

Peroxisome proliferator-activated receptor γ is expressed in airways and inhibits features of airway remodeling in a mouse asthma model

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Background: Allergic asthma is associated with persistent functional and structural changes in the airways and involves many different cell types. Peroxisome proliferator-activated receptor γ (PPAR- γ), a member of the nuclear hormone receptor superfamily, is predominantly expressed in adipose tissue and plays a major role in regulating adipocyte differentiation and glucose metabolism. Recently, PPAR- γ has been shown to play an important role in the control of inflammatory responses, including within the lung, acting on both immune and nonimmune cells.

Objective: Our aim was to assess the anti-inflammatory potential of a PPAR- γ agonist locally delivered by means of nebulization.

Methods: We used a mouse model of asthma induced by sensitization and airway challenge with ovalbumin. Ciglitazone, a PPAR- γ agonist, was administered by means of nebulization alone at the time of antigen challenge or by means of gavage and nebulization. Treatments with both ciglitazone and GW9662, a specific antagonist, were also performed to verify that ciglitazone's effects were mediated through PPAR- γ activation.

Results: Our results show that PPAR- γ is mainly expressed in airway epithelium on antigen sensitization. Treatment with ciglitazone reduced PPAR- γ levels in the lung, whereas combined treatment with GW9662 abrogated this inhibition. Importantly, nebulization with ciglitazone decreased airway hyperresponsiveness, basement membrane thickness, mucus production, collagen deposition, and TGF- β synthesis. A significant correlation was also found between airway hyperresponsiveness, basement membrane thickness, and TGF- β levels.

Conclusion: These results demonstrate that inhaled agonistic ligands of PPAR- γ might have new therapeutic potential for airway asthmatic inflammation. (*J Allergy Clin Immunol* 2004;113:882-8.)

Key words: Peroxisome proliferator-activated receptors, asthma, airway remodeling, airway hyperresponsiveness, IgE, eosinophils, trachea, TGF- β

Abbreviations used

AHR: Airway hyperresponsiveness
HRP: Horseradish peroxidase
OVA: Ovalbumin
PAS: Periodic acid-Schiff
PPAR: Peroxisome proliferator-activated receptor
RT: Room temperature

Asthma remains one of the most common chronic inflammatory diseases, and its incidence has markedly increased over the past 2 decades. Bronchial asthma is associated with persistent functional and structural changes within the airways that include airway hyperresponsiveness (AHR), increased subepithelial fibrosis, epithelial metaplasia, and mucus production.^{1,2} Much evidence suggests that bronchial fibrosis correlates with airway function.²⁻⁴ Various mediators, such as TGF- β , GM-CSF, and epidermal growth factor, were reported to regulate airway remodeling.^{1,3} Among these factors, TGF- β is an important profibrotic cytokine. It promotes fibroblast differentiation into myofibroblasts, which secrete interstitial collagens, as well as growth factors, such as endothelin 1 and vascular endothelial growth factor, both of which are mitogenic for smooth muscle and endothelial cells.⁵ In most studies TGF- β expression correlates with basement membrane thickness, fibroblast numbers, and disease severity.^{2,6,7} Nuclear receptors are ligand-activated transcription factors.⁸ The glucocorticoid receptor α (GR- α) mediates the anti-inflammatory effects of corticosteroids.⁹ Inhaled corticosteroids are now a first-line treatment for chronic asthma.^{10,11} However, glucocorticoids, particularly at high doses, have significant and severe adverse effects. Furthermore, a subgroup of patients with severe asthma exhibited a poor response to treatment with glucocorticoids and might indeed be glucocorticoid resistant.^{12,13} Peroxisome proliferator-activated receptors (PPARs) were also reported to regulate inflammatory responses.¹⁴⁻¹⁶ Three subtypes of PPARs (α , β/δ , and γ) have been identified.¹⁷ One of the natural ligands for PPAR- γ is 15-deoxy- Δ -prostaglandin J₂, the major metabolite from prostaglandin D₂.¹⁸ One group of synthetic PPAR- γ agonistic ligands is the glitazones, drugs used in the treatment of diabetic patients.¹⁹

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Several studies have clearly demonstrated that PPAR- γ plays an important role in the control of inflammatory responses,^{20,21} acting on T cells,^{22,23} macrophages, dendritic cells,²⁴ and mast cells.²⁵ In monocytes and macrophages PPAR- γ agonists inhibit the expression of proinflammatory cytokines, such as TNF- α , IL-1 β , and IL-6.^{26,27} In smooth muscle cells PPAR- γ activation has additional anti-inflammatory effects compared with the effects of corticosteroids.²⁸ We demonstrated that PPAR- γ was expressed by eosinophils and that PPAR- γ agonists were able to inhibit eosinophil chemotaxis and antibody-dependent cellular cytotoxicity reactions *in vitro*.²⁹ Furthermore, in a murine model of asthma, treatment with a PPAR- γ agonist also inhibited the development of allergic inflammation, including pulmonary eosinophilia and airway AHR.²⁹ However, target cells for PPAR- γ agonists within the airways and the mechanisms by which they inhibit allergic inflammation are poorly characterized. We reasoned that PPAR- γ agonist nebulization might not only affect inflammatory cells, such as eosinophils, mast cells, and T_H2 cells, but also could directly act on airway epithelium and smooth muscle and thus more efficiently contribute to a decreased AHR and remodeling.

In this study we thus examined the effect of PPAR- γ agonist nebulization on the regulation of airway remodeling and TGF- β production in a murine asthma model.

METHODS

Murine asthma model

Specific pathogen-free female BALB/c mice were purchased from Iffa Credo. Experiments were carried out in accordance with ethical guidelines from the Comité d'Ethique en Expérimentation Animale de la Région Nord-Pas de Calais. Six- to 8-week-old mice were sensitized by means of intraperitoneal injection of ovalbumin (OVA; 50 μ g, Sigma) in 100 μ L of alum (Imject, Pierce) or received alum only and were challenged for 20 minutes on days 14, 15, 16, 18, 19, and 20 by means of aerosol nebulization with OVA (1% in PBS) by using an ultrasonic nebulizer (System). Ciglitazone (5×10^{-5} mol/L, Alexis) in PBS or ciglitazone (5×10^{-5} mol/L) and GW9662 (5×10^{-5} mol/L, Cayman) in PBS (or vehicle) were administered by means of nebulization for 20 minutes immediately before and during each OVA challenge. For some experiments, ciglitazone was also administered orally (20 mg/kg in 1% carboxymethylcellulose 6 hours before nebulization on day 13, 14, 15, 16, 18, 19, and 20), and nebulization was performed as described above. Serum was collected on day 21.

AHR

AHR to increasing concentrations of methacholine (1.5–12 mg/mL) was measured on unrestrained conscious mice by using whole-body plethysmography (Emka)³⁰ on day 22, 48 hours after the last nebulization. Enhanced pause values were directly obtained from these analyses.

OVA-specific IgE measurement

Anti-OVA IgE concentrations in the serum were measured by means of ELISA with anti-IgE (Pharmingen) as a capture antibody

and biotinylated OVA and horseradish peroxidase (HRP)–conjugated streptavidin (Amersham Pharmacia) for detection. Two-fold serial dilutions were prepared for each serum (starting dilution, 1:25 for titrations). Antibody titers were calculated as the dilution corresponding to twice the mean absorbance value obtained for nonsensitized mouse sera.²⁹

Histology

Lung and whole trachea samples were fixed with Immunohistofix (Intertiles) for 7 days at 4°C. After dehydration in graded alcohol baths, embedding was performed by using 3 successive immersions in Immunohisto wax (Intertiles) at 37°C. Tracheas were positioned vertically during embedding for horizontal sectioning at the level of the main carina. Sections (5 μ m thickness) were dewaxed in acetone for 5 minutes, washed with PBS, and then stained either with May-Grünwald-Giemsa or Periodic acid–Schiff (PAS) for detection of mucopolysaccharide accumulation or Sirius Red for detection of collagen deposition.

Morphometry

Mucus accumulation was determined on transverse sections from the upper left lobe of the lung. The mucus index was calculated as follows:

$$\begin{aligned} & \left(\frac{\text{[average intensity of epithelial PAS staining]} \times \text{[area of epithelial PAS staining]}}{\text{[total area of conducting airway epithelium]}} \right) \end{aligned}$$

by using Mac Scope software (Mitani Corp).

For measurement of tracheal basement membrane thickness, care was taken to select exactly the same area for each trachea. Images were captured with a digital camera (using a 100 $\times$ immersion objective). Basement membrane (reticulin-stained zone) thickness was measured by tracing with the Metavue software (Universal Imaging Corp). For each sample, the entire length of basement membrane was considered. Three measures were taken, and the average basement membrane thickness was calculated.

Immunohistochemistry

Dewaxed and PBS-washed sections were incubated with 3% H₂O₂ in PBS for 15 minutes to block endogenous peroxidases and then in PBS-Blocking Reagent (Boehringer) for 30 minutes at room temperature (RT). Sections were incubated with anti-mouse PPAR- γ (SC7196, 10 μ g/mL; Santa Cruz Biotechnology) for 2 hours at RT. After washing, sections were incubated with biotin-conjugated anti-rabbit IgG (H+L, Jackson ImmunoResearch) for 30 minutes at RT. The biotinylated reagents were detected with ABCcomplex HRP (Dako). Sections were counterstained with hematoxylin.

Collagen quantification

Collagen lung content was determined by quantifying soluble collagen with the Sircol Collagen Assay Kit (Biocolor), according to the manufacturer's instructions. Briefly, lungs were extracted in 1.5 mL of 0.5 mol/L acetic acid containing 10 mg of pepsin (Sigma). Each sample was incubated overnight at RT during stirring. After centrifugation, 100 μ L of each supernatant was mixed for 30 minutes with 1 mL of Sircol dye reagent. After centrifugation, the pellet was suspended in 1 mL of alkali reagent, and absorbance was measured at 570 nm.

Western blotting

Western blot analysis of PPAR- γ expression in the lung was performed as previously described.²⁹ A single band was detected at 54 to 57 kD, as previously evidenced on adipose tissue. Lung samples

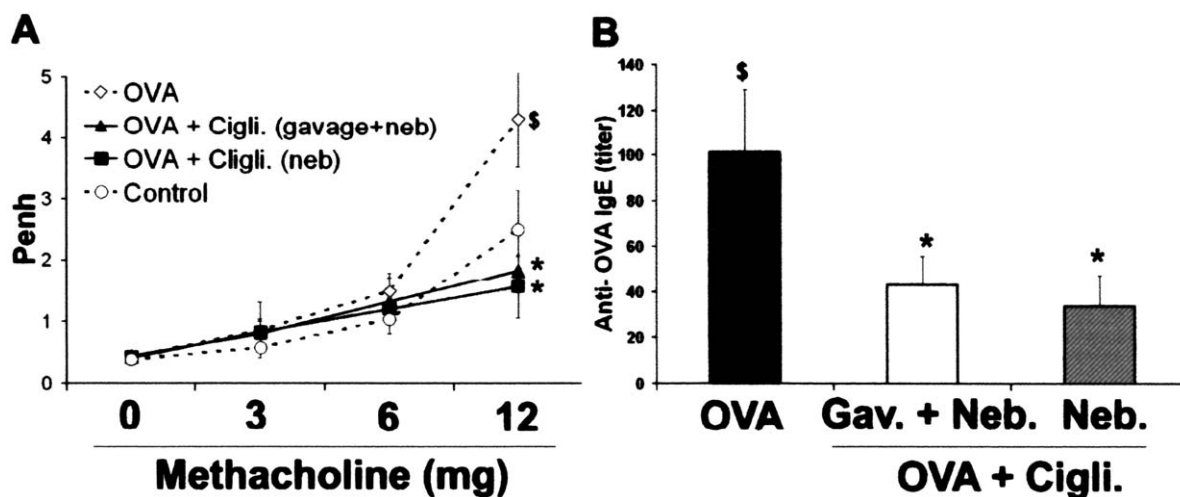


FIG 1. Effect of ciglitazone nebulization or oral administration and nebulization on antigen-induced AHR and IgE production. OVA-sensitized and OVA-challenged mice with (■) or without (◇) nebulization by ciglitazone or by ciglitazone nebulization and oral administration (▲) are shown. Unsensitized mice are shown as ○. **A**, Enhanced pause (*Penh*) on nebulization with increasing doses of methacholine. **B**, OVA-specific IgE titers in serum ($n = 4-5$ animals per group; error bars are SEM). * $P < .05$, OVA-sensitized and ciglitazone-treated versus OVA-sensitized mice. \$ $P < .05$, sensitized and challenged versus unsensitized but challenged mice. *Cigli*, Ciglitazone; *Neb*, nebulization; *Gav*, gavage.

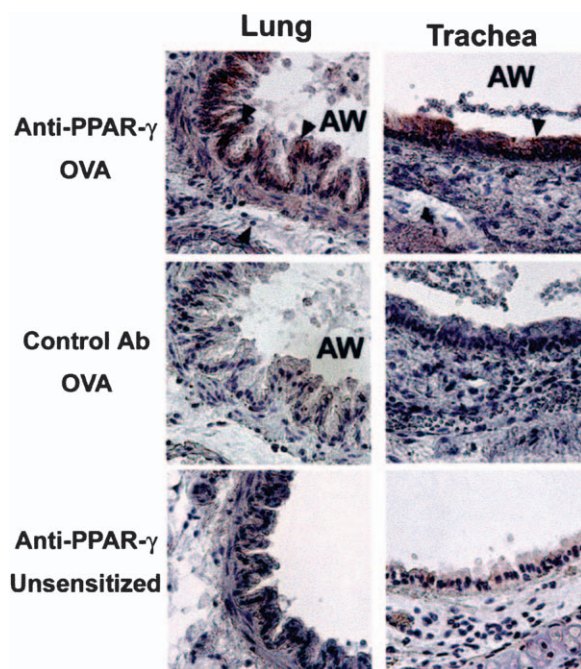


FIG 2. PPAR- γ expression in the airways: immunohistochemical detection of PPAR- γ expression in the lung and trachea. Arrowheads show PPAR- γ expression in the epithelium and smooth muscle (original magnification 250 $\times$). AW, Airway; Ab, antibody.

and spleen tissue, which was used as a positive control, were homogenized with an Ultra-turrax in 400 μ L of buffer (per right lung) containing 1% NP40, 150 mmol/L NaCl, 50 mmol/L Hepes, 10 mmol/L NaF, 0.5 mmol/L Na_3VO_4 , 1 mmol/L phenylmethylsulfonyl fluoride, and complete protease inhibitor cocktail (Roche) and centrifuged at 13,000 rpm for 10 minutes. Protein quantification was

performed on supernatants before gel loading to equalize for the amount of material. Twenty micrograms of protein was loaded for each sample on a 10% SDS-PAGE gel and transferred onto a nitrocellulose membrane. Blots were incubated with anti-PPAR- γ -specific antisera (Biomol). Immunoblot signals were detected with an HRP-conjugated antibody (Jackson ImmunoResearch Laboratories) and visualized by using the ECL system (Amersham Pharmacia Biotech).

TGF- β ELISA

Murine TGF- β was measured in 1% NP40 lung protein extracts, prepared as for Western blot analyses, by using a standardized sandwich ELISA technique. TGF- β -specific purified capture and biotinylated detection antibodies recognizing human, rat, and mouse proteins (clones A75 2.1 and 3.1, respectively) were purchased from PharMingen. HRP-conjugated streptavidin (Amersham Pharmacia) and TMB (Sigma) were used for revelation. ELISA was performed after sample acidification, according to the manufacturer's instructions. Human TGF- β (0-2000 pg/mL, R&D) was used as a standard. The lower detection limit was 125 pg/mL.

Statistical analysis

Values were expressed as means $\pm$ SEMs. Data were compared by using the unpaired t test, except for plethysmography data, which were analyzed by means of ANOVA. A P value of less than .05 was considered significant.

RESULTS

In a classical model of mouse asthma, we compared the effects of ciglitazone, a PPAR- γ agonist, delivered by means of nebulization only or in association with oral administration. Both ciglitazone treatments were equally efficient at reducing AHR to methacholine, as measured on the basis of whole-body plethysmography (Fig 1, A) and antigen-specific IgE production associated with the

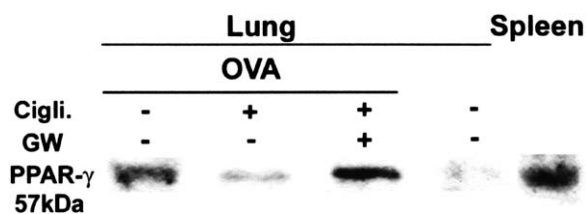


FIG 3. Ciglitazone treatment decreases lung PPAR- γ expression. Western blot analysis of PPAR- γ expression in mice lung extracts. *Cigli*, Ciglitazone; *GW*, GW9662.

development of this asthma-like reaction (Fig 1, B), as well as eosinophil numbers in bronchoalveolar lavage fluid and in peribronchial and perivascular inflammatory infiltrates, as previously reported.²⁹ Airways thus appear as a relevant target for PPAR- γ agonists.

Because several different cell types, among which some already known to express PPAR- γ , are present within the inflammatory lung, we thus aimed at more precisely locating PPAR- γ expression in mouse airways after OVA sensitization and challenge. Immunohistochemistry of lung and trachea sections revealed that PPAR- γ was mainly expressed in the epithelium but was also detected in airway smooth muscle, mast cells, and some inflammatory cells (Fig 2). The extensive surface covered by epithelial cells compared with other cell types and the predominant PPAR- γ expression in epithelial cells might therefore account for the efficiency of nebulization as a route for ciglitazone treatment. On the other hand, no expression was detected by using this technique in the airways from unsensitized but nebulized mice (Fig 2), suggesting that antigen-induced inflammation promotes PPAR- γ expression in the lung.

PPAR- γ expression was also investigated by means of Western blotting on lung extracts. In broad agreement with immunohistochemical detection, PPAR- γ protein was detected in lung extracts from OVA-sensitized and OVA-challenged mice but was barely detectable in lungs from unsensitized challenged animals (Fig 3). Interestingly, treatment with ciglitazone significantly reduced PPAR- γ levels (Fig 3). This effect was abrogated when animals were treated with both ciglitazone and GW9662, a specific PPAR- γ antagonist, showing that ciglitazone's effects on PPAR- γ levels were mediated through interaction with PPAR- γ (Fig 3). This suggests that ligand-induced PPAR- γ downmodulation accompanies the reduction of inflammation.

Airway remodeling and mucus accumulation are important factors leading to AHR. To investigate whether PPAR- γ agonist nebulization was effective at reducing these parameters, we assessed mucus accumulation in the lung, collagen deposition, and basement membrane thickness in the trachea. Mucus hypersecretion in the airways was observed in the OVA-sensitized and OVA-challenged animals but was barely detectable in unsensitized animals. Mucus gland hyperplasia and occlusion of airway diameter caused by mucus hypersecretion were reduced by 75% on ciglitazone nebulization (Fig 4). Sirius Red

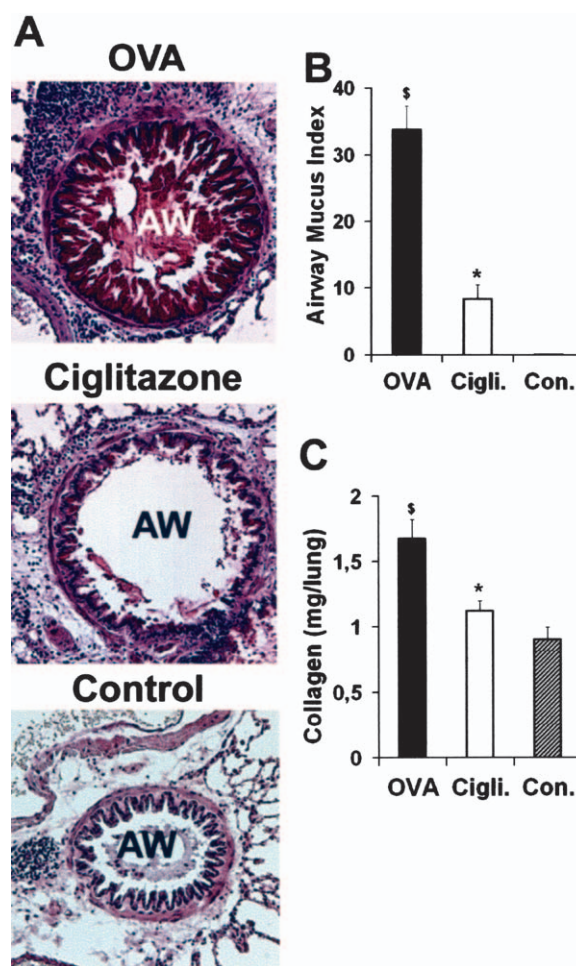


FIG 4. Decreased mucus production and collagen deposition in lungs from ciglitazone-nebulized mice. **A**, Tissue stained with PAS (original magnification, 100X). **B**, Mucus index. **C**, Collagen quantification. * $P < .05$, OVA-sensitized and ciglitazone-treated versus only OVA-sensitized mice. \$ $P < .05$, sensitized and challenged versus unsensitized but challenged mice. *AW*, Airway; *Cigli*, ciglitazone; *Con*, control.

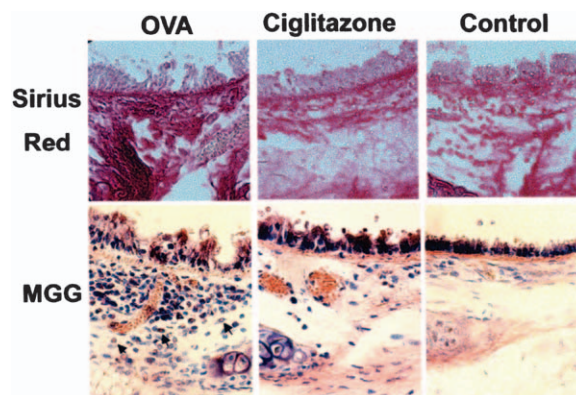


FIG 5. Decreased eosinophilic inflammation and deposition of collagen in trachea from ciglitazone-nebulized mice: *upper row*, Sirius Red staining revealing collagen deposits (original magnification, 250X); *lower row*, May-Grünwald-Giemsa staining. *Arrows* indicate eosinophilic infiltrates in the subepithelial layer (original magnification, 250X). *MGG*, May-Grünwald-Giemsa.

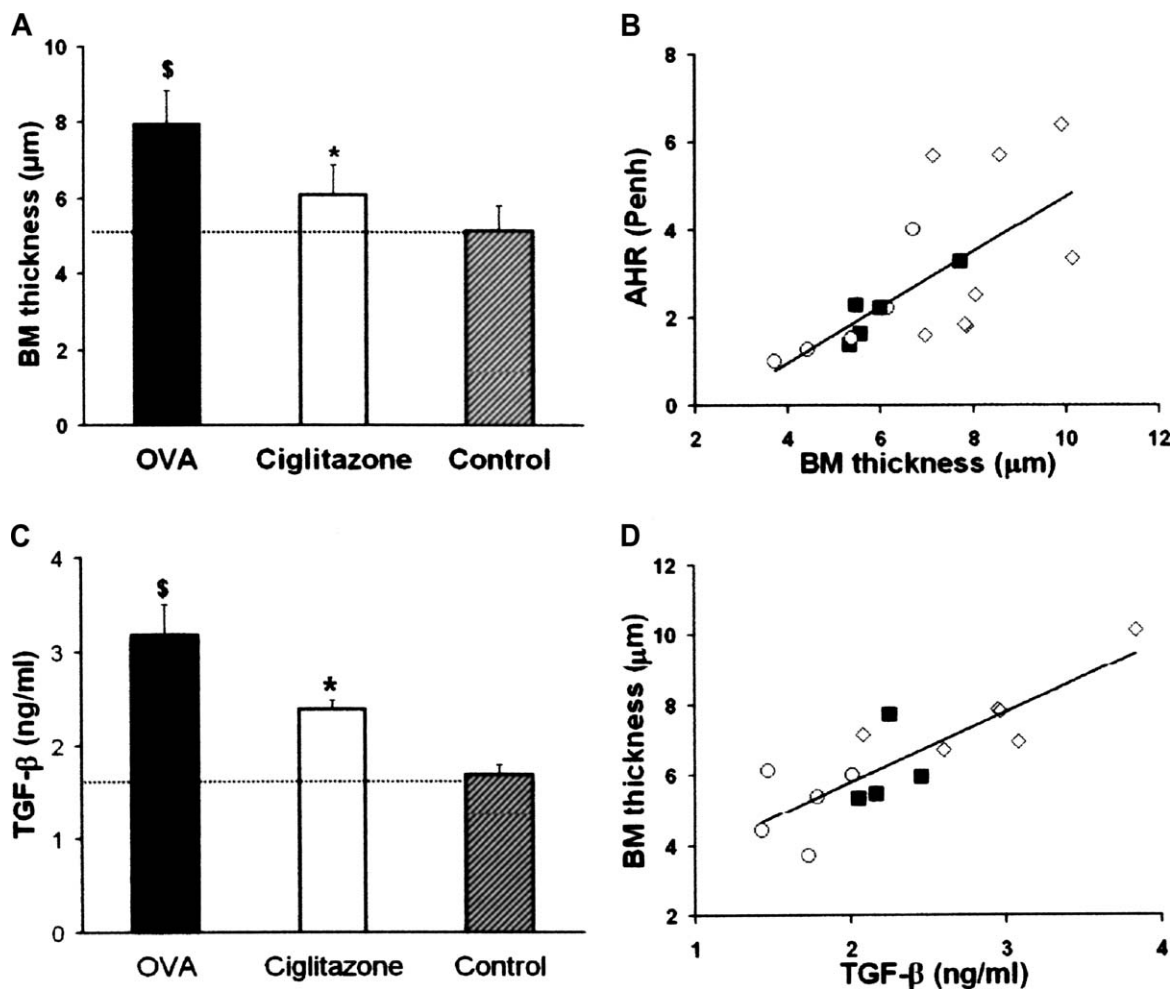


FIG 6. Decreased tracheal basement membrane thickness and TGF- β concentrations in ciglitazone-nebulized mice. **A**, Basal membrane thickness ($n = 4-5$ animals per group; error bars are SEM). **B**, Correlations between enhanced pause (methacholine, 12 mg/mL) and tracheal basement membrane thickness ($r = 0.670$). OVA-sensitized and challenged mice with (■) or without (◇) nebulization by ciglitazone are shown. Control mice are shown as ○. **C**, TGF- β levels in lung extracts ($n = 5-7$ animals per group; error bars are SEM). **D**, Correlations between tracheal basement membrane and TGF- β concentrations in lung extracts ($r = 0.836$). * $P < .05$, OVA-sensitized and ciglitazone-treated versus OVA-sensitized mice. \$ $P < .05$, sensitized and challenged versus unsensitized but challenged mice. BM, Basement membrane; Penh, enhanced pause.

staining revealed that OVA-sensitized mice displayed airway wall thickening with collagen deposition. Likewise, nebulization with ciglitazone decreased collagen deposition in the lung by 72% (Fig 4), as well as in the trachea (Fig 5). May-Grünwald-Giemsa staining also revealed that ciglitazone treatment also affected inflammatory cell recruitment. Indeed, eosinophilic infiltrates in the subepithelial layer of the trachea were reduced in ciglitazone-treated mice compared with in untreated animals (Fig 5).

Furthermore, the antigen-induced increase in tracheal basement membrane thickness was also inhibited by 65% on ciglitazone treatment (OVA-sensitized and OVA-challenged mice, $7.98 \pm 1.27 \mu\text{m}$; OVA-sensitized,

OVA-challenged, and ciglitazone-treated mice, $6.12 \pm 1.10 \mu\text{m}$; unsensitized mice, $5.13 \pm 1.04 \mu\text{m}$; $P < .05$; Fig 6, A). A strong correlation was found between basement membrane thickness and enhanced pause values ($r = 0.670$; Fig 6, B), confirming the importance of tracheal integrity for normal airway function.

As expected, levels of the profibrotic cytokine TGF- β were increased in lung extracts from OVA-sensitized mice compared with that seen in tissues from unsensitized animals. More importantly, ciglitazone nebulization was also effective at significantly inhibiting this antigen-induced increase by 54% (Fig 6, C). TGF- β concentrations in lung extracts were fully correlated with basement membrane thickness ($r = 0.836$; Fig 6, D).

Thus in this model ciglitazone nebulization appears to be very effective at inhibiting several key parameters of airway inflammation.

DISCUSSION

We have demonstrated, in a murine model of asthma, that PPAR- γ was involved in the regulation of antigen-induced AHR, associated pulmonary eosinophilia, and IgE production.²⁹

We show that besides eosinophils, PPAR- γ is expressed in airway epithelium and smooth muscle on antigen sensitization and challenge and that PPAR- γ agonist nebulization reduces tracheal eosinophilic infiltrate, basement membrane thickening, and collagen deposition, as well as mucus accumulation and TGF- β levels in the lungs.

It has been shown that PPAR- γ is expressed in an epithelial cell line, where it is able to regulate IL-8 production,³¹ and in human airway epithelium as smooth muscle cells.³² This expression is increased in biopsy specimens from asthmatic patients compared with those from healthy donors and is positively correlated with basement membrane thickness and negatively correlated with lung functions.³² In our animal model of asthma, PPAR- γ expression in the lung remained undetectable by means of immunohistochemistry and was only evidenced as a very faint band by means of Western blotting in the absence of antigen sensitization. However, consistent with the study performed in human subjects,³² expression levels were greatly increased in inflammatory lung tissue on antigen sensitization and challenge and hence were reduced after nebulization of a PPAR- γ agonist, which decreases inflammation.

TGF- β can be synthesized by several cell types, such as macrophages, epithelial cells, fibroblasts, and eosinophils.^{2,6,7,33} Smad proteins, c-Jun NH2-terminal kinase, and mitogen-activated protein kinase are reported to be involved in TGF- β signaling.^{33,34} These factors are targets for PPAR- γ regulation.^{21,35} It has also been demonstrated that PPAR- γ inhibits TGF- β -induced connective tissue growth factor expression in human aortic smooth muscle cells by directly interfering with the Smad3 signaling pathway.³⁶ It is thus likely that PPAR- γ activation downregulates TGF- β production in the airways.

In addition to TGF- β , T_H2 cytokines also affect airway function and remodeling and play a key role in promoting asthmatic disease.³⁷ For instance, CCR10-driven overexpression of IL-13 in the lung-airway caused a profound phenotype characterized by eosinophilic and mononuclear inflammation, with mucus metaplasia, subepithelial fibrosis, airway obstruction, and AHR.³⁸ Similarly, IL-4, IL-5, and IL-9 caused extensive mucus metaplasia, whereas IL-9 and IL-5 induced subepithelial fibrosis and AHR.³⁹⁻⁴¹ In our previous study we showed that ciglitazone treatment reduced the pulmonary eosinophilia and the production of cytokines associated with inflammation (IL-6), eosinophilia (IL-5), and T_H2-driven

humoral immune responses (IL-4 and IL-13) in lung extracts.²⁹ Expression of these cytokines is in part under the control of known ubiquitously expressed PPAR- γ targets, such as MAP kinases, c-jun and c-fos, or nuclear factor κ B. This latter transcription factor indeed plays a key role in the development of allergic airway inflammation.^{42,43} Taken together, PPAR- γ activation in this model appears to regulate airway function and remodeling by downregulating TGF- β and T_H2 cytokines and by directly acting on several cellular targets within the airways: epithelial cells, smooth muscle cells, T lymphocytes, and eosinophils.

Finally, we have demonstrated that nebulization of ciglitazone is as effective at inhibiting AHR as combined treatment by means of oral administration and nebulization. Our results thus provide evidence for the therapeutic potential of inhaled PPAR- γ agonists in the treatment of airway asthmatic inflammation because of the multiplicity of their cellular targets to their lesser side effects compared with those of corticosteroids and because of the possibility of delivering them easily locally.

REFERENCES

1. Elias JA, Zhu Z, Chupp G, Homer RJ. Airway remodeling in asthma. *J Clin Invest* 1999;104:1001-6.
2. Minshall EM, Leung DY, Martin RJ, Song YL, Cameron L, Ernst P, et al. Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1997;17:326-33.
3. Hoshino M, Nakamura Y, Sim JJ. Expression of growth factors and remodelling of the airway wall in bronchial asthma. *Thorax* 1998;53:21-7.
4. Chetta A, Foresi A, Del Donno M, Bertorelli G, Pesci A, Olivieri D. Airways remodeling is a distinctive feature of asthma and is related to severity of disease. *Chest* 1997;111:852-7.
5. Richter A, Puddicombe SM, Lordan JL, Bucchieri F, Wilson SJ, Djukanovic R, et al. The contribution of interleukin (IL)-4 and IL-13 to the epithelial-mesenchymal trophic unit in asthma. *Am J Respir Cell Mol Biol* 2001;25:385-91.
6. Vignola AM, Chanez P, Chiappara G, Merendino A, Pace E, Rizzo A, et al. Transforming growth factor-beta expression in mucosal biopsies in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1997;156:591-9.
7. Ohno I, Nitta Y, Yamauchi K, Hoshi H, Honma M, Woolley K, et al. Transforming growth factor beta 1 (TGF beta 1) gene expression by eosinophils in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* 1996;15:404-9.
8. Corton JC, Anderson SP, Stauber A. Central role of peroxisome proliferator-activated receptors in the actions of peroxisome proliferators. *Annu Rev Pharmacol Toxicol* 2000;40:491-518.
9. Escoubet-Lozach L, Glass CK, Wasserman SI. The role of transcription factors in allergic inflammation. *J Allergy Clin Immunol* 2002;110:553-64.
10. Beato M, Herrlich P, Schutz G. Steroid hormone receptors: many actors in search of a plot. *Cell* 1995;83:851-7.
11. Barnes PJ, Pedersen S, Busse WW. Efficacy and safety of inhaled corticosteroids. New developments. *Am J Respir Crit Care Med* 1998;157(suppl):S1-53.
12. Covar RA, Leung DY, McCormick D, Steelman J, Zeitler P, Spahn JD. Risk factors associated with glucocorticoid-induced adverse effects in children with severe asthma. *J Allergy Clin Immunol* 2000;106:651-9.
13. Adcock IM. Steroid resistance in asthma. Molecular mechanisms. *Am J Respir Crit Care Med* 1996;154(suppl):S58-61.
14. Moore KJ, Rosen ED, Fitzgerald ML, Randow F, Andersson LP, Altshuler D, et al. The role of PPAR-gamma in macrophage differentiation and cholesterol uptake. *Nat Med* 2001;7:41-7.

15. Setoguchi K, Misaki Y, Terauchi Y, Yamauchi T, Kawahata K, Kadowaki T, et al. Peroxisome proliferator-activated receptor-gamma haploinsufficiency enhances B cell proliferative responses and exacerbates experimentally induced arthritis. *J Clin Invest* 2001;108:1667-75.
16. Cunard R, Ricote M, DiCampi D, Archer DC, Kahn DA, Glass CK, et al. Regulation of cytokine expression by ligands of peroxisome proliferator-activated receptors. *J Immunol* 2002;168:2795-802.
17. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* 1999;20:649-88.
18. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* 1995;83:803-12.
19. Willson TM, Cobb JE, Cowan DJ, Wiethe RW, Correa ID, Prakash SR, et al. The structure-activity relationship between peroxisome proliferator-activated receptor gamma agonism and the antihyperglycemic activity of thiazolidinediones. *J Med Chem* 1996;39:665-8.
20. Zhang X, Young HA. PPAR and immune system—what do we know? *Int Immunopharmacol* 2002;2:1029-44.
21. Delerive P, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors in inflammation control. *J Endocrinol* 2001;169:453-9.
22. Clark RB. The role of PPARs in inflammation and immunity. *J Leukoc Biol* 2002;71:388-400.
23. Harris SG, Phipps RP. The nuclear receptor PPAR gamma is expressed by mouse T lymphocytes and PPAR gamma agonists induce apoptosis. *Eur J Immunol* 2001;31:1098-105.
24. Faveeuw C, Fougeray S, Angeli V, Fontaine J, Chinetti G, Gosset P, et al. Peroxisome proliferator-activated receptor gamma activators inhibit interleukin-12 production in murine dendritic cells. *FEBS Lett* 2000;486:261-6.
25. Sugiyama H, Nonaka T, Kishimoto T, Komoriya K, Tsuji K, Nakahata T. Peroxisome proliferator-activated receptors are expressed in mouse bone marrow-derived mast cells. *FEBS Lett* 2000;467:259-62.
26. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 1998;391:79-82.
27. Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* 1998;391:82-6.
28. Patel HJ, Belvisi MG, Bishop-Bailey D, Yacoub MH, Mitchell JA. Activation of peroxisome proliferator-activated receptors in human airway smooth muscle cells has a superior anti-inflammatory profile to corticosteroids: relevance for chronic obstructive pulmonary disease therapy. *J Immunol* 2003;170:2663-9.
29. Woerly G, Honda K, Loyens M, Papin J-P, Auwerx J, Staels B, et al. Peroxisome proliferator-activated receptors alpha and gamma down-regulate allergic inflammation and eosinophil activation. *J Exp Med* 2003;198:411-21.
30. Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, et al. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 1997;156:766-75.
31. Wang AC, Dai X, Luu B, Conrad DJ. Peroxisome proliferator-activated receptor-gamma regulates airway epithelial cell activation. *Am J Respir Cell Mol Biol* 2001;24:688-93.
32. Benayoun L, Letuve S, Druilhe A, Boczkowski J, Dombret MC, Mechighel P, et al. Regulation of peroxisome proliferator-activated receptor gamma expression in human asthmatic airways: relationship with proliferation, apoptosis, and airway remodeling. *Am J Respir Crit Care Med* 2001;164:1487-94.
33. Hovevar BA, Brown TL, Howe PH. TGF-beta induces fibronectin synthesis through a c-Jun N-terminal kinase-dependent, Smad4-independent pathway. *EMBO J* 1999;18:1345-56.
34. Yamaguchi K, Shirakabe K, Shibuya H, Irie K, Oishi I, Ueno N, et al. Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. *Science* 1995;270:2008-11.
35. Desreumaux P, Dubuquoy L, Nutton S, Peuchmaur M, Englaro W, Schoonjans K, et al. Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor gamma (PPARgamma) heterodimer. A basis for new therapeutic strategies. *J Exp Med* 2001;193:827-38.
36. Fu M, Zhang J, Zhu X, Myles DE, Willson TM, Liu X, et al. Peroxisome proliferator-activated receptor gamma inhibits transforming growth factor beta-induced connective tissue growth factor expression in human aortic smooth muscle cells by interfering with Smad3. *J Biol Chem* 2001;276:45888-94.
37. Walter DM, McIntire JJ, Berry G, McKenzie AN, Donaldson DD, DeKruyff RH, et al. Critical role for IL-13 in the development of allergen-induced airway hyperreactivity. *J Immunol* 2001;167:4668-75.
38. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 1999;103:779-88.
39. Temann UA, Geba GP, Rankin JA, Flavell RA. Expression of interleukin 9 in the lungs of transgenic mice causes airway inflammation, mast cell hyperplasia, and bronchial hyperresponsiveness. *J Exp Med* 1998;188:1307-20.
40. Rankin JA, Picarella DE, Geba GP, Temann UA, Prasad B, DiCosmo B, et al. Phenotypic and physiologic characterization of transgenic mice expressing interleukin 4 in the lung: lymphocytic and eosinophilic inflammation without airway hyperreactivity. *Proc Natl Acad Sci U S A* 1996;93:7821-5.
41. Lee JJ, McGarry MP, Farmer SC, Denzler KL, Larson KA, Carrigan PE, et al. Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. *J Exp Med* 1997;185:2143-56.
42. Yang L, Cohn L, Zhang DH, Homer R, Ray A, Ray P. Essential role of nuclear factor kappaB in the induction of eosinophilia in allergic airway inflammation. *J Exp Med* 1998;188:1739-50.
43. Das J, Chen CH, Yang L, Cohn L, Ray P, Ray A. A critical role for NF-kappa B in GATA3 expression and TH2 differentiation in allergic airway inflammation. *Nat Immunol* 2001;2:45-50.