Down-regulated Peroxisome Proliferator-activated Receptor γ (PPARγ) in Lung Epithelial Cells Promotes a PPARγ Agonist-reversible Proinflammatory Phenotype in Chronic Obstructive Pulmonary Disease (COPD)§

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Background: The mechanistic role of peroxisome proliferator-activated receptor γ (PPARγ) in chronic obstructive pulmonary disease (COPD) is poorly understood.

Results: COPD and cigarette smoke exposure down-regulated PPARγ and produced inflammation that PPARγ agonists reversed through multiple pathways.

Conclusion: PPARγ plays a pivotal role in COPD.

Significance: PPARγ agonists may be the first effective treatment for COPD.

Chronic obstructive inflammatory conditions are the principal cause of death in the United States and worldwide. It is a progressive disease that, due to lack of effective treatment (1), is a major public health concern. With an estimated 154 million cases in 2003, COPD may be responsible for more than 1 million deaths per year (2). Recent advances have attributed such glucocorticoid ineffectiveness to decreased activity of the corepressor histone deacetylase 2 (HDAC2) (3), an essential component of a major mechanism of glucocorticoid action. Oxidative stress (4, 5) and cigarette smoke (6) reduce HDAC2 levels in airway epithelial cells, thereby impairing the anti-inflammatory effectiveness of glucocorticoid receptor (GR-α) activation. The ligand-activated transcription factor peroxisome proliferator-activated receptor α (PPARα), a member of the nuclear hormone receptor superfamily, exerts strong anti-inflammatory and antioxidant effects (7, 8) by down-regulating activity of nuclear factor-κB (NF-κB) and other pro-inflammatory transcription factors via multiple mechanisms. These actions might be pathophysiologically or therapeutically relevant to COPD, but the potential roles of PPARγ and its agonists in responses to chronic obstructive inflammatory conditions remain uncertain (3).

PPARγ plays a pivotal role in COPD. Treating epithelial cells with synthetic (rosiglitazone) or endogenous (10-nitro-oleic acid) PPARγ agonists strongly up-regulated PPARγ expression and activity, suppressed CSE-induced production and secretion of inflammatory cytokines, and reversed its activation of NF-κB by inhibiting the IκB kinase pathway and by promoting direct inhibitory binding of PPARγ to NF-κB. In contrast, PPARγ knockdown via siRNA augmented CSE-induced chemokine release and decreases in HDAC activity, suggesting a potential anti-inflammatory role of endogenous PPARγ. The results imply that down-regulation of pulmonary epithelial PPARγ by cigarette smoke promotes inflammatory pathways and diminishes glucocorticoid-mediated anti-inflammatory activity.

This article has been retracted by the publisher. The right pAcK-H3 and H3 immunoblots from Fig. 6A were reused in Fig. 1A as PPARγ. The right Lamin B1 immunoblot from Fig. 3C was reused in Fig. 4C as the right H3 immunoblot. In Fig. 5E, the first lane from the lower β-actin immunoblot was reused in the right pMSK1 immunoblot.

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The abbreviations used are: COPD, chronic obstructive pulmonary disease; HDAC2, histone deacetylase 2; PPARγ, peroxisome proliferator-activated receptor γ; GR-α, glucocorticoid receptor; HBE, human bronchial epithelial; CSE, cigarette smoke extract; Rosi, rosiglitazone; OA-NO₂, 10-nitro-oleic acid; IKK, IκB kinase; ROS, reactive oxygen species; p, phosphorylated.
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cigarette smoke exposure and COPD have previously been poorly characterized. Known PPARγ agonists include the synthetic thiazolidinediones, used to treat type 2 diabetes, and various endogenous compounds. Physiologically relevant endogenous PPARγ agonists remain to be identified, but plausible candidates include nitrated fatty acids, which constitute one of the largest blood-borne pools of biologically active nitrogen compounds (9) and circulate in concentrations sufficient to activate PPARγ (10).

Here we assessed the anti-inflammatory potential of PPARγ in pulmonary epithelial cells of people with and without COPD and on smoke-induced epithelial responses. We also explored its mechanistic relationships with key transcription/signaling factors including the NF-κB pathway, GR-α, and HDAC2. We found that PPARγ expression and activity are down-regulated in human bronchial epithelial (HBE) cells from COPD patients and those exposed to cigarette smoke extract (CSE) in vitro, whereas proinflammatory pathways are up-regulated. Treating lung epithelial cells with either the thiazolidinedione rosiglitazone (Rosi) or the endogenous PPARγ agonist 10-nitro-oleic acid (OA-NO2) reversed these CSE effects and the accompanying decreases in GR-α and HDAC2. PPARγ agonists also blocked CSE-induced inflammatory cytokine and chemokine production and ROS production by reversing the CSE-induced increase in NF-κB activity through multiple PPARγ-mediated mechanisms. Conversely, PPARγ knockdown augmented CSE responses. These findings raise the possibility that PPARγ agonists may be useful for treating COPD, and furthermore, may reverse patients’ resistance to anti-inflammatory steroid therapy by restoring impaired HDAC2 activity.

EXPERIMENTAL PROCEDURES

Cells—H292 cells were obtained from the ATCC (Rockville, MD) and maintained in RMPI-1640 medium supplemented with 10% FBS, 10,000 units/ml penicillin, 10,000 units/ml streptomycin (HyClone, Logan, UT). Normal and diseased human bronchial epithelial cells were obtained from Lonza (Walkersville, MD) and maintained in RPMI medium supplemented with 10% FBS, 0.4% bovine pituitary extract, 0.1% insulin, 0.1% human EGF, 0.1% hydrocortisone, 0.1% GA-1000, 0.1% retinoic acid, 0.1% transferrin, 0.1% triiodothyronine, epinephrine, penicillin, and streptomycin (HyClone). Cells were cultured at 37 °C in a humidified atmosphere of 5% CO2, 95% air in tissue culture flasks, plates, or dishes. Monolayer cultures at 90% confluence were deprived of serum for 24 h prior to treatment.

Patient Lung Tissue Samples—Human lung tissues were obtained from excess pathologic tissue after lung transplantation and organ donation, under a protocol approved by the University of Pittsburgh Institutional Review Board. COPD lung tissues were obtained from explanted lungs of subjects with advanced COPD, and control lungs were donated lungs not suitable for transplantation from the Center for Organ Recovery and Education (CORE). Lung tissues were obtained from 3R4F; Kentucky Tobacco Research and Development Centre, University of Kentucky, Lexington, KY) into 10 ml of medium according to the Federal Trade Commission (FTC) protocol,

FIGURE 1. PPARγ expression and activity are decreased in COPD. A, Western blots for PPARγ in tissue extracts of pathologically normal (non-COPD; n = 6) and COPD (n = 6) lung (top panel) and in whole-cell extracts of HBE cells obtained from normal (n = 6) and COPD (n = 6) subjects (bottom left panel) followed by densitometric analysis (bottom center panel). B, Western blots for HDAC2 and p65 from lung (top panel) and in whole-cell extracts of HBE cells (middle left panel) as described in A. Bottom left panel and bottom right panel, HDAC deacetylase (bottom left panel) and p65 DNA binding activities (bottom right panel) measured using ELISA-based assays, as described in A. C, intracellular ROS levels in normal and COPD HBE cells, assessed by confocal microscopy. Data are representative of three independent experiments; *** p < 0.001.
each puff being of 2-s duration and 35-ml volume. The pH of CSE was adjusted to 7.4 and sterilized by filtration through a 0.22-μm filter (EMD Millipore, Billerica, MA). The extract, defined as 100% CSE, was diluted to the indicated concentrations and used within 10 min of preparation. For control experiments, air was bubbled into 10 ml of medium, which was then treated as for CSE.

**Measurement of Cytokine and Chemokine Levels in Culture Medium**—Cell culture medium from different treatment groups was collected and stored at −80 °C. Levels of TNF-α, IL-6, and IL-8 were measured using ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

**Determination of Cellular ROS**—H2O2 production in cell culture media was determined using the Amplex Red hydrogen peroxide assay kit (Molecular Probes, Eugene, OR). Production of intracellular ROS in live cells was determined using the Cell Meter fluorimetric intracellular total ROS activity assay kit (AAT Bioquest, Sunnyvale, CA). Samples were mounted on glass slides with VECTASHIELD mounting medium (Vector Laboratories, Burlingame, CA). The slides were viewed by an Olympus Fluoview FV1000 confocal microscope (Olympus, Center Valley, PA) using a 60 × fluorescence lens along with FluoView confocal software (FV10-ASW v1.7, Olympus).

**HDAC Activity and Transcription Factor DNA Binding Activity Assays**—Nuclear proteins were extracted using a nuclear extraction kit (Active Motif, Carlsbad, CA), and their concentrations were determined using the BCA protein assay kit (Pierce). Nuclear extracts were used to quantify HDAC activity and DNA binding activity of PPARγ, GR-α, and the p65 subunit of NF-κB using ELISA-based kits (56210, 40196, 45496, and 40096; Active Motif).

**Western Blotting**—Total protein extracts were prepared, and Western blotting was performed as described previously (11). Antibodies against PPARγ, p65, COX-2, NOX4, GR-α, HDAC2, p300, β-actin, and lamin B1 were from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against p-Ser,
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Figure 3. CSE down-regulates anti-inflammatory transcription factors PPARγ and GR-

A

B

C

D

p-IKKα, p-1xβ, p-MSK1, acetylated lysine (Ac-Lys), phospho-histone H3 (Lys-9/Ser-10), acetyl-histone H3, and histone H4 were from Cell Signaling Technology (Beverly, MA). Antibody against nitrotyrosine was from Oxis International (Beverly, MA). Antibody against 4-hydroxy-2-nonenal was from Oxis International, Inc. (Beverly Hills, CA). Antibody against 4-hydroxy-2-nonenal.

Immunoprecipitation—Nuclear extracts were prepared and were immunoprecipitated using the Dynabeads protein G immunoprecipitation kit (Invitrogen). Antibodies were bound to Dynabeads protein G, and Dynabeads-antibody complex was used to precipitate target proteins from the nuclear extracts. Unbound proteins were washed away, and complexes were eluted. All samples (20 µg/lane) were separated by electrophoresis on SDS-polyacrylamide gels and transferred to PVDF membranes, and Western blotting was performed.

ChIP assay—The ChIP assay was performed using the SimpleChIP enzymatic chromatin immunoprecipitation kit with magnetic beads (Cell Signaling Technology). Briefly, cellular chromatin was cross-linked with 1% formaldehyde for 10 min at room temperature, the cross-linking was stopped with 0.125 M glycine, and cells were washed twice with ice-cold PBS. Nuclei were pelleted and digested by micrococcal nuclease. Following sonication and centrifugation, equal amounts of sheared chromatin were incubated overnight at 4°C with antibodies, IgG as negative control, and RNA polymerase II as positive

Transfecting Small Interfering RNA into Normal HBE Cells—Normal HBE cells were incubated for 8 h with a liposome complex containing 100 nm of small interfering RNA (siRNA) targeted to PPARγ or scrambled control (Dharmacon, Lafayette, CO; supplemental Table 2) and Lipofectamine 2000 (Invitrogen) under serum- and antibiotic-free conditions. After 8 h, fresh medium with 10% FBS was added, and the cells were incu-
bated for a further 16 h. After a 24-h incubation, cells were treated with CSE as described.

**Statistical Analysis**—Data are presented as mean ± S.D. Differences between groups were analyzed using an unpaired t test or analysis of variance followed by a Bonferroni’s multiple comparison test using GraphPad Prism 5.03 (GraphPad Software, La Jolla, CA). A p < 0.05 was considered significant.

**RESULTS**

PPARγ Down-regulation in COPD Is Associated with Reduction of HDAC2 and Activation of NF-κB—To assess the potential pathophysiological role of PPARγ in COPD, we tested whether PPARγ expression and function are altered in lung tissue samples of COPD patients when compared with those from individuals without COPD. This PPARγ down-regulation was associated with the previously reported (3) up-regulated expression and activity of the pro-inflammatory transcription factor NF-κB and down-regulation of the corepressor HDAC2 (Fig. 1B), a factor that participates in GR-α-mediated anti-inflammatory activity. HBE cells from COPD patients also exhibited oxidative stress consistent with their proinflammatory state, as shown by immunostaining for ROS followed by confocal microscopy (Fig. 1C).

CSE Induces Inflammatory Responses and Oxidative Stress in Human Epithelial Cells—Cigarette smoking, the major risk factor for COPD, produces lung inflammation and oxidative stress. To assess the mechanisms by which smoke down-regu-
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lates epithelial PPARγ and induces inflammation, we determined the time courses and concentration-response relationships of CSE-induced proinflammatory proteins, transcriptional mediators, and ROS in H292 human lung epithelial cells. Treating cells with varying concentrations of CSE for 6 h or with 10% CSE for various times up-regulated expression and release of the inflammatory cytokines TNF-α and IL-6 and the chemokine IL-8 (Fig. 2, A and B). Production of H2O2 was also increased (Fig. 2C) as were intracellular ROS levels seen by immunofluorescence after a 6-h exposure to 10% CSE (Fig. 2D). CSE exposure likewise activated the proinflammatory transcription factor NF-κB, seen via increased nuclear NF-κB p65 levels (Fig. 2F) and DNA binding activity (Fig. 2G), and increased expressed protein levels of its transcriptional targets COX-2 and NADPH oxidase 4 (NOX4) (Fig. 2H).

We also measured changes in inhibitor of NF-κB (IκB), which down-regulates activity of NF-κB by preventing its translocation to the nucleus, and in IκB kinase (IKK), which drives ubiquitination and degradation of IκB, thereby increasing NF-κB activity (14). CSE treatment increased the levels of phosphorylated IκB and phosphorylated (activated) IKK (Fig. 2E) and those of the activated form of mitogen- and stress-activated protein kinase 1 (MSK1), which phosphorylates IKK (Fig. 2F). MSK1 also promotes inflammatory gene transcription by phosphorylating NF-κB itself, allowing it to recruit coactivators by phosphorylating histone H3, allowing it to induce chromatin loosening (15). The time courses and concentration-response relationships of all these CSE-induced responses are similar, pointing to a broadly coordinate response program in lung epithelial cells.

CSE Down-regulates PPARγ and Enhancing Chromatin Acetylation—Based on the CSE-induced proinflammatory profile we saw in H292 cells and the suppression of PPARγ activation (13), we tested whether CSE influences PPARγ activity in lung epithelial cells. Supporting our hypothesis, exposing H292 cells to CSE decreased GR-α phosphorylation, an inhibitory post-translational modification (Fig. 3, A and B). To test the idea that CSE-induced down-regulation of GR-α might contribute to the ineffectiveness of glucocorticoids in COPD, we tested the effects of CSE on GR-α. Supporting our hypothesis, exposing H292 cells to CSE down-regulated both expression and activity of GR-α and also induced its lysine acetylation (Fig. 3, C and D), an inhibitory post-translational modification.

Transcription factors attract coactivators with histone acetyltransferase activity, which acetylates specific lysines in histones H3 and H4 and thereby loosens chromatin structure so as to allow RNA polymerase to bind and initiate transcription. GR-α suppresses proinflammatory gene expression in part by associating with NF-κB and attracting the corepressor HDAC2, which inhibits transcriptional activation by deacetylating histones (16). We tested the influence of CSE on this system. CSE decreased the nuclear localization and activity of HDAC2 (Fig. 4, A and B) and thus increased the acetylation of histones H3 and H4, in a time- and concentration-dependent manner (Fig. 4C). CSE-induced suppression of HDAC activity reflected not only its down-regulated expression, but also induction of multiple concentration- and time-dependent post-translational modifications including serine phosphorylation, tyrosine nitrosylation, and cysteine alkylation by 4-hydroxy-2-nonenal, a specific marker of oxidative stress (Fig. 4D).

PPARγ Activation Reduces CSE-induced Inflammation and Oxidative Stress while Up-regulating GR-α and HDAC2—To determine whether PPARγ activation can suppress the CSE-induced proinflammatory profile and resulting oxidative stress in epithelial cells, we treated H292 cells with either the synthetic agonist Rosi (1 μM) or the endogenous agonist OA-NO2 (100 nM) for the 30 min preceding a 6-h exposure to 10% CSE. Both PPARγ agonists reduced the CSE-induced increases in...
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FIGURE 6. PPARγ activation reverses CSE-induced changes in PPARγ, GR-α, and HDAC2. A, Western blots showing the levels of PPARγ, GR-α, and HDAC2 with Rosi (1 μM) or OA-NO2 (100 nM) followed by CSE treatment (10%, 6 h) of H292 cells. B, Western blots showing phosphorylation in immunoprecipitated (IP) PPARγ, acetylation of histone H3 (Lys-9, Lys-12, and Ser-10), and Lamin B1 (to control for loading, which was maintained). C, Quantitation of Figure 6A. D, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. E, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. F, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. G, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. H, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. I, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. J, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. K, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. L, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. M, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. N, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. O, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. P, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. Q, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. R, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. S, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. T, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. U, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. V, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. W, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. X, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. Y, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. Z, Western blots showing the levels of HDAC2 in immunoprecipitated HDAC2. [Image 128x642 to 217x652] [Image 128x654 to 217x663] [Image 128x666 to 217x675] [Image 128x592 to 217x602] [Image 128x630 to 217x639] [129x279]/H9251 OA-NO2 MARCH 7, 2014 • VOLUME 289 • NUMBER 10 JOURNAL OF BIOLOGICAL CHEMISTRY 6389...
in wild-type cells. Thus, whereas PPARγ activation reversed the deleterious effects of CSE, reducing PPARγ greatly exaggerated them.

To assess the potential relevance of agonist-induced PPARγ activation to the effects of COPD we saw in HBE cells (Fig. 1), we tested the abilities of Rosi- and OA-NO2-induced PPARγ activation to inhibit CSE responses in HBE cells. As in H292 cells (Figs. 5–7), both agonists abrogated CSE-induced suppression of PPARγ, GR-α, and HDAC2 levels and its up-regulation of NF-κB (Fig. 8E). These effects of Rosi and OA-NO2 were accompanied by reduced NF-κB binding to the promoter region of its target gene IL-8 (Fig. 8F) and decreased CSE-stimulated IL-8 release (Fig. 8G). These effects in human lung cells thus show that our results are generalizable beyond H292 cells and support their relevance for the smoke-exposed human airway in vivo.

**DISCUSSION**

Our studies lead to two central conclusions. First, PPARγ expression and DNA binding activity are down-regulated in lung tissue samples and bronchial epithelial cells from COPD patients. These observations are linked to down-regulation of GR-α and its associated corepressor HDAC2, whereas the transcription factor NF-κB is up-regulated. The net effect of these findings is the enhanced airway inflammation typically observed in COPD. CSE treatment of human lung epithelial
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Mechanistic studies supported multiple paths through which PPARγ activation, with consequent increases in its expression and activity, blocks the ability of cigarette smoke to up-regulate NF-κB and thus induce inflammation. Co-immunoprecipitation demonstrated binding of PPARγ to NF-κB, which can inhibit that transcription factor in at least two different ways (17, 18). Furthermore, treatment with either PPARγ agonist reversed the CSE-induced phosphorylation of IKK, thus blocking degradation of IκB and consequent nuclear localization of NF-κB (14). This extends to airway epithelial cells and an additional PPARγ agonist previous observations that the thiazolidinedione pioglitazone blocks IKK activation through a PPARγ-dependent mechanism in IL-1β-stimulated vascular smooth muscle cells (19). The agonist-induced reduction in phosphorylation we see presumably reflects the accompanying decrease in activating phosphorylation of MSK1. The ability of PPARγ to attack inflammation via multiple pathways thus enhances its attractiveness as a potential therapeutic target in COPD.

It is also unclear whether the PPARγ down-regulation and inhibition we observe directly reflect inflammatory signaling, are due to inflammation-associated oxidative stress, or both. Blanquicett et al. (20) have reported that H2O2 induces a prolonged down-regulation of PPARγ mRNA expression in human vascular endothelial cells. The authors attributed this effect to oxidative stress activation of the transcription factor activator protein 1 (AP1). The inhibitory post-translational phosphorylation we also see appears to reflect a different mechanism, however, since it is driven by activation of mitogen-activated protein kinases (MAPKs). Previous studies have shown that such phosphorylation is mediated by members of the MAPK family (21–23) or c-Jun N-terminal kinase (21, 24). There is redundancy in the pathways involved because PPARγ can be phosphorylated by either extracellular signal-related kinase (21–23) or c-Jun N-terminal kinase (21, 24). Both are members of the MAPK family that can be activated by cigarette smoke (25).

The limited effectiveness of glucocorticoids represents a major therapeutic challenge in COPD. We demonstrated that CSE reduces nuclear localization and activity of both GR-α and the HDAC2 corepressor that it utilizes, with accompanying post-translational modifications of these proteins, and that
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**Expression**

- PPARγ
- GR-α
- HDAC2

**COPD**

- Increased expression of NF-κB dependent genes

**COPD + PPARγ Ligands**

- Histone Deacetylation
- Sequence of events:
  - Activation
  - NF-κB Sites
  - RNA Pol II
  - TFs

**FIGURE 9. Schematic summary of how PPARγ, GR-α, and HDAC2 affect inflammatory signaling, which leads to degradation and post-translational modifications. Top panel, cigarette smoke-induced inflammatory signaling, which leads to degradation and post-translational modifications. Middle panel, activation of PPARγ and GR-α, results in histone deacetylation and chromatin unwinding. This allows NF-κB activation and binding, NF-κB activity is further reduced by binding to PPARγ and GR-α and, through GR-α, by binding to coactivators. Bottom panel, activation of PPARγ and GR-α results in histone deacetylation and chromatin unwinding. This allows NF-κB activation and binding. NF-κB activity is further reduced by binding to PPARγ and GR-α and, through GR-α, by binding to coactivators.**

these effects are reversed by PPARγ activation. PPARγ activation suggests that PPARγ agonists may restore normal epithelial cell sensitivity in lung epithelial cells and potentially in COPD patients, raising the possibility that joint administration of PPARγ agonists and corticosteroids may be therapeutically appropriate. PPARγ activation also increases expression of GR-α, as has been observed by others (28). While this PPARγ-GR-α binding is direct or indirect remains unclear, however. It has been suggested that it may represent binding to common coactivators (26).

A prior study found that PPARγ levels in lung tissues were up-regulated in patients with mild COPD, but in line with our present findings, were down-regulated in those with moderate or severe disease (27). The cell types involved were not identified. Because PPARγ levels were recently found to be unaltered in alveolar macrophages of COPD patients (28), our findings clearly point to lung epithelial cells as a key locus of PPARγ down-regulation and target for its potential therapeutic activation in COPD patients. Other previous investigations have shown that PPARγ activation can attenuate inflammation either in animal models of COPD or following CSE exposure in vitro, but have not addressed the signaling pathways we investigated. Both the thiazolidinedione pioglitazone (29) and the endogenous PPARγ agonist 15-deoxy-D_12,14_-prostaglandin J_2 (15d-PGJ_2) (30) have proven effective in the LPS-induced model of COPD, while both Rosi and pioglitazone were effective in a smoke-induced model (28). In vitro, Lee et al. (31) attributed the ability of Rosi to inhibit CSE-induced TNF-α and NF-κB expression in H292 cells to up-regulation of phosphorylated protein kinase B and tensin homolog deleted on chromosome 10 (PTEN), with consequent down-regulation of the Akt signaling pathway. However, Rosi did not block CSE-induced cytokine production in a monocyte-macrophage cell line in which CSE disrupts the association between PPARγ and NF-κB (32). Taken together with the previously mentioned study by Lea et al. (28), this suggests that the role of PPARγ in COPD may differ between macrophages and epithelial cells.

Our results show that down-regulation of epithelial cell PPARγ expression and activity plays an important role in cigarette smoke-induced inflammation and the pathophysiology of COPD (Fig. 9, top panel). Among its other effects, this down-regulation decreases HDAC2 expression and activity, which contributes to increased acetylation of chromatin histones and a looser, unwound chromatin conformation. This conformational change facilitates binding of NF-κB (p50 + p65, expression and activity of which are up-regulated in COPD) and its p300 coactivator to the genes’ promoter regions. Acetylation by p300 further loosens chromatic structure, allowing binding of RNA polymerase II and increased transcription of these genes. These changes are reversed by PPARγ activation (Fig. 9, bottom panel). This leads to up-regulation of HDAC2 expression and activity, with removal of histone acetyl groups and rewinding of the chromatin. NF-κB, now bound to PPARγ, GR-α, and HDAC2, no longer binds to the promoter regions of its target proteins. Furthermore, activated PPARγ itself attracts the coactivators that NF-κB requires (33), limiting the activity of
any remaining promoter-bound transcription factor. We also show that PPARγ and GR-α simultaneously bound to NF-κB, as suggested by our finding that these two nuclear hormone receptors co-immunoprecipitate.

Our studies thus provide major new insights into the mechanisms by which COPD-induced down-regulation of PPARγ expression and inhibition of its activity contribute to the pro-inflammatory phenotype characteristic of this disease and the ways in which PPARγ agonists reverse these effects. They also illuminate the mechanisms underlying the glucocorticoid insensitivity seen in COPD and imply that PPARγ agonists could restore sensitivity. Taken together, these results support the possibility that PPARγ agonists might prove effective treatments for this common and deadly disease.

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