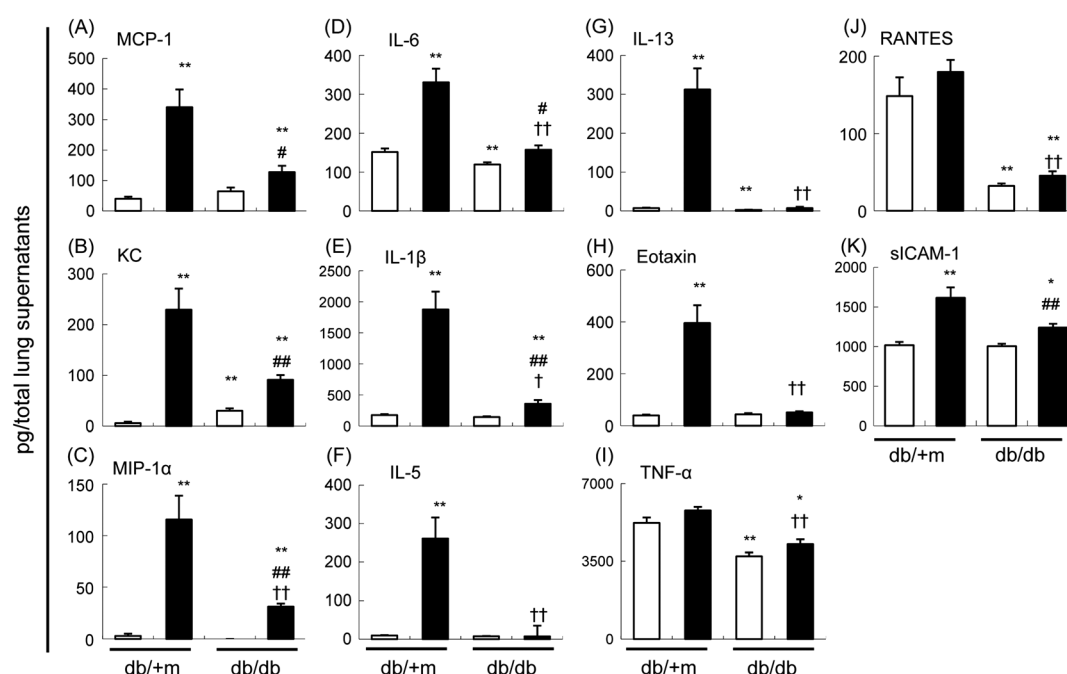


Figure 3. Protein levels of cytokines, chemokines and sICAM-1 in the lungs following exposure to DEP. Twenty-four hours after final intratracheal instillation, the supernatants of homogenized lungs were collected and measured using ELISA. (A) MCP-1; (B) KC; (C) MIP-1 α ; (D) IL-6; (E) IL-1 β ; (F) IL-5; (G) IL-13; (H) Eotaxin; (I) TNF- α ; (J) RANTES; (K) sICAM-1. White and black columns indicate vehicle- and DEP-exposed specimens, respectively. Data represent means $\pm$ SEM of 13–17 animals per group. * $P < 0.05$ versus vehicle-exposed db/+m mice, ** $P < 0.01$ versus vehicle-exposed db/+m mice, # $P < 0.05$ versus vehicle-exposed db/db mice, ## $P < 0.01$ versus vehicle-exposed db/db mice, † $P < 0.05$ versus DEP-exposed db/+m mice, †† $P < 0.01$ versus DEP-exposed db/+m mice. DEP, diesel exhaust particles; IL, interleukin; KC, keratinocyte chemoattractant; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; RANTES, regulated upon activation normal T cell expressed and secreted (chemokine); sICAM, soluble intercellular adhesion molecule; TNF, tumor necrosis factor.

effects. In addition, PM exposure may adversely affect individuals with obesity, metabolic syndrome and/or type 2 diabetes. Dubowsky *et al.* (2006) reported that individuals with systemic inflammation, such as obesity, diabetes and hypertension, might

have increased susceptibility to air pollutants. Obesity is also known to increase the risk of pulmonary diseases, including asthma. Therefore, it is plausible that obese individuals have a higher susceptibility to PM such as DEP, which may act to

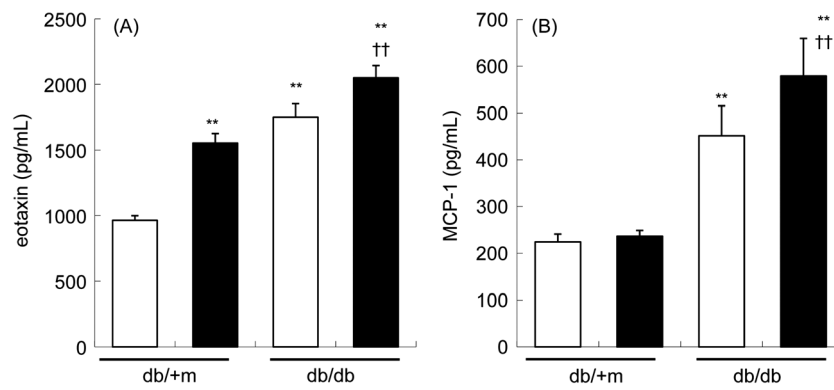


Figure 4. Levels of eotaxin and MCP-1 in the serum following exposure to DEP. Serum (A) eotaxin and (B) MCP-1 were measured 24 h after the last intratracheal administration using ELISA. White and black columns indicate vehicle- and DEP-exposed specimens, respectively. Data represent means $\pm$ SEM of 18–22 animals per group. ** $P < 0.01$ versus vehicle-exposed db/+m mice, †† $P < 0.01$ versus DEP-exposed db/+m mice. DEP, diesel exhaust particles; MCP, monocyte chemoattractant protein.

exacerbate asthmatic responses via systemic inflammation; however, this finding remains to be established. In this study, we elucidated the association between obesity and airway inflammation induced by DEP exposure and investigated the changes in systemic and local inflammation to clarify the pathological factors that attribute to differences in susceptibility to DEP between db/db and db/+m mice. The present results demonstrated that DEP exposure resulted in marked eosinophilic airway inflammation in db/+m mice, while db/db mice demonstrated mild neutrophilic inflammation accompanied by attenuated eosinophilic inflammation (Fig. 1).

Accumulation and subsequent activation of eosinophils in bronchial tissues play a critical role in the pathogenesis of airway inflammation. Eotaxin is an important inflammatory chemokine in eosinophil chemotaxis and acts by inducing recruitment of eosinophils into the lung. In addition, Th2 cytokines such as IL-5 and IL-13 play an important role in eosinophilic inflammation into the airways (Ok *et al.*, 2009). IL-5 is also the most important cytokine in the mediation of eosinophil differentiation, maturation, activation and survival (Sanderson, 1992). IL-13 also plays a key role in airway inflammation and hyperresponsiveness (Grünig *et al.*, 1998; Wills Karp *et al.*, 1998). In addition, an *in vivo* study has shown that chronic exposure to DEP aggravates airway inflammation in mice, which is accompanied by eosinophil infiltration, mucus secretion and respiratory resistance (Sagai *et al.*, 1996). In the present study, exposure to DEP in db/+m mice resulted in airway eosinophilic inflammation, which was paralleled by the generation of Th2 cytokines (IL-5, IL-6 and IL-13) and chemokines (eotaxin and MCP-1), suggesting that DEP can enhance airway eosinophilic inflammation via the expression of inflammatory molecules. In contrast, db/db mice demonstrated attenuated airway eosinophilic inflammation and showed slight neutrophilic inflammation. Eotaxin, IL-13 and IL-5 levels in the lungs remained at lower levels in db/db mice than in db/+m mice following exposure to DEP. From these results, it is plausible that a reduction in eotaxin led to the attenuation of eosinophil chemotaxis into the lung and a subsequent decrease in levels of IL-5 and IL-13 secreted by the eosinophils. In animal studies, a decrease in allergen-induced airway eosinophilia and Th2 cytokine secretion was observed in mice fed a high-fat diet as well as db/db mice (Johnston *et al.*, 2007; de Vries *et al.*, 2009; Calixto *et al.*, 2010). On the other hand, recent epidemiologic studies showed that weight loss in obese asthmatics is associated with improvements in lung

function and airway responsiveness (Maniscalco *et al.*, 2008; Haselkorn *et al.*, 2009; Sideleva *et al.*, 2012) without significant improvements in eosinophilic airway inflammation and cytokine expression (Juel *et al.*, 2012; Sideleva *et al.*, 2012), suggesting that asthma in obesity is not associated with airway inflammation. The association between obesity and respiratory disorders remains controversial. Thus, further investigation is necessary to clarify the impact on airway hyperresponsiveness following exposure to DEP in our model; however, our data may partially support that obesity may not enhance eosinophilic airway inflammation in asthmatic subjects.

Our current study found that DEP exposure in db/+m mice caused a significant increase in eotaxin and MCP-1 levels in the lungs compared to those in db/db mice, whereas vehicle exposure had no significant effect in either db/+m and db/db mice (Figure 3A, H). On the other hand, serum eotaxin and MCP-1 levels in db/db mice were much higher in db/db mice than in db/+m mice, regardless of exposure to DEP (Figure 4). Johnston *et al.* (2007) have suggested that differences in the production of eotaxin can contribute to the decreased influx of eosinophils in the lungs of db/db mice. An epidemiological study reported that MCP-1 levels in the serum of obese children are higher than in that of normal weight children (Breslin *et al.*, 2012). These studies suggest that the higher level of circulating chemokines in obese individuals compared with that in lean individuals gives rise to constitutive systemic inflammation resulting remained eosinophils in the blood rather than migrating to the airways. Furthermore, DEP exposure may play a potential role in the enhancement of systemic inflammation in obese subjects.

TNF- α is a proinflammatory cytokine with many biological effects. It can directly induce the release of chemical mediators (Horie *et al.*, 1996) and mediate eosinophil recruitment into the airways (Lukacs *et al.*, 1995). Tsukahara *et al.* (1999) suggested that TNF- α is associated with eosinophil survival and plays a critical role in the enhancement of eosinophilic inflammation. RANTES, a member of the CC chemokine family, also possesses selective chemotactic activity for eosinophils (Chiba *et al.*, 2005). In the present study, TNF- α and RANTES levels in db/db mouse lungs were lower than those in db/+m mouse lungs in the absence and presence of DEP (Fig. 3I,J). Therefore, constitutive depletion of TNF- α and RANTES in the lungs of db/db mice may result in the attenuation of transmigration and survival of eosinophils in our model.

Numerous *in vitro* and *in vivo* studies have demonstrated the essential roles of cell adhesion molecules in eosinophil adhesion

and transmigration. To evaluate the role of cell adhesion molecules in eosinophil migration into the airways, we evaluated soluble ICAM-1 expression in the lungs. DEP exposure particularly elevated soluble ICAM-1 levels, and to a greater extent in db/+m mice than in db/db mice. The decrease in ICAM-1 in the lungs of db/db mice may also contribute to the attenuation of eosinophil transmigration into the airways.

In the present study, DEP exposure increased neutrophilic infiltration into db/db mouse lungs, which was paralleled by the protein expression of MCP-1, KC, MIP-1 α , IL-6 and IL-1 β . KC, MIP-1 α (neutrophil chemoattractants), IL-6 and IL-1 β (neutrophilic cytokines) are known to be associated with neutrophilic inflammation in animal models (Tsai *et al.*, 1998; Yoshida *et al.*, 2001). Zhu *et al.* have reported that ozone-induced acute lung injury is consistent with the increases in neutrophils and IL-6 and KC levels in BALF (Zhu *et al.*, 2010). MCP-1 is critical for the recruitment of monocytes and causes neutrophil recruitment directly (Balamayooran *et al.*, 2011). Our previous report suggested that intratracheal administration of DEP aggravates lipopolysaccharide-related acute lung injury in mice, which is characterized by neutrophil influx, interstitial edema and an increase in proinflammatory molecules such as IL-1 β , MCP-1, KC and MIP-1 α (Takano *et al.*, 2002). These results suggest that DEP may result in neutrophilic airway inflammation via secretion of chemoattractants rather than in eosinophilic inflammation in obese individuals. On the other hand, a recent report has shown that lipopolysaccharide-induced acute lung injury, such as the early phase of neutrophil recruitment and neutrophil cytokine responses, is attenuated in db/db or diet-induced obese mice (Kordonowy *et al.*, 2012). These paradoxical results may be due to the differences between acute and chronic responses; however, further investigations are needed to define the mechanisms of neutrophilic airway inflammation in obese subjects.

Obesity is generally found to be more consistently associated with asthma in women than men (Boulet, 2013). Obese women seem to have a higher risk of developing asthma suggesting a possible influence of sex hormones on this relationship. Jensen *et al.* (2013) reported that the prevalence of non-eosinophilic asthma is higher in obese females than in obese males. Another report described that obese females with asthma enhance neutrophilic airway inflammation (Scott *et al.*, 2011). These results may help to interpret our current data observed in female db/db mice. The association between obesity and asthma requires further investigation, including sex differences.

Conclusion

This study demonstrated that DEP exposure results in eosinophilic airway inflammation via generation of Th2 cytokines, chemokines and soluble ICAM-1 in db/+m mice, whereas in db/db mice, there was mild neutrophilic inflammation with attenuation of eosinophilic infiltration in the lungs. These findings may be explained by a decrease in Th2-type inflammation, local expression of soluble ICAM-1 and an increase in serum chemokine levels. Therefore, obesity can affect the susceptibility to DEP-related airway inflammation, presumably because of differences in systemic and local inflammatory responses between obese and lean individuals.

Conflicting Interests

The Authors did not report any conflict of interest.

Acknowledgements

The authors thank Naoko Ueki and Satomi Abe for technical assistance. The authors would like to thank Enago (www.enago.jp) for the English language review. This study was supported in part by grants from the National Institute for Environmental Studies (0708AE367 to R.Y.).

References

- Akerman MJH, Calacanis CM, Madsen MK. 2004. Relationship between asthma severity and obesity. *J. Asthma* **41**: 521–526.
- Balamayooran G, Batra S, Balamayooran T, Cai S, Jeyaseelan S. 2011. Monocyte chemoattractant protein 1 regulates pulmonary host defense via neutrophil recruitment during *Escherichia coli* infection. *Infect. Immun.* **79**: 2567–2577.
- Boulet LP. 2013. Asthma and obesity. *Clin. Exp. Allergy* **43**: 8–21.
- Breslin W, Johnston C, Strohacker K, Carpenter K, Davidson T, Moreno J, Foreyt J, McFarlin B. 2012. Obese Mexican American children have elevated MCP-1, TNF- α , monocyte concentration, and dyslipidemia. *Pediatrics* **129**: e1180–e1186.
- Brook RD. 2008. Cardiovascular effects of air pollution. *Clin. Sci. (Lond.)* **115**: 175–187.
- Brook RD, Rajagopalan S, Pope CA, 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, Holguin F, Hong Y, Luepker RV, Mittleman MA, Peters A, Siscovick D, Smith SC, Jr, Whitsel L, Kaufman JD. 2010. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation* **121**: 2331–2378.
- Calixto MC, Lintomen L, Schenka A, Saad MJ, Zanesco A, Antunes E. 2010. Obesity enhances eosinophilic inflammation in a murine model of allergic asthma. *Br. J. Pharmacol.* **159**: 617–625.
- Chiba T, Kamada Y, Saito N, Oyama H, Ueki S, Kobayashi Y, Kayaba H, Chihara J. 2005. RANTES and eotaxin enhance CD11b and CD18 expression on eosinophils from allergic patients with eosinophilia in the application of whole blood flow cytometry analysis. *Int. Arch. Allergy Immunol.* **137** (Suppl 1): 12–16.
- Dong GH, Qian Z, Liu MM, Wang D, Ren WH, Fu Q, Wang J, Simckes M, Ferguson TF, Trevathan E. 2013. Obesity enhanced respiratory health effects of ambient air pollution in Chinese children: the Seven North-eastern Cities study. *Int. J. Obes. (Lond)* **37**: 94–100.
- Dubowsky S, Suh H, Schwartz J, Coull B, Gold D. 2006. Diabetes, obesity, and hypertension may enhance associations between air pollution and markers of systemic inflammation. *Environ. Health Perspect.* **114**: 992–998.
- Field AE, Coakley EH, Must A, Spadano JL, Laird N, Dietz WH, Rimm E, Colditz GA. 2001. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. *Arch. Intern. Med.* **161**: 1581–1586.
- Goldberg MS, Burnett RT, Yale JF, Valois MF, Brook JR. 2006. Associations between ambient air pollution and daily mortality among persons with diabetes and cardiovascular disease. *Environ. Res.* **100**: 255–267.
- Grünig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, Sheppard D, Mohrs M, Donaldson DD, Locksley RM, Corry DB. 1998. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* **282**: 2261–2263.
- Haselkorn T, Fish J, Chipps B, Miller D, Chen H, Weiss S. 2009. Effect of weight change on asthma-related health outcomes in patients with severe or difficult-to-treat asthma. *Respir. Med.* **103**: 274–283.
- Horie S, Gleich GJ, Kita H. 1996. Cytokines directly induce degranulation and superoxide production from human eosinophils. *J. Allergy Clin. Immunol.* **98**: 371–381.
- Jensen M, Gibson P, Collins C, Wood L. 2013. Airway and systemic inflammation in obese children with asthma. *Eur. Respir. J.*, doi:10.1183/09031936.00124912.
- Johnston RA, Zhu M, Rivera-Sanchez YM, Lu FL, Theman TA, Flynt L, Shore SA. 2007. Allergic airway responses in obese mice. *Am. J. Respir. Crit. Care Med.* **176**: 650–658.
- Juel CT, Ali Z, Nilas L, Ulrik CS. 2012. Asthma and obesity: does weight loss improve asthma control? A systematic review. *J. Asthma Allergy.* **5**: 21–26.
- Kokuludag A, Sin A, Terzioğlu E, Saydam G, Sebik F. 2002. Elevation of serum eosinophil cationic protein, soluble tumor necrosis factor receptors and soluble intercellular adhesion molecule-1 levels in acute bronchial asthma. *J. Invest. Allergol. Clin. Immunol.* **12**: 211–214.

- Kordonowy LL, Burg E, Lenox CC, Gauthier LM, Petty JM, Antkowiak M, Palvinskaya T, Ubags N, Rincón M, Dixon AE, Vernooy JH, Fessler MB, Poynter ME, Suratt BT. 2012. Obesity is associated with neutrophil dysfunction and attenuation of murine acute lung injury. *Am. J. Respir. Cell Mol. Biol.* **47**: 120–127.
- Lukacs NW, Strieter RM, Chensue SW, Widmer M, Kunkel SL. 1995. TNF-alpha mediates recruitment of neutrophils and eosinophils during airway inflammation. *J. Immunol.* **154**: 5411–5417.
- Mancuso P. 2009. Obesity and lung inflammation. *J. Appl. Physiol.* **108**: 722–728.
- Maniscalco M, Zedda A, Faraone S, Cerbone M, Cristiano S, Giardiello C, Sofia M. 2008. Weight loss and asthma control in severely obese asthmatic females. *Respir. Med.* **102**: 102–108.
- McClean KM, Kee F, Young IS, Elborn JS. 2008. Obesity and the lung: 1. Epidemiology. *Thorax* **63**: 649–654.
- Miller KA, Siscovick DS, Sheppard L, Shepherd K, Sullivan JH, Anderson GL, Kaufman JD. 2007. Long-term exposure to air pollution and incidence of cardiovascular events in women. *N. Engl. J. Med.* **356**: 447–458.
- Ok I-S, Kim S-H, Kim B-K, Lee J-C, Lee Y-C. 2009. Pinellia ternata, Citrus reticulata, and their combinational prescription inhibit eosinophil infiltration and airway hyperresponsiveness by suppressing CCR3+ and Th2 cytokines production in the ovalbumin-induced asthma model. *Mediators Inflamm.* **2009**: 413270–413270.
- O'Neill MS, Veves A, Sarnat JA, Zanobetti A, Gold DR, Economides PA, Horton ES, Schwartz J. 2007. Air pollution and inflammation in type 2 diabetes: a mechanism for susceptibility. *Occup. Environ. Med.* **64**: 373–379.
- Pope CA, 3rd, Burnett RT, Thurston GD, Thun MJ, Calle EE, Krewski D, Godleski JJ. 2004. Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease. *Circulation* **109**: 71–77.
- Sagai M, Saito H, Ichinose T, Kodama M, Mori Y. 1993. Biological effects of diesel exhaust particles. I. In vitro production of superoxide and in vivo toxicity in mouse. *Free Radic. Biol. Med.* **14**: 37–47.
- Sagai M, Furuyama A, Ichinose T. 1996. Biological effects of diesel exhaust particles (DEP). III. Pathogenesis of asthma like symptoms in mice. *Free Radic. Biol. Med.* **21**: 199–209.
- Sanderson CJ. 1992. Interleukin-5, eosinophils, and disease. *Blood* **79**: 3101–3109.
- Scott HA, Gibson PG, Garg ML, Wood LG. 2011. Airway inflammation is augmented by obesity and fatty acids in asthma. *Eur. Respir. J.* **38**: 594–602.
- Sideleva O, Suratt B, Black K, Tharp W, Pratley R, Forgione P, Dienz O, Irvin C, Dixon A. 2012. Obesity and asthma: an inflammatory disease of adipose tissue not the airway. *Am. J. Respir. Crit. Care Med.* **186**: 598–605.
- Sun Q, Yue P, Deiuliis JA, Lumeng CN, Kampfrath T, Mikolaj MB, Cai Y, Ostrowski MC, Lu B, Parthasarathy S, Brook RD, Moffatt-Bruce SD, Chen LC, Rajagopalan S. 2009. Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. *Circulation* **119**: 538–546.
- Takano H, Yanagisawa R, Ichinose T, Sadakane K, Yoshino S, Yoshikawa T, Morita M. 2002. Diesel exhaust particles enhance lung injury related to bacterial endotoxin through expression of proinflammatory cytokines, chemokines, and intercellular adhesion molecule-1. *Am. J. Respir. Crit. Care Med.* **165**: 1329–1335.
- Thomson CC, Clark S, Camargo CA. 2003. Body mass index and asthma severity among adults presenting to the emergency department. *Chest* **124**: 795–802.
- Tomaru M, Takano H, Inoue K, Yanagisawa R, Osakabe N, Yasuda A, Shimada A, Kato Y, Uematsu H. 2007. Pulmonary exposure to diesel exhaust particles enhances fatty change of the liver in obese diabetic mice. *Int. J. Mol. Med.* **19**: 17–22.
- Tsai WC, Strieter RM, Wilkowski JM, Bucknell KA, Burdick MD, Lira SA, Standiford TJ. 1998. Lung-specific transgenic expression of KC enhances resistance to Klebsiella pneumoniae in mice. *J. Immunol.* **161**: 2435–2440.
- Tsukahara K, Nakao A, Hiraguri M, Mii S, Mamura M, Saito Y, Iwamoto I. 1999. Tumor necrosis factor-alpha mediates antiapoptotic signals partially via p38 MAP kinase activation in human eosinophils. *Int. Arch. Allergy Immunol.* **120** (Suppl 1): 54–59.
- de Vries A, Hazlewood L, Fitch PM, Seckl JR, Foster P, Howie SE. 2009. High-fat feeding redirects cytokine responses and decreases allergic airway eosinophilia. *Clin. Exp. Allergy* **39**: 731–739.
- Wills Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, Donaldson DD. 1998. Interleukin-13: central mediator of allergic asthma. *Science* **282**: 2258–2261.
- Xu X, Yavar Z, Verdin M, Ying Z, Mihai G, Kampfrath T, Wang A, Zhong M, Lippmann M, Chen LC, Rajagopalan S, Sun Q. 2010. Effect of early particulate air pollution exposure on obesity in mice: role of p47phox. *Arterioscler. Thromb. Vasc. Biol.* **30**: 2518–2527.
- Yan Y-H, Chou C, Lee C-T, Liu J-Y, Cheng T-J. 2011. Enhanced insulin resistance in diet-induced obese rats exposed to fine particles by instillation. *Inhal. Toxicol.* **23**: 507–519.
- Yoshida K, Matsumoto T, Tateda K, Uchida K, Tsujimoto S, Iwakura Y, Yamaguchi K. 2001. Protection against pulmonary infection with Klebsiella pneumoniae in mice by interferon-gamma through activation of phagocytic cells and stimulation of production of other cytokines. *J. Med. Microbiol.* **50**: 959–964.
- Zanobetti A, Schwartz J. 2001. Are diabetics more susceptible to the health effects of airborne particles? *Am. J. Respir. Crit. Care Med.* **164**: 831–833.
- Zanobetti A, Schwartz J. 2002. Cardiovascular damage by airborne particles: are diabetics more susceptible? *Epidemiology* **13**: 588–592.
- Zhu M, Hug C, Kasahara D, Johnston R, Williams A, Verbout N, Si H, Jastrab J, Srivastava A, Williams E, Ranscht B, Shore S. 2010. Impact of adiponectin deficiency on pulmonary responses to acute ozone exposure in mice. *Am. J. Respir. Cell Mol. Biol.* **43**: 487–497.