Adiponectin-deficient mice are protected against tobacco-induced inflammation and increased emphysema

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Miller M, Pham A, Cho JY, Rosenthal P, Broide DH. Adiponectin-deficient mice are protected against tobacco-induced inflammation and increased emphysema. Am J Physiol Lung Cell Mol Physiol 299: L834–L842, 2010. First published October 8, 2010; doi:10.1152/ajplung.00326.2009.—Adiponectin is a cytokine with both proinflammatory and anti-inflammatory properties that is expressed in epithelial cells in the airway in chronic obstructive pulmonary disease- emphysema (COPD-E). To determine whether adiponectin modulates levels of lung inflammation in tobacco smoke-induced COPD-E, we used a mouse model of COPD-E in which either adiponectin-deficient or wild-type (WT) mice were exposed to tobacco smoke for 6 mo. Outcomes associated with tobacco smoke-induced COPD-E were quantitated including lung inflammation [bronchoalveolar lavage (BAL) and total and differential cell count], lung mediators of inflammation (cytokines and chemokines), air space enlargement (i.e., linear intercept), and lung function (tissue elastance) in the different groups of mice. Whereas exposure of WT mice to tobacco smoke for 6 mo induced significant lung inflammation (increased total BAL cells, neutrophils, and macrophages), adiponectin-deficient mice had minimal BAL inflammation when exposed to tobacco smoke for 6 mo. In addition, whereas chronic tobacco-exposed WT mice had significantly increased levels of lung mediators of inflammation [i.e., TNF-α, keratinocyte-derived chemokine (KC), and adiponectin] as well as significantly increased air space enlargement (increased linear intercept) and decreased tissue elastance, exposure of adiponectin-deficient mice to chronic tobacco smoke resulted in no further increase in lung mediators, air space enlargement, or tissue elastance. In vitro studies demonstrated that BAL macrophages derived from adiponectin-deficient mice incubated in media containing tobacco smoke expressed minimal TNF-α or KC compared with BAL macrophages from WT mice. These studies suggest that adiponectin plays an important proinflammatory role in tobacco smoke-induced COPD-E.

macrophage; neutrophil; tumor necrosis factor-α; keratinocyte-derived chemokine

ADIPONECTIN IS AN APPROXIMATELY 28-kDa molecular mass cytokine that is highly expressed in adipocytes and has been extensively investigated for its anti-diabetic and antiatherogenic effects (6, 17, 19). Studies of adiponectin in diabetes and atherosclerosis have demonstrated that release of adiponectin from adipocytes is an endogenous anti-inflammatory pathway in the metabolic syndrome, diabetes, and atherosclerosis (6, 17, 19). We have recently demonstrated that adiponectin is highly expressed in bronchoalveolar lavage (BAL) in the emphysematous form of chronic obstructive pulmonary disease (COPD-E), suggesting that adiponectin may play a previously unrecognized role in COPD-E (12). In these studies, BAL fluids from subjects with COPD-E and control subjects were screened, and adiponectin was noted to be highly expressed in COPD-E but not in control subjects using an adiponectin ELISA assay (12). In addition, we demonstrated that adiponectin is highly expressed in airway epithelium (a cell type not previously appreciated to express adiponectin) (12) in COPD-E. The expression of adiponectin by airway epithelial cells in COPD-E has the potential to elicit functional responses in the lung through either autocrine pathways [airway epithelial cells express adiponectin R1 (AdipoR1) receptors that are functional and release IL-8 on incubation with adiponectin] (12) or through paracrine pathways by adiponectin released from airway epithelium binding to adiponectin receptors expressed by other cell types in the lung (i.e., macrophages express AdipoR1) (2). In previous studies using wild-type (WT) mice in a model of COPD-E, we demonstrated that tobacco smoke exposure induced both evidence of COPD-E as well as increased levels of adiponectin in BAL fluid and increased adiponectin expression by airway epithelial cells (12). In this study, we have used adiponectin-deficient mice in a model of tobacco smoke-induced emphysema to investigate whether tobacco smoke-induced expression of adiponectin in COPD-E plays a role in lung inflammation and/or disease pathogenesis. In studies unrelated to the lung, adiponectin has predominantly been noted to exert anti-inflammatory effects (6, 17, 19), although some studies have demonstrated proinflammatory effects of adiponectin (5, 15). As macrophages are considered to be a key proinflammatory cell involved in the pathogenesis of COPD (1), the known anti-inflammatory influence of adiponectin on macrophages (which express high levels of AdipoR1) could play an important role in inhibiting macrophage activation in COPD-E. Adiponectin is known to inhibit the synthesis of macrophage-derived proinflammatory cytokines (i.e., TNF-α) (20) as well as induce the production of macrophage-derived anti-inflammatory cytokines [i.e., IL-10 and IL-1 receptor antagonist (IL-1Ra)] (6, 17, 19). In contrast to the aforementioned studies demonstrating an anti-inflammatory role for adiponectin, the ability of adiponectin to stimulate airway epithelial cells to express chemokines such as the neutrophil chemoattractant IL-8 (12) also provides evidence that adiponectin receptors expressed by airway epithelial cells are functional and may exert potential proinflammatory effects in the airway. This observation is consistent with several studies indicating that adiponectin may also exert proinflammatory effects (5, 15).

Although initial studies of adiponectin-deficient mice did not demonstrate a phenotype (6, 17, 19), more recent studies of the lungs of adiponectin-deficient mice not exposed to tobacco smoke have demonstrated that, at 6–18 days of age, adiponectin-deficient mice have air space enlargement (without increased numbers of total BAL cells, macrophages, neutrophils,
or lymphocytes) (16) suggesting a role for adiponectin in lung development (18). In the absence of tobacco smoke exposure, the size of the air spaces in adiponectin-deficient mice decreases from 6 days to 3 mo of age, although not to levels observed in WT mice (16). Based on our observation that adiponectin is induced to be expressed in airway epithelial cells in vivo in WT mice exposed to tobacco smoke, we investigated whether adiponectin-deficient mice exposed to tobacco smoke for 6 mo would have enhanced levels of airway inflammation and COPD-E compared with WT mice. Interestingly, our results demonstrate the contrary, that adiponectin-deficient mice have significantly reduced levels of airway inflammation on exposure to tobacco smoke as well as no increased levels of tobacco smoke-induced COPD-E, suggesting that adiponectin expression in tobacco smoke-exposed WT mice normally plays a significant proinflammatory role in the lung, which contrasts with the well-described anti-inflammatory pathway of adiponectin in the metabolic syndrome, diabetes, and atherosclerosis (6, 17, 19).

METHODS

Mouse Model of COPD-E

In this study, we used 8- to 10-wk-old adiponectin-deficient mice (kindly provided by Philipp E. Scherer, PhD, the University of Texas Southwestern Medical Center, Dallas, TX) (14) and WT mice on a background of C57 black. To induce COPD-E, different groups of mice (n = 12/group) were exposed for 6 mo to either tobacco smoke or room air as previously described by this laboratory (12). In brief, mice were subjected to chronic tobacco smoke exposure (mainstream tobacco smoke from 2 cigarettes/day, 5 days/wk) generated by burning 2R4F reference cigarettes (2.45 mg nicotine/cigarette; purchased from Tobacco and Health Research Institute, University of Kentucky, Lexington, KY) using a smoking machine (McChesney-Jaeger CSM-SSM Single Cigarette Smoking Machine; CH Technologies, Westwood, NJ) regulated by programmable controls provided with JASPER Windows 9x/2000 software over RS-232 communication ports (CH Technologies). The smoking machine settings delivered a puff volume of 35 ml, a frequency of 1 puff/25 s, and a total burning time for 1 cigarette of 35 ml, a measurement of the linear intercept (Lm) in lung sections stained with hematoxylin and eosin. The Lm, a measurement of the distance between the opposing walls of a single alveolus (12), was quantitated in micrometers using a light microscope (Leica DMLS; Leica Microsystems, Depew, NY) attached to an image-analysis system (Image-Pro Plus; Media Cybernetics, Silver Spring, MD). In each mouse, the Lm was quantitated in 10 alveoli/field, in each of 5 random lung fields, at ×20 magnification.

Quantitation of Lung Mediators of Inflammation

Levels of lung adiponectin as well as levels of lung mediators of inflammation expressed in COPD-E [i.e., TNF-α and keratinocyte-derived chemokine (KC)] (1) and known to be regulated by adiponectin (i.e., TNF-α and KC) (6, 17, 19) were measured in lung by ELISA (R&D Systems, Minneapolis, MN). To quantitate levels of mediators in the lung, lung tissue was homogenized in lysis buffer, and lung supernatants (obtained by centrifugation at 10,000 g for 20 min) were passaged through a 0.8-μm pore size filter and frozen at −80°C in polypropylene tubes until used in assays as previously described by this laboratory (9). Lung protein levels were quantitated using a BCA protein assay (Pierce, Rockford, IL). Results are expressed as picograms of mediator per milligram total lung protein.

Lung Elastance

Lung elastance was assessed 24 h after the final tobacco smoke exposure in intubated and ventilated mice (flexiVent ventilator; SCI-REQ, Montréal, Québec, Canada) anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) intraperitoneally as previously described (12). Tissue elastance and dynamic elastance (centimeters of water per milliliter) were determined using SCIREQ software. The ventilator settings were 10 ml/kg tidal volume and 150/min frequency. Lung elastance was measured at settings of positive end-expiratory pressure (PEEP) ranging from 3 to 9 cmH2O.

Effect of Tobacco Smoke on In Vitro BAL Macrophage Cytokine Expression by WT vs. Adiponectin-Deficient Cells

BAL macrophages were obtained from WT and adiponectin-deficient mice (8 wk old) by inserting a 20-gauge catheter into the trachea and lavaging the lungs with 1 ml of PBS/EDTA, which was repeated on 5 occasions (4). The BAL fluid was then centrifuged at 1,000 rpm for 5 min. The number of cells in the BAL cell pellet was counted, and the cells were resuspended in RPMI 1640 media (Invitrogen, Carlsbad, CA) containing 10% HI-FBS (Cellgro, Manassas, VA), 2 mM L-glutamine (Sigma), 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen). BAL cells were plated 4 × 10^5/well in a final volume of 500 μl of media in a flat-bottom, 48-well plate (Corning, Lowell, MA). The BAL cell cultures were treated with media containing either tobacco smoke (13) or LPS (100 ng/ml) for 24 h at 37°C. Adherent BAL cell supernatants were assayed by ELISA for TNF-α (31 pg/ml assay sensitivity) or KC (16 pg/ml assay sensitivity; all from R&D Systems). To generate media containing tobacco smoke, one 2R4F reference cigarette was smoked for 5 min using a smoking machine as described above for in vivo

Quantitation of Air Space Enlargement

Lungs in the different groups of mice were equivalently inflated with a similar volume of 4% paraformaldehyde solution (Sigma, St. Louis, MO) to preserve the pulmonary architecture as previously described by this laboratory (12). Inflated lungs were fixed for 48 h before embedding in paraffin. Six-micrometer-thin sections of lung from each paraffin block were deparaffinized with xylene and hydrated in ethanol and PBS at pH 7.4. The extent of COPD-E was assessed by quantitating the linear intercept (Lm) in lung sections stained with hematoxylin and eosin. The Lm, a measurement of the distance between the opposing walls of a single alveolus (12), was quantitated in micrometers using a light microscope (Leica DMLS; Leica Microsystems, Depew, NY) attached to an image-analysis system (Image-Pro Plus; Media Cybernetics, Silver Spring, MD). In each mouse, the Lm was quantitated in 10 alveoli/field, in each of 5 random lung fields, at ×20 magnification.
tobacco smoke exposure experiments. The tubing from the tobacco smoking machine was immersed in 25 ml of complete RPMI 1640 media using a previously described method to generate media containing tobacco smoke for in vitro experiments (4). The media containing tobacco smoke was then filtered through a 0.2-μm filter to remove any particles (4). Nicotine levels in the media containing tobacco smoke were measured by ELISA (Bio-Quant, San Diego, CA) and noted to be 12 μg/ml. LPS levels in the media containing tobacco smoke were measured by limulus amebocyte lysate (LAL) assay according to the manufacturer’s instructions (0.01 ng/ml sensitivity of LPS assay; Lonza, Walkersville, MD).

Fig. 1. Levels of bronchoalveolar lavage (BAL) total cells, neutrophils, and alveolar macrophages in adiponectin-deficient and wild-type (WT) mice exposed to chronic tobacco smoke. Different groups of adiponectin-deficient or WT mice were exposed to room air or tobacco smoke for 6 mo (n = 12 mice/group). The number of total BAL cells (A), total BAL macrophages (B), and total BAL neutrophils (C) were quantitated in Wright-Giemsa-stained BAL cytopsins. In WT mice, tobacco smoke exposure significantly increased levels of total BAL cells (P < 0.0001; A), total BAL macrophages (P = 0.0003; B), and total BAL neutrophils (P < 0.0001; C) compared with non-tobacco-exposed WT mice. Adiponectin-deficient mice exposed to tobacco smoke had significantly reduced levels of total BAL cells (P = 0.002; A), total BAL macrophages (P = 0.001; B), and total BAL neutrophils (P = 0.001; C) compared with tobacco-exposed WT mice. Adipo, adiponectin; KO, knockout.

Fig. 2. Levels of lung mediators of inflammation [i.e., TNF-α, keratinocyte-derived chemokine (KC), and adiponectin] in adiponectin-deficient and WT mice exposed to tobacco smoke. Levels of lung mediators of inflammation (i.e., adiponectin, TNF-α, and KC) were measured by ELISA in lung tissue derived from adiponectin-deficient and WT mice exposed to room air or tobacco smoke for 6 mo (n = 12 mice/group). Results are expressed as picograms of mediator per milligram total lung protein. WT mice exposed to tobacco smoke had a significant increase in levels of lung adiponectin (P < 0.01 vs. WT no tobacco smoke; A), lung KC (P < 0.003 vs. WT no tobacco smoke; B), and lung TNF-α (P < 0.05 vs. WT no tobacco smoke; C). In contrast, adiponectin-deficient mice exposed to chronic tobacco smoke had no significant increase in lung adiponectin (A), lung KC (B), or lung TNF-α [C; P = not significant (NS) vs. adiponectin-deficient no tobacco smoke].
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In vitro. In these experiments, we incubated purified preparations of bone marrow-derived macrophages (10⁶ cells/500 μl) from either WT or adiponectin-deficient mice with 30 μg of globular adiponectin (R&D Systems) for 24 h at 37°C. Bone marrow-derived macrophages were generated as previously described by this laboratory (8). Supernatants were assayed for KC by ELISA.

In vivo. In these experiments, WT or adiponectin-deficient mice (n = 3 mice/group) were administered 30 μg of intranasal globular adiponectin under isoflurane anesthesia, and levels of BAL neutrophils and BAL fluid KC were quantitated 8 h later.

**Statistical Analysis**

Results in the different groups were compared by ANOVA using the nonparametric Kruskal-Wallis test followed by posttesting using Dunn multiple comparison of means. All results are presented as means ± SE. A statistical software package (GraphPad Prism, San Diego, CA) was used for the analysis. P values of <0.05 were considered statistically significant.

**RESULTS**

**BAL Inflammation in Tobacco Smoke-Exposed WT vs. Adiponectin-Deficient Mice**

Exposure of WT mice to tobacco smoke induced an increase in the total number of BAL cells (P < 0.0001; Fig. 1A), BAL macrophages (P = 0.0003; Fig. 1B), and BAL neutrophils (P < 0.0001; Fig. 1C; WT tobacco smoke vs. WT no tobacco smoke).

In contrast to WT mice, adiponectin-deficient mice exposed to tobacco smoke had reduced levels of total BAL cells (3.0 ± 0.4 vs. 6.5 ± 0.9 total BAL cells × 10⁴; adiponectin-deficient mice tobacco smoke vs. WT tobacco smoke; P = 0.002; Fig. 1A), BAL macrophages (2.6 ± 0.4 vs. 6.3 ± 0.9 total BAL macrophages × 10⁴; adiponectin-deficient mice tobacco smoke vs. WT tobacco smoke; P = 0.001; Fig. 1B), and BAL neutrophils (0.4 ± 0.1 vs. 2.1 ± 0.4 total BAL neutrophils × 10⁴; adiponectin-deficient mice tobacco smoke vs. WT tobacco smoke; P = 0.001; Fig. 1C) compared with WT mice exposed to tobacco smoke.

**Lung Mediators of Inflammation in Tobacco Smoke-Exposed WT vs. Adiponectin-Deficient Mice**

Levels of adiponectin were increased in the lungs of WT mice exposed to tobacco smoke for 6 mo (1,291 ± 152 vs. 722 ± 109 pg adiponectin/mg lung protein; WT mice tobacco smoke vs. WT mice no tobacco smoke; P = 0.01; Fig. 2A). In contrast, neither tobacco-exposed nor non-tobacco-exposed,
adiponectin-deficient mice expressed adiponectin in the lung (Fig. 2A).

In WT mice, exposure to tobacco smoke increased lung levels of KC ($P = 0.003$; Fig. 2B) and TNF-$\alpha$ ($P = 0.03$; Fig. 2C; WT mice tobacco smoke vs. WT mice no tobacco smoke). In contrast, in adiponectin-deficient mice, there was no difference between tobacco-exposed and non-tobacco-exposed mice in levels of KC ($P = $ not significant (NS); Fig. 2D) or TNF-$\alpha$ ($P = $ NS; Fig. 2C; adiponectin-deficient mice tobacco smoke vs. adiponectin-deficient mice no tobacco smoke).

Air Space Enlargement in Tobacco Smoke-Exposed WT vs. Adiponectin-Deficient Mice

WT mice exposed to tobacco smoke for 6 mo had evidence of emphysema characterized histologically by destruction of the alveolus (Fig. 3A) as well as by measurement of an increased $L_m$ ($28.0 \pm 0.3 \mu m$ vs. $19.4 \pm 0.2 \mu m$; WT mice exposed to tobacco smoke for 6 mo vs. WT mice no exposure to tobacco smoke for 6 mo; $P < 0.0001$; Fig. 3B).

Adiponectin-deficient mice exposed to tobacco smoke for 6 mo had no increase in $L_m$ compared with adiponectin-deficient mice not exposed to tobacco smoke ($25.3 \pm 0.3$ vs. $24.8 \pm 0.4 \mu m$; adiponectin-deficient mice exposed to tobacco smoke for 6 mo vs. adiponectin-deficient mice no exposure to tobacco smoke for 6 mo; $P = $ NS; Fig. 3B).

Adiponectin-deficient mice not exposed to tobacco smoke have previously been noted to have an increased $L_m$ compared with WT mice (as early as 18 days of age, which persisted through 3 mo of age) (16), which we demonstrated to be present at 6 days of age and persisted to 8 mo of age (Fig. 3, C and D). Although the $L_m$ is increased in adiponectin-deficient mice not exposed to tobacco from 6 days to at least 8 mo of age, the $L_m$ decreases from 6 days to 8 mo of age ($33.6 \pm 0.7$ vs. $24.8 \pm 0.4 \mu m$; adiponectin-deficient mice not exposed to tobacco smoke 6 days vs. adiponectin-deficient mice no exposure to tobacco smoke age 8 mo; $P < 0.0001$; Fig. 3D). The $L_m$ also decreases in non-tobacco-exposed WT mice from 6 days to 6 mo of age (Fig. 3D).

Lung Elastance in Tobacco Smoke-Exposed WT vs. Adiponectin-Deficient Mice

Lung function in WT mice exposed to tobacco smoke for 6 mo demonstrated decreased tissue elastance and dynamic elastance compared with WT mice not exposed to chronic tobacco smoke (WT mice exposed to tobacco smoke for 6 mo vs. WT mice no exposure to tobacco smoke for 6 mo; $P$ values range from $<0.05$ to $<0.0001$ for tissue elastance and dynamic elastance measured at PEEP of 1, 3, 5, 7, and 9 cmH$_2$O; Fig. 4, A and C). In contrast, chronic tobacco-exposed, adiponectin-deficient mice did not have a statistically significant change in tissue elastance or dynamic elastance when exposed to chronic tobacco smoke (adiponectin-deficient mice exposed to tobacco smoke for 6 mo vs. adiponectin-deficient mice no exposure to tobacco smoke for 6 mo; $P = $ NS for tissue elastance and dynamic elastance measured at PEEP of 1, 3, 5, 7, and 9 cmH$_2$O; Fig. 4, B and D).

Fig. 4. Lung elastance in adiponectin-deficient and WT mice exposed to tobacco smoke. WT or adiponectin-deficient mice were exposed to room air for 6 mo ($n = 12$ mice/group). Lung tissue elastance (H) and dynamic elastance (E) were assessed 24 h after the final tobacco smoke exposure in intubated and ventilated mice (flexiVent) at settings of positive end-expiratory pressure (PEEP) ranging from 3 to 9 cmH$_2$O. WT mice exposed to tobacco smoke for 6 mo demonstrated significantly decreased H (A) and E (C) compared with WT mice not exposed to chronic tobacco smoke ($P$ values range from $<0.05$ to $<0.0001$ for H measured at PEEP of 1, 3, 5, 7, and 9 cmH$_2$O). In contrast, chronic tobacco-exposed, adiponectin-deficient mice did not have a statistically significant change in H (B) or E (D) when exposed to chronic tobacco smoke ($P = $ NS for E measured at PEEP of 1, 3, 5, 7, and 9 cmH$_2$O).
Effect of Tobacco Smoke on In Vitro BAL Macrophage Cytokine Expression by WT vs. Adiponectin-Deficient Cells

Incubation of BAL macrophages from either WT or adiponectin-deficient mice with media alone did not induce release of TNF-α or KC (Fig. 5, A and C). Incubation of WT BAL macrophages with media containing tobacco smoke-induced expression of TNF-α (1,129 ± 171 vs. <31 pg/ml; \( P < 0.001; \) Fig. 5A) and KC (1,081 ± 9 vs. <16 pg/ml; \( P < 0.001; \) Fig. 5C) compared with WT BAL macrophages incubated in media alone. In contrast, incubation of BAL macrophages from adiponectin-deficient mice with media containing tobacco smoke did not induce release of TNF-α (Fig. 5A) and only minimal amounts of KC (Fig. 5C) that were not statistically different from levels in adiponectin-deficient BAL macrophages cultured in media alone. Adiponectin-deficient BAL macrophages were not unresponsive to all stimuli, as they generated similar increased levels as WT mice of TNF-α (1,672 ± 63 vs. 1,367 ± 210 pg/ml; \( P = \) NS; Fig. 5B) and KC (1,147 ± 97 vs. 1,212 ± 93 pg/ml; \( P = \) NS; Fig. 5D) when stimulated with LPS. Levels of LPS in the tobacco smoking media (0.1 ng/ml) were lower than concentrations of LPS used to stimulate macrophages in vitro (100 ng/ml).

Effect of Exogenous Adiponectin on Inflammation In Vitro and In Vivo

These studies demonstrated that adiponectin had proinflammatory properties inducing KC release from bone marrow-derived macrophages derived from both WT and adiponectin-deficient mice in vitro (Fig. 6A). Adiponectin-deficient macrophages tended to release more KC than WT macrophages incubated with adiponectin, but this was not statistically significant. Similarly, in vivo studies demonstrated that administration of adiponectin was proinflammatory inducing a BAL neutrophil response in both WT and adiponectin-deficient mice (Fig. 6B). As with the in vitro studies, in vivo studies demonstrated that adiponectin-deficient mice had a greater BAL neutrophil and KC response compared with WT mice to exogenous adiponectin, although this trend was not statistically significant (Fig. 6, B and C).

DISCUSSION

In this study, we have demonstrated that adiponectin is induced in the lung in a model of tobacco smoke-induced COPD-E and plays a significant role in mediating BAL inflammation in a mouse model of COPD-E. Levels of total BAL cells as well as levels of BAL cells considered important to the pathogenesis of COPD-E (i.e., macrophages and neutrophils) (1) were significantly lower in adiponectin-deficient mice compared with WT mice exposed to tobacco smoke for 6 mo. Indeed, levels of total BAL cells as well as total BAL macrophages and total BAL neutrophils in tobacco smoke-exposed, adiponectin-deficient mice were reduced to levels very similar to non-tobacco-exposed, adiponectin-deficient mice. One of the potential mechanisms by which adiponectin may contribute...
to airway inflammation is by influencing levels of mediators known to be important to recruitment of neutrophils and mononuclear cells to the airway and also known to be expressed in COPD-E (i.e., IL-8 and TNF-α) (1). We demonstrated that WT mice with tobacco smoke-induced COPD-E had significantly increased levels of lung KC (murine equivalent of IL-8) (7) as well as significantly increased levels of lung TNF-α compared with WT mice not exposed to tobacco smoke, whereas adiponectin-deficient mice exposed to tobacco smoke had no significant increase in levels of lung KC or lung TNF-α compared with adiponectin-deficient mice not exposed to tobacco smoke. Interestingly, in vitro studies with BAL macrophages derived from WT and adiponectin-deficient mice demonstrated that tobacco smoke induced significantly increased levels of TNF-α and KC in WT mice but did not do so in BAL macrophages derived from adiponectin-deficient mice providing in vitro support to our in vivo observations. BAL macrophages from adiponectin-deficient mice were not unresponsive to all stimuli as LPS induced similar levels of TNF-α and KC in adiponectin-deficient BAL macrophages as in WT BAL macrophages. The potential importance of IL-8 and TNF-α to the pathogenesis of COPD is suggested from studies demonstrating increased levels of IL-8 and TNF-α expression in COPD (1) as well as studies in TNF receptor-deficient mice, which have significantly reduced levels of emphysema and inflammation (e.g., neutrophils and macrophages) on chronic exposure to tobacco smoke (3). Although the significantly reduced levels of lung inflammation in tobacco-exposed, adiponectin-deficient mice are associated with reduced lung KC and TNF-α responses in the lungs in tobacco-exposed, adiponectin-deficient mice, further studies are needed to determine whether there is a causal relationship between the reduced levels of these mediators and the reduced lung inflammatory response in tobacco-exposed, adiponectin-deficient mice. As we investigated a chronic 6-mo response to cigarette smoke exposure in adiponectin-deficient mice, further studies are needed to determine whether responses of these mice to acute exposure to tobacco smoke differs from chronic exposure as the lungs might adapt differently over time to the chosen protocol.

In previous studies, we have examined which cell types express adiponectin in COPD-E (12). Although macrophages are a known source of adiponectin (6, 17, 19), we (12) have previously demonstrated in a mouse model of tobacco smoke-induced COPD-E as well as in human subjects with COPD-E that airway epithelial cells are a key cell type immunostaining positive for adiponectin. We (12) have also used lung epithelial cell lines in vitro to confirm that epithelial cells express adiponectin mRNA as well as to demonstrate that adiponectin is inducible in epithelial cells in response to stimulation with cytokines such as TNF-α. Thus TNF-α released into the airway in tobacco smoke-exposed WT mice with COPD-E may induce lung epithelial cells to express adiponectin. Lung epithelial cells not only express adiponectin following cytokine stimulation, but also respond to adiponectin by releasing proinflammatory mediators such as IL-8 (12). There are two adiponectin receptor subtypes (i.e., AdipoR1 and AdipoR2) that share 66% amino acid identity (17). Lung epithelial cells express functional AdipoR1 receptors that, when stimulated in vitro with adiponectin, release significant amounts of IL-8 (12). Transfection of lung epithelial cells in vitro with AdipoR1-specific small interfering RNA (siRNA) inhibits the ability of adiponectin to induce IL-8 release from lung epithelial cells (12). Thus WT mice exposed to chronic tobacco smoke express adiponectin, which may through autocrine pathways bind to AdipoR1 expressed by lung epithelial cells and induce release of neutrophil chemoattractants such as KC. In contrast, adiponectin-deficient mice are not able to express adiponectin, which may potentially explain the reduced lung KC and reduced neutrophil recruitment to the lungs in tobacco smoke-exposed, adiponectin-deficient mice we have observed.

In addition to exhibiting significantly reduced lung inflammation when exposed to chronic tobacco smoke exposure, adiponectin-deficient mice also had significantly reduced tobacco smoke-induced changes in air space enlargement (measured as $L_m$) as well as significantly reduced tobacco smoke-induced changes in lung function (measured as lung elastance) compared with WT mice exposed to tobacco smoke.
smoke. Adiponectin-deficient mice have enlarged air spaces compared with WT mice that are evident soon after birth (16), which suggests a role for adiponectin in alveolar development (18). Although the adiponectin-deficient mice have constitutively enlarged airways at birth, the size of the air spaces in adiponectin-deficient mice significantly decreases by ~26% from 6 days to 8 mo of age in the absence of tobacco smoke exposure. Thus one would expect that the air spaces in adiponectin-deficient mice could reach the size of the enlarged air spaces present at 6 days of age if induced to do so by a stimulus such as tobacco smoke. In addition, the lack of changed air space enlargement in adiponectin-deficient mice in response to chronic tobacco smoke exposure is also supported by the lung physiology data, which demonstrated no significant difference in lung elastance in tobacco-exposed and air-exposed, adiponectin-deficient mice. In contrast, in WT mice, chronic tobacco smoke exposure induced both significant air space enlargement and significant reductions in lung tissue elastance.

Studies with exogenous administration of adiponectin demonstrated that adiponectin has proinflammatory effects in vitro (e.g., increased KC) and in vivo (e.g., increased BAL neutrophils) with the response trending to being greater in adiponectin-deficient compared with WT mice. As adiponectin-deficient mice like WT mice express adiponectin receptors R1 and R2, they are able to respond to exogenous adiponectin. Based on the trend for increased response of adiponectin-deficient compared with WT mice to exogenous adiponectin, we considered that adaptive upregulation of adiponectin receptors in adiponectin-deficient mice could have explained these results. However, we did not find evidence of differences in levels of adiponectin R1 or R2 receptors in WT compared with adiponectin-deficient mice as assessed using immunohistochemistry (data not shown). It is still possible that adiponectin-deficient mice have an up- or downregulation of a signaling or receptor pathway that makes them trend to be more responsive than WT mice to exogenous adiponectin.

In summary, in this study, we have demonstrated that adiponectin plays an important proinflammatory role in tobacco smoke-induced COPD-E in a mouse model. This is evident from the significantly reduced lung inflammatory response (for total BAL cells, BAL neutrophils, and BAL macrophages) in chronic tobacco-exposed, adiponectin-deficient mice as well as the reduced changes in air space enlargement and tissue elastance in these mice when exposed to chronic tobacco smoke. Adiponectin-deficient mice exposed to chronic tobacco smoke have reduced levels of TNF-α and KC, which may contribute to reduced lung inflammation in these mutant mice. In vitro studies with BAL macrophages derived from adiponectin-deficient mice demonstrate that they express significantly reduced levels of TNF-α and KC when incubated with tobacco smoke containing media supporting the in vivo observations. This proinflammatory role of adiponectin in a mouse model of COPD-E contrasts with the well-established anti-inflammatory role of adiponectin in diabetes and atherosclerosis (6, 17, 19). Studies using adiponectin-deficient mice in a mouse model of ovalbumin-induced asthma have also demonstrated that such mice have increased levels of BAL eosinophils, monocytes, chemokines, and remodeling, suggesting an anti-inflammatory role for adiponectin (11). In contrast, studies in rheumatoid arthritis (5) like our studies in COPD-E suggest a proinflammatory role for adiponectin. Thus the specific immune and inflammatory response in different diseases associated with adiponectin expression (e.g., COPD-E, asthma, diabetes, atherosclerosis, and rheumatoid arthritis) may regulate which cell types express adiponectin and adiponectin receptors and as a consequence may influence whether adiponectin plays a proinflammatory (e.g., COPD-E and rheumatoid arthritis) or anti-inflammatory (e.g., asthma, diabetes, atherosclerosis, and metabolic syndrome) role in disease pathogenesis. Further studies are thus needed to determine whether adiponectin (which has been detected to be expressed in the lung in subjects with COPD-E) (5) plays a proinflammatory role in humans with COPD-E.

REFERENCES


