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Adiponectin and Functional Adiponectin Receptor 1 Are Expressed by Airway Epithelial Cells in Chronic Obstructive Pulmonary Disease

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We screened bronchoalveolar lavage (BAL) fluids from COPD-E (chronic obstructive pulmonary disease-Emphysema) and control subjects using a 120 Ab cytokine array and demonstrated that adiponectin was highly expressed in BAL in COPD-E. An adiponectin ELISA confirmed that adiponectin was highly expressed in BAL in COPD-E compared with smokers and healthy control subjects. Immunohistochemistry studies of lung sections from subjects with COPD-E demonstrated that airway epithelial cells expressed significant levels of adiponectin and adiponectin receptor (AdipoR) 1 but not AdipoR2. In vitro studies with purified populations of human lung A549 epithelial cells demonstrated that they expressed both adiponectin and AdipoR1, whereas AdipoR2 as assessed by RT-PCR, Western blot, and immunohistochemistry. Lung A549 epithelial AdipoR1 were functional as incubation with adiponectin induced release of IL-8, which was inhibited by small interfering RNA to AdipoR1. Using a mouse model of COPD, tobacco smoke exposure induced both expression and increased levels of adiponectin in BAL fluid and increased adiponectin expression by airway epithelial cells. As adiponectin expression in adipocytes is dependent upon NF-eB, we determined levels of adiponectin in tobacco smoke exposed CC10-Cre^{+/−}/Ikkβ^{−/−} mice (deficient in the ability to activate NF-eB in airway epithelium). These studies demonstrated that CC10-Cre^{+/−}/Ikkβ^{−/−} and wild-type mice had similar levels of BAL adiponectin and airway epithelial adiponectin immunostaining. Overall, these studies demonstrate the novel observation that adiponectin and functional AdipoR1 are expressed by lung epithelial cells, suggesting a potential autocrine and/or paracrine pathway for adiponectin to activate epithelial cells in COPD-E.

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease of the lung with features including, increased numbers of inflammatory cells (neutrophils, macrophages, CD8+ T lymphocytes) in the airway and parenchyma, increased levels of expression of lung chemokines and cytokines, and increased extracellular matrix components (1, 2). There are likely multiple mechanisms that mediate inflammation in COPD including a direct effect of cigarette smoke on recruitment of inflammatory cells, an indirect effect of cigarette smoke inducing epithelial cells to release inflammatory cell chemotransactants, and the effect of intercurrent infections on increasing inflammation (1–3). Neutrophils accumulate in the lung within hours of acute cigarette exposure (4). Elastase released from neutrophils mediates elastin degradation, suggesting an important role for neutrophils in emphysema (2, 4). In contrast to the rapid increase in neutrophil numbers in response to cigarette exposure, macrophage numbers increase progressively over weeks to months of cigarette smoke exposure (5). Accumulation of these inflammatory cells seems to persist to some degree indefinitely even once the inciting stimulus, cigarette smoke, has been removed (2). In addition to the presence of inflammation in emphysema, emphysema is also characterized by decreased lung elastin content and increased lung collagen content (1, 2). Serine proteases (e.g., elastase), matrix metalloproteases, and profibrotic growth factors (e.g., TGFβ) are all considered to play an important role in the structural changes induced by inflammation in emphysema.

Adiponectin, an anti-inflammatory cytokine that is highly expressed in adipocytes, has been extensively investigated for its anti-diabetic and anti-atherogenic effects (6–8) but has not yet been the focus of studies in COPD. 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 (6–8). Adiponectin was first cloned in mice (9) and subsequently cloned in humans (10) and has sequence homology with a family of proteins that are characterized by a C-terminal globular domain (6–8). Human adiponectin is a 244 amino acid protein of ~28 kDa, which shares 83% amino acid identity with mouse adiponectin (7). Adiponectin can exist either as a full length protein or as a proteolytic cleavage fragment consisting of the globular C-terminal domain (known as globular adiponectin) (6). Proteolytic enzymes released from macrophages and neutrophils are thought to mediate the cleavage of adiponectin to generate the globular fragment of adiponectin (6). The adiponectin receptors AdipoR1 and AdipoR2 have been cloned and share 66% amino acid identity (6). AdipoR1 has been detected to be expressed on human macrophages (11). AdipoR1 is most highly expressed in skeletal muscle, whereas AdipoR2 is most abundantly expressed in hepatocytes in the liver (6–8). Globular adiponectin activates adiponectin receptors (mainly AdipoR1), whereas AdipoR2 engages...
mainly full length adiponectin (12). Adiponectin receptors are predicted to be seven transmembrane spanning receptors. However, the topology of AdipoR is opposite to all other G-protein coupled receptors with N terminus internal and the C-terminal external (11). Activation of AdipoR1 and/or AdipoR2 by adiponectin stimulates the activation of intracellular cytoplasmic signaling kinases (including AMP-activated protein kinase and p38 MAPK), the nuclear transcription factor peroxisome proliferators activated receptor-α, as well as NF-κB and AP-1 (6).

In this study, we have made several novel observations regarding adiponectin including the demonstration a) that adiponectin is not only expressed in adipocytes, but is also highly expressed in airway epithelium, b) that not only adiponectin, but also adiponectin receptors are expressed by airway epithelial cells, c) that AdipoR1 on lung epithelial cells are functional and release IL-8 upon incubation with adiponectin, and d) using CC10-CreR1/IIκBβVA mice (deficient in the ability to activate NF-κB in airway epithelium) we demonstrated that adiponectin expression in airway epithelium in vivo is NF-κB independent, which differs from results of studies in adipocytes in which expression of adiponectin is NF-κB dependent (13–15). Finally, we demonstrated that adiponectin is highly expressed in the lungs of human subjects with the emphysematous form of COPD (COPD-E). In contrast, exposure to tobacco smoke in subjects without evidence of COPD (COPD-E) was not associated with the expression of adiponectin in airway epithelium in vivo.

Materials and Methods

COPD and control subjects

Study subjects with a chest computerized tomography (CT) scan diagnosis of COPD-E (n = 16) and fifteen non-COPD control subjects with a normal chest CT scan (nonsmokers n = 7 or current smokers n = 8) underwent bronchoscopy to determine their levels of biomarkers in bronchoalveolar lavage (BAL) fluid in a protocol approved by the University of California-San Diego Human Subjects Protection Committee. The COPD-E study and control subjects were recruited as part of a National Institutes of Health Biomarker study, and their clinical characteristics have previously been described in detail (16). In brief, subjects with COPD-E were recruited based on chest CT scan evidence of emphysema, which had to be present to include the subjects in the study, while control subjects had to have a normal chest CT scan. Subjects also completed pulmonary function studies, the clinical history (history of chronic cough, sputum production, dyspnea), and a standardized COPD questionnaire (i.e., St. George Questionnaire) (16). The COPD-E cohort enrolled was all subjects with GOLD Stage Ib moderate COPD (forced expiratory volume in 1 s 30–50%) with chest CT scan evidence of significant emphysema (16). As previously reported, COPD-E subjects had a significantly higher level in BAL of mediators noted to be elevated in COPD including myeloperoxidase, LTβ, and IL-8 compared with either the normal nonsmoking group or the current smoker group (16). Normal individuals were nonsmokers who had no evidence of disease on history and physical examination, and who had a normal chest CT scan, as well as normal pulmonary function. Current smokers (>20 pack year) who had no evidence of disease on history and physical examination, and who had a normal chest CT scan, as well as normal pulmonary function were also recruited (16). All the study subjects had blood cotinine levels to verify their smoking status. None of the study subjects used oral or inhaled bronchodilators and were in a clinically stable condition. For Western blots, whole A549 cell lysates (TNF-stimulated or unstimulated) were prepared using a lysis buffer (Invitrogen). The cell lysates were separated by electrophoresis on a 10% SDS-PAGE. The gels were transferred to a polyvinylidene difluoride membrane (Invitrogen), which was blocked in 5% nonfat dry milk diluted in Tris-buffered saline. Blots were incubated with either anti-adiponectin (R&D Systems), anti-AdipoR1, or anti-AdipoR2 (both AdipoR Abs from Abcam) at 4°C overnight. Washed blots were incubated for 60 min with a secondary biotinylated Ab (Vector Laboratories), washed and incubated with HRP (1/200) (R&D Systems), followed by diaminobenzidine detection. Equivalent loading of lanes in gels was confirmed by anti-GAPDH (Sigma-Aldrich).

Effect of adiponectin on lung epithelial IL-8 expression

To determine whether the AdipoR1 receptors detected on lung epithelial cells were functional, A549 lung epithelial cells were incubated with or without adiponectin (0.01, 0.5, and 1 μg/ml) for 24 h and levels of IL-8 determined in supernatants by ELISA (R&D Systems) having a sensitivity of 32.5 pg/ml.
not induce nonspecific effects on gene expression. Both siRNA were obtained from Ambion. Following transfection, A549 cells were cultured for 72 h and then serum starved for 12 h. A549 cells were then cultured in the presence or absence of adiponectin (1 μg/ml for 24 h), and supernatants were assayed for IL-8 by Elisa.

**AP-1 activation assay**

As adiponectin induces activation of the transcription factor AP-1 (comprised of c-Fos and Jun protein families) (18), we investigated whether adiponectin induced activation of c-Fos, c-Jun, and JunB in the A549 human lung epithelial cell line. In these experiments, we incubated A549 epithelial cells with adiponectin (1 μg/ml) for 4 h and measured c-Fos, c-Jun, and JunB nuclear binding activity in nuclear extracts using a TransAM AP-1 ELISA kit (Active Motif) according to the manufacturer’s protocol.

**Mouse model of COPD demonstrating adiponectin expression in airway epithelium**

To induce COPD, different groups of mice were exposed for 6 mo to either tobacco smoke (n = 19 mice) or room air (n = 20 mice). Mice (C57BL/6 8-wk-old females) were subjected to chronic inhalation exposures (mainstream tobacco smoke from two cigarettes/day, 5 days/wk) generated by burning 2R4F reference cigarettes (2.45 mg nicotine per cigarette; purchased from Tobacco Research Institute) using a smoking machine (McChesney-Jaeger CSM-SSM Single Cigarette Machine; CH Technologies USA) regulated by programmable controls provided with JASPER Windows 9×2000 software over RS-232 communication ports (CH Technologies USA). The smoking machine settings delivered a puff volume of 35 ml, a frequency of one puff every 25 s, a total burning time for one cigarette of 5 min, with no resting time between two cigarettes. The volume of the smoke chamber was 500 ml (3). The mice were exposed to the tobacco smoke in a 12 port nose-only directed flow inhalation exposure system (Jaeger-NYU 12 port). Nose ports were monitored for total suspended particulates was 173 (Jaeger-NYU 12 port). Nose ports were monitored for total suspended particulates was 173 (Jaeger-NYU 12 port).

The extent of COPD was assessed by quantitating the linear intercept (Lm) in lung sections stained with H&E. The Lm, a measurement of the distance between the opposing walls of a single alveolus (21), was quantitated in μm using a light microscope (Leica DMLS; Leica Microsystems) attached to an image-analysis system (Image-Pro Plus; Media Cybernetics). In each mouse the Lm was quantitated in 10 alveoli/field, in each of 5 random lung fields, at ×20 magnification. The number of neutrophils was quantitated in Wright-Giemsa stained BAL fluid of COPD-E and control mice. Levels of leukocyte chemotacticants KC and MIP-2 were measured in BAL fluid by ELISA and the area of adiponectin immunostaining assessed in lung airway epithelial cells by immunohistochemistry (Fig. 1). COPD-E subjects had significantly higher levels of adiponectin compared with healthy normal controls (p = 0.03). Smokers without COPD had significantly reduced levels of BAL adiponectin (p = 0.003).

**Statistical analysis**

Results in the different groups were compared by ANOVA using the non-parametric Kruskal-Wallis test followed by post-testing using Dunn’s multiple comparison of means. All results are presented as mean ± SEM. A statistical software package (Graph Pad Prism) was used for the analysis. The p values of <0.05 were considered statistically significant.

**Results**

**Detection of cytokines in BAL in COPD using a cytokine Ab array membrane**

As anticipated the cytokine Ab array detected cytokines already known to be highly expressed in BAL in COPD (i.e., IL-8, MCP-1, TNF-α). However, interestingly, one of the most highly expressed BAL cytokines detected by the array was adiponectin, a cytokine not previously reported to be expressed in lung diseases or COPD. The level of BAL adiponectin expression detected by the cytokine array was 3.45 times higher in BAL fluid in COPD compared with levels noted in healthy nonsmokers.

**Measurement of adiponectin in BAL in COPD and control study subjects by ELISA**

To confirm the cytokine array data we used an ELISA to quantitate levels of adiponectin in BAL samples of COPD-E and control subjects (Fig. 1). COPD-E subjects had significantly higher levels of adiponectin in BAL compared with healthy normal controls who were nonsmokers (6.2 ± 1.8 vs 3.3 ± 0.6 ng/ml adiponectin, p = 0.03). Interestingly, in contrast to COPD-E subjects who had increased levels of BAL adiponectin, current smokers without COPD had significantly reduced levels of BAL adiponectin (6.2 ± 1.8 vs 0.2 ± 0.1 ng/ml adiponectin, p = 0.003).

**Immunohistochemistry demonstrating epithelial adiponectin expression in COPD subjects**

To address which lung cells express adiponectin, lung tissue samples were obtained from a different cohort of COPD-E patients and non-COPD control subjects. Immunostaining of lung sections from subjects with COPD-E demonstrated significant expression of adiponectin, which was predominantly noted in airway epithelial cells (Fig. 2A). Significant adiponectin expression was not noted in the lungs from non-COPD controls. We also examined the expression of AdipoR1 and AdipoR2 in lung sections of COPD-E patients and control subjects. Airway epithelial cells of COPD-E subjects had significant levels of expression of AdipoR1 compared...
with non-COPD control subjects (Fig. 2B). We did not detect significant levels of AdipoR2 expression in lung sections from either COPD-E or non-COPD control subjects (Fig. 2C). There was no nonspecific staining of lung tissue with either anti-adiponectin, anti-AdipoR1, or anti-AdipoR2 isotype control Abs (Fig. 2).

**RT-PCR, immunohistochemistry, and Western blot demonstrating adiponectin and adiponectin receptor expression in pure populations of A549 lung epithelial cells**

To determine whether purified populations of lung epithelial cells expressed adiponectin and adiponectin receptors, we used the A549 airway epithelial cell line. A549 lung epithelial cells stimulated with TNF expressed significant levels of adiponectin as assessed by immunohistochemistry (Fig. 3A), RT-PCR (Fig. 3D), and Western blot (Fig. 3E). Unstimulated A549 lung epithelial cells did not express significant adiponectin (Fig. 3A).

We also used immunohistochemistry, Western blots, and RT-PCR to determine whether adiponectin receptors (AdipoR1 and AdipoR2) were expressed by A549 lung epithelial cells. AdipoR1 was strongly expressed by stimulated A549 lung epithelial cells as assessed by immunohistochemistry (Fig. 3B), Western blot (Fig. 3E), and RT-PCR (Fig. 3D). Unstimulated A549 lung epithelial cells did not express AdipoR1 (Fig. 3B). In contrast to the expression of AdipoR1 by A549 lung epithelial cells, neither stimulated nor unstimulated A549 lung epithelial cells expressed AdipoR2 (Fig. 3, C–E).

**Adiponectin receptors expressed on lung epithelial cells are functional**

To determine whether adiponectin receptors expressed by airway epithelial cells are functional, we incubated A549 cells with different concentration of globular adiponectin and quantitated levels of the epithelial expressed chemokine IL-8. A549 cells had dose-dependent expression of IL-8 in response to stimulation with 0.01 μg/ml adiponectin (p = 0.05 vs control), 0.5 μg/ml adiponectin (p = 0.04 vs control), and 1 μg/ml adiponectin (p < 0.001 vs control) (Fig. 4A).

**siRNA inhibition of AdipoR1 function in lung epithelial cells**

To confirm the role of AdipoR1 in mediating IL-8 release from A549 lung epithelial cells, we inhibited AdipoR1 by using RNA interference. In the absence of siRNA, adiponectin induced significant release of IL-8 from A549 cells (397.1 ± 15.5 pg/ml vs 212.4 ± 6.3 pg/ml) (adiponectin vs no adiponectin) (p = 0.05). A549 cells transfected with AdipoR1 specific siRNA, and then stimulated with adiponectin, had significantly reduced levels of IL-8 (204.8 ± 7.3 pg/ml) compared with A549 cells stimulated with adiponectin alone in the absence of transfection (397.1 ± 15.5 pg/ml) (p = 0.02), or compared with A549 cells transfected with control siRNA and stimulated with adiponectin (400.2 ± 11.9 pg/ml) (p = 0.02) (Fig. 4B).

**Mouse model of COPD demonstrating adiponectin expression in airway epithelium**

To determine whether exposure to tobacco smoke modulated levels of airway adiponectin, we exposed wild-type (WT) mice to tobacco smoke for either 1 mo (a time period that is not sufficient to induce emphysema in mice) or 6 mo (a time period that induces emphysema). WT mice exposed to tobacco smoke for 1 mo did not have evidence of emphysema as assessed by measurement of the linear intercept (Lm) (data not shown). In contrast, WT mice exposed to...
tobacco smoke for 6 mo had significant evidence of emphysema characterized histologically by destruction of the alveolus (Fig. 5B) as well as by measurement of a significantly increased Lm (53.3 ± 0.5 vs 28.7 ± 0.5 μm) (WT mice exposed to tobacco smoke for 6 mo vs WT mice no exposure to tobacco smoke for 6 mo) (p < 0.005) (Fig. 5B).

WT mice exposed to tobacco smoke for 6 mo had a significant increase in BAL neutrophils compared with WT mice that were not exposed to tobacco smoke (916.2 ± 154.2 vs 208.3 ± 14.9 BAL neutrophils) (p < 0.001) (Fig. 5C). The levels of BAL KC (4.5 ± 0.6 pg/ml vs 0 ± 0 pg/ml) (p = 0.05) and MIP-2α (17.0 ± 1.5 pg/ml vs 1.3 ± 0.8 pg/ml) (p < 0.0001) were also significantly higher in WT mice exposed to 6 mo tobacco smoke compared with WT mice that were not exposed to tobacco smoke (Fig. 5, D and E, respectively).

WT mice exposed to tobacco smoke for 1 mo had reduced levels of BAL adiponectin compared with nontobacco smoke exposed control WT mice (0.6 ± 0.1 vs 1.1 ± 0.2 ng/ml BAL adiponectin) (p = 0.001) (Fig. 5F). In contrast, WT mice that were exposed to tobacco smoke for 6 mo had significantly higher levels of BAL adiponectin compared with nontobacco smoke exposed control WT mice (4.2 ± 0.9 vs 1.1 ± 0.2 ng/ml BAL adiponectin) (p < 0.0005) (Fig. 5F) and also demonstrated significantly increased levels of expression of adiponectin in lung epithelial cells as assessed by immunohistochemistry (Fig. 5G). In contrast, nontobacco smoke exposed WT mice or WT mice exposed to short-term tobacco smoke exposure for 1 mo did not have significant levels of expression of adiponectin in airway epithelium as assessed by immunohistochemistry (data not shown).

Adiponectin expression in airway epithelium in a mouse model of COPD-E is largely NF-κB independent

Both WT mice (p = 0.03, WT tobacco smoke vs WT room air) and CC10-Cre+/IkββΔ/Δ mice (p = 0.01, CC10-Cre+/IkββΔ/Δ tobacco smoke vs CC10-Cre+/IkββΔ/Δ room air) that were exposed to tobacco smoke for 6 mo had significantly increased levels of BAL adiponectin compared with nontobacco smoke exposed control mice (Fig. 6A). Although CC10-Cre+/IkββΔ/Δ mice exposed to tobacco smoke for 6 mo had a slight decrease in BAL adiponectin compared with WT mice this was not statistically significant (Fig. 6A). Similar results were noted with immunohistochemistry demonstrating significant adiponectin expression in both CC10-Cre+/IkββΔ/Δ and WT mice exposed to tobacco smoke for 6 mo (Fig. 6, B and C). Image analysis quantitation of the area of adiponectin immunoreactivity in airway epithelial demonstrated that both WT mice (2.3 ± 0.2 vs 0.3 ± 0.1 μm²/μm area of adiponectin epithelium immunoreactivity) (WT tobacco smoke vs WT room air) (p = 0.001) and CC10-Cre+/IkββΔ/Δ (1.9 ± 0.1 vs 0.8 ± 0.1 μm²/μm area of adiponectin epithelium immunoreactivity) (CC10-Cre+/IkββΔ/Δ tobacco smoke vs CC10-Cre+/IkββΔ/Δ room air)
Airway epithelial adiponectin immunoreactivity was significantly increased in mice exposed to tobacco smoke compared with control mice (Fig. 6C). Although tobacco smoke exposure increased airway epithelial adiponectin immunoreactivity compared with nontobacco smoke exposed mice, this was not statistically significant (Fig. 6C).

**Adiponectin activates AP-1 in lung epithelial cells**

To determine whether adiponectin activates the AP-1 (c-Fos, c-Jun, and JunB) signaling pathway in human lung epithelial cells, we stimulated A549 cell line with globular adiponectin. Adiponectin significantly increased DNA binding activity for c-Fos in A549 cells (0.14 AU vs 0.08 AU; p = 0.04; adiponectin vs no adiponectin) (Fig. 7A). Adiponectin also induced c-Jun (0.10 AU vs 0.04 AU; p = 0.05; adiponectin vs no adiponectin) (Fig. 7B) and JunB (0.50 AU vs 0.26 AU; p = 0.05; adiponectin vs no adiponectin) (Fig. 7C).
inhibitory effect of tobacco smoke on adiponectin expression by airway epithelial cells that we have noted. In adipocytes, reactive oxygen species reduced the transcriptional activity of the adiponectin gene, and the antioxidant N-acetyl cysteine reverses these effects (27).

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 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 (17, 23). Thus, adiponectin expression by airway epithelial cells could have both anti-inflammatory and proinflammatory effects in the airway in COPD. Which effect is dominant in COPD will require further in vivo studies in which adiponectin is neutralized. Interestingly, a recent study demonstrated that adiponectin-deficient mice spontaneously develop emphysema in the absence of tobacco smoke exposure (28), suggesting that adiponectin may play a role in alveolar development.

The potential disease relevance of adiponectin expression by airway epithelium is also suggested from our studies demonstrating that human subjects with COPD-E, as well as mice exposed for 6 mo to tobacco smoke to develop COPD, express increased levels of adiponectin. Interestingly, short-term exposure of mice to tobacco smoke suppresses adiponectin expression. We have noted similar observations in human smokers who have suppressed levels of adiponectin. In contrast, mice exposed to long-term tobacco smoke develop COPD and this is associated with increased adiponectin expression. Similar observations are noted in subjects with Gold Stage II COPD who have increased levels of BAL adiponectin. The molecular mechanism by which tobacco smoke exposure down-regulates adiponectin expression, and the development of COPD up-regulates adiponectin expression is unknown. We hypothesize that in subjects without COPD, one or several of the 4000 constituents of tobacco smoke induces an epithelial signaling pathway to inhibit transcription of adiponectin. Oxidative stress and reactive oxygen species are known to inhibit adiponectin expression in adipocytes (6–8, 27). With the development of

FIGURE 7. A549 epithelial cells were incubated with adiponectin (1 μg/ml) for 4 h and (A) c-Fos, (B) c-Jun, and (C) JunB nuclear binding activity assessed in nuclear extracts using a TransAM AP-1 ELISA.
COPD, we hypothesize that an additional mediator (not adiponectin) is expressed by epithelial cells and/or nonepithelial cells (that is not induced in smokers alone). This mediator up-regulates adiponectin expression and counteracts the inhibitory effect of tobacco smoke on suppressing adiponectin expression. At present, there is no experimental data to support or refute this hypothesis.

Our immunohistology studies demonstrate that adiponectin is expressed by airway epithelial cells as opposed to alveolar cells the predominant site of disease in COPD-E. This suggests that adiponectin expressed by airway epithelium has alveolar effects by indirect mechanisms through binding to adiponectin receptors expressed by macrophages and/or airway epithelium. Thus, adiponectin released by airway epithelium may inhibit macrophage/epithelial production of proinflammatory mediators (matrix metalloprotease-9, TGF-β), as well as induce macrophages to express anti-inflammatory cytokines (IL-10, IL-1Rα), which consequently influence the extent of alveolar inflammation, alveolar injury, airspace enlargement, and repair. Interestingly, our studies using C57L-CreERT2/Hkkβ+Δ/Δ mice in whom NF-κB is inactivated in airway epithelial cells demonstrate that NF-κB signaling in airway epithelium is not required for the expression of adiponectin by airway epithelial cells. This contrasts with the importance of NF-κB signaling to adiponectin expression by adipocytes (13–15).

As not all subjects exposed to tobacco smoke develop COPD, it is likely that COPD is an example of a disease that manifests because of gene environment interactions (29). One such gene polymorphism (i.e., adiponectin) interacting with an environmental stimulus (i.e., tobacco smoke) is potentially one of several gene environment interactions that may explain why only a subset of individuals exposed to tobacco smoke develop COPD. In the case of adiponectin, the SNP 276 adiponectin polymorphism is associated with significantly reduced adiponectin levels, diminished anti-inflammatory responses, and permit the more rapid progression of tobacco smoke induced inflammation to COPD.

In summary, in this study we have made the novel observation that lung epithelial cells express adiponectin and functional AdipoR1. The potential disease relevance of adiponectin expression by lung epithelium is suggested from our studies demonstrating that adiponectin is highly expressed in the lungs of human subjects with the emphysematous form of COPD who have stopped smoking. In contrast, exposure to tobacco smoke in subjects without COPD down-regulates adiponectin expression. Further studies are needed to determine whether subjects with moderate severe COPD who continue to smoke also have increased levels of adiponectin. Overall, these studies suggest that adiponectin expression by airway epithelium may modulate the inflammatory response in COPD through autocrine or paracrine pathways.

Disclosures

The authors have no financial conflict of interest.

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