

Symposium: Nutrients, Nuclear Receptors, Inflammation, and Immunity

Nutrients, Nuclear Receptors, Inflammation, Immunity Lipids, PPAR, and Allergic Asthma¹⁻³

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ABSTRACT The peroxisome proliferator-activated receptors (PPARs) belong to the larger superfamily of steroid/thyroid nuclear receptors. PPAR γ is expressed in a number of hematopoietic cells, including dendritic cells, eosinophils, macrophages, and T cells. A number of lipids and synthetic compounds interact with PPAR γ , that, depending on the cell type, results in the regulation of specific genes. There is now a large body of data indicating that allergic asthma is the result of a predominant type-2 helper T cell immune response including IL-4, -5 and -13, eosinophilic inflammation in the lungs, mucous production, and airway hyperresponsiveness (AHR). Targeting the production of these type-2 helper T cell mediated cytokines has been proposed as a way to regulate this disease. Because PPAR γ ligands can affect T cell cytokine production in vitro, we have examined whether these ligands affect symptoms of allergic asthma in a murine model of this disease. We discuss data showing that ciglitazone and GW1929, two agonistic ligands for PPAR γ , significantly inhibited airway inflammation during allergic asthma induction. Oral treatment with ciglitazone and GW1929 inhibited airway inflammation, with less of an effect on AHR. By contrast, intranasal exposure to GW1929 significantly reduced AHR following exposure to allergen, while GW9662, a PPAR γ antagonist, had no effect. In vitro, T cells from ciglitazone-treated mice secreted significantly less IL-4 and IFN- γ in response to restimulation. These data suggest that PPAR γ agonists may be useful for the treatment of allergic asthma. J. Nutr. 136: 695–699, 2006.

KEY WORDS: • allergy • asthma • lung • PPAR • transcription factors

The peroxisome proliferator-activated receptors (PPARs)⁵ are members of the nuclear-receptor superfamily and includes three family members, PPAR α , PPAR β/δ , and PPAR γ (1). Ligands for the PPAR family tend to be fatty acids or metabolites, including prostaglandins, and interaction with these

receptors regulates the transcription of specific genes (2). Although expression of PPAR α and PPAR β/δ can be observed in a variety of cell types, PPAR γ seems to have a more preferential expression, and in particular, is expressed in myeloid cells that include dendritic cells, macrophages, and eosinophils, as well as T cells (3–6). Embryos lacking PPAR γ do not develop past 10.5–11.5 d postcoitus, however, those carrying 1 copy of the PPAR γ gene exhibit increased B cell responses, and when exacerbated experimentally they induce arthritis (7–9). A number of synthetic compounds have been developed that act as agonists or antagonists of PPAR γ . These include the thiazolidinediones, rosiglitazone, ciglitazone, and triglitazone, and GW1929, which all act as agonists, and GW9662, which acts as an antagonist (2). Agonists of PPAR γ have been shown to regulate lipid and sugar metabolism by transcriptionally regulating cells of the adipose tissue (2).

The identification of PPAR γ receptors in several different cell types of the immune system has prompted the investigation of PPAR γ as an immune-system regulator. One putative endogenous ligand for PPAR γ , 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂, as well as many synthetic ligands, have been shown to regulate macrophage activation by IFN- γ (5). In vitro, PPAR γ agonists

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⁵ Abbreviations: AHR, airway hyperresponsiveness; OVA, ovalbumin; PPAR, peroxisome proliferator-activated receptor; TCR, T cell receptor; T_{H1}, type-1 helper T cell; T_{H2}, type-2 helper T cell.

inhibit macrophage production of the inflammatory cytokine tumor necrosis factor- α and the induction of inducible nitric oxide synthetase (5). In addition, PPAR γ agonists have been shown to downregulate the production of type-1 helper T cell (T_H1) cytokines IFN- γ and IL-2, and to inhibit T cell proliferation in vitro (3,10). In vivo, PPAR γ ligands have been shown to suppress the symptoms of immune-mediated diseases in murine models, including experimental autoimmune encephalomyelitis, collagen-induced arthritis, and inflammatory bowel disease, all T_H1-mediated diseases (11–13). Thus PPAR γ agonists seem to have potent anti-inflammatory effects on T_H1-mediated immune responses and diseases.

The observation that PPAR γ is expressed in T cells and may be able to modulate T-cell cytokine production prompted us to examine its role in a murine model of a type-2 helper T cell (T_H2)-mediated disease, allergic asthma. In humans, allergic asthma is a chronic inflammatory disease of the airways. At least 26 million Americans are reported to have some symptoms of asthma, with 8.6 million under the age of 18. Allergic asthma is known to involve an enhanced T_H2 response to airborne allergens (14). This response includes increased presence of T_H2 cytokines IL-4, -5, and -13 in lung and bronchoalveolar lavage, increased eosinophil infiltrate in the lung and bronchoalveolar lavage due to IL-5 (and perhaps IL-4), goblet-cell hyperplasia, and increased mucous production due to the presence of IL-13 (15). In murine models of allergic asthma, introduction of antigen-specific T_H2 cells alone, or IL-4 and IL-13 alone, results in airway hyperresponsiveness (AHR), and a blockade of these cytokines prevents the development of AHR in mice (15,16). Although an increase in overall and antigen-specific serum IgE is commonly observed, a role for IgE in this disease is controversial, and murine models of this disease do not have a requirement for IgE (17). These events culminate in AHR, and murine models have been reported that exhibit most of these symptoms (17).

Although there is no cure for this disease, a number of treatments are currently used in the clinic to relieve or reduce the incidence of asthmatic attacks exhibited as AHR with difficult breathing. These treatments include corticosteroids, β 2 adrenoreceptor agonists, and leukotriene antagonists (14). However, long-term use of some of these drugs has been associated with significant side effects including an increased susceptibility to infectious diseases. Better approaches for treating asthma will require a better understanding of the mechanisms underlying disease development. Recent efforts have targeted T_H2 cytokines, and clinical studies in humans have shown that blockade of the T_H2 cytokine IL-4 can relieve some of the symptoms of asthma, although efforts at targeting other T_H2 cytokines, such as IL-5, have led to disappointing results, perhaps due to an inadequate reduction in the levels of this cytokine in the lung (16).

We have examined whether, by regulating specific cytokine production in T cells, ligands for PPAR γ can regulate the development of this TH2-mediated disease in a murine model of allergic asthma. The data discussed here indicates that agonistic ligands for PPAR γ significantly inhibit the symptoms of allergic asthma. We found that orally delivered PPAR γ agonists ciglitazone and GW1929 significantly prevented airway inflammation. Furthermore, oral treatment with ciglitazone suppressed experimental asthma after symptoms had already developed. There was not a significant effect on AHR from oral treatment with either ciglitazone or GW1929. In contrast, intranasal exposure to GW1929 significantly reduced AHR and other markers of experimental asthma when delivered following exposure to an allergen. The PPAR γ antagonist GW9662 had no effect on experimental allergic asthma. In

vitro, T cells from ciglitazone-treated mice secreted significantly less IL-4 and IFN- γ . These data suggest that PPAR γ agonists may be useful for the suppression of TH2-driven diseases like asthma.

MATERIALS AND METHODS

Mice. Groups of age- and sex-matched BALB/c mice (Jackson Laboratories) at 9–11 wk were used for the experiments. All mice were fed synthetic diets as described in a previous paper (18). T cell receptor (TCR) transgenic mice (OT-II, C57BL/6), specific for ovalbumin (OVA) were also obtained from Jackson Laboratories. These experiments were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University.

Chemical and reagents. PPAR γ ligands agonists Ciglitazone and GW1929, antagonist GW1929, and Methylcholine were obtained from Sigma.

Induction of allergic asthma. Mice were induced to develop allergic asthma as described in (18,19). Alternatively, we used OT-II TCR transgenic mice to induce allergic asthma in a 5-day abbreviated protocol as follows. OT-II transgenic mice were exposed intranasally to 40 μ g of OVA in sterile saline, every day for 4 d. Twenty-four hours after the last intranasal challenge, mice were analyzed for AHR as described below. Control mice were challenged intranasally with PBS alone but were otherwise treated in the same way.

Analysis of AHR. AHR was analyzed using a single-chamber Buxco whole-body plethysmograph, and data was analyzed using the BioSystem XA software for Windows (20).

Drug treatment protocols. We used 2 different Ciglitazone treatment protocols as described in (18). We used 3 different GW1929 treatment protocols. In the first protocol, mice were given oral doses of GW1929 in their diets, which included 0.12 μ M GW1929/d per mouse, with control mice fed the same diets without GW1929 for 1 wk prior to the induction of allergic asthma. The mice were continued on these diets for the remainder of the study. In the second protocol, mice were primed with OVA in the absence of GW1929, then, on the day of the first intranasal challenge, they were exposed intraperitoneally to 5 mg/kg GW1929 in PBS, followed in 4 h by intranasal challenge with OVA as described above. In the third protocol, mice were primed with OVA in the absence of GW1929, then, on the day of the first intranasal challenge, they were exposed intranasally to 5 mg/kg GW1929 in PBS, followed in 4 h by intranasal challenge with OVA as described above. Mice treated with PPAR γ antagonist GW9662 were exposed in a similar manner to the third GW1929 protocol, using 5 mg/kg GW9662 per dose per mouse. Control mice received vehicle alone.

Proliferation assays and ELISAs. Analysis of T cell proliferation and cytokine secretion was determined as previously described (18).

Analysis of histopathology. Histopathology was performed and analyzed as previously described (18).

Analysis of serum IgE levels by ELISA. IgE levels in serum was analyzed as previously described (18).

Statistics. The data were analyzed by ANOVA, with treatment as a between-subject factor. Fisher's post-hoc test was used to determine significance. The level of significance was set at $P < 0.05$. Data were analyzed using StatView (SAS Institute).

RESULTS

Regulation of airway inflammation by oral PPAR γ agonist. Clark et al. (3) have previously reported that PPAR γ ligands could affect IL-2 secretion by purified T cells stimulated in vitro with anti-TCR antibodies. We have shown that PPAR γ agonists inhibit the secretion of the T_H2 cytokine IL-5 in vitro (18). We therefore examined the effect of ciglitazone (EC₅₀ 3 μ M), an orally available agonist of PPAR γ , on a murine model of TH2-mediated disease, allergic asthma. Mice were immunized with OVA plus alum (which preferentially generates a T_H2 type response) followed by intranasal challenge

with OVA (or PBS as a control) over a 3-d period (19). Lungs were isolated from these animals, fixed and stained to determine inflammatory cell infiltration, epithelial hyperplasia, and mucous production, followed by scoring as described (18). Mice challenged with OVA in this manner developed increased inflammation and epithelial-cell hyperplasia in the lung (average histopathology score 3.0 ± 0.7 , $n = 6$) (17,18). By contrast, mice that had been fed diets containing $0.12 \mu\text{M}$ ciglitazone prior to disease induction developed significantly less lung inflammation (average histopathology score 1.0 ± 0.3 , $P < 0.05$, $n = 6$). In addition to inflammation, the asthmatic lung is characterized by increased production of mucous by goblet cells that occur as a result of IL-13 secretion by T_H2 cells (15). We analyzed mucous secretion in the lungs of the mice, and found that the PPAR γ agonist-treated mice had less mucous (average score 1.6 ± 0.5 , $n = 6$) in the lungs compared with the lungs from control treated mice (average score 2.6 ± 0.5 , $n = 6$), although the difference was not statistically significant. These data suggest that the PPAR γ agonist ciglitazone significantly decreased the amount of cellular infiltrates and decreased mucous production in the lungs of mice induced to develop allergic asthma compared with controls.

In order to determine whether PPAR γ agonist treatment was effective after mice had already been primed to develop experimental asthma (immunized with the allergen), we also set up experiments where mice were immunized with OVA first and then fed diets containing $0.12 \mu\text{M}$ ciglitazone during the intranasal exposure to OVA. We found that late treatment with ciglitazone also resulted in significantly reduced airway inflammation in these mice (average histopathology score 0.5 ± 0.3 , $P < 0.05$, $n = 6$). By contrast, late treatment with ciglitazone did not affect mucous production (average score 2.3 ± 0.5 , $n = 6$). Together these data suggest that ciglitazone can reduce lung inflammation when given during priming, as well as after priming during intranasal challenge, although late exposure does not affect mucous production.

Suppression of T cell cytokine production following in vivo ciglitazone exposure. The development of allergic asthma in this model is accompanied by antigen-specific secretion of IL-4 following in vitro restimulation [reviewed in (15)]. As we were observing reduced lung inflammation in mice that were fed diets containing ciglitazone, we examined cytokine secretion and proliferation of T cells in response to restimulation in vitro with OVA. Splenocytes from mice treated as described above (i.e., induced to develop allergic asthma with or without ciglitazone in vivo) were stimulated in vitro with OVA in the absence of further ciglitazone treatment, and cytokine production and proliferation was determined. We found that T cells from mice treated with ciglitazone secreted significantly reduced levels of IFN- γ (4031 ± 215 vs. 1996 ± 393 pg/mL, $n = 6$, $P < 0.05$) and IL-4 (463 ± 103 vs. 95 ± 37 pg/mL, $n = 6$, $P < 0.05$). Analysis of splenocytes from mice treated with late ciglitazone did not show reduced secretion of these cytokines (data not shown), suggesting that ciglitazone may affect priming of T cells for cytokine secretion, and that treatment of mice with ciglitazone after priming does not affect their ability to secrete cytokine in the absence of ciglitazone. In contrast, antigen-specific proliferation was not affected in any of the treatments, which suggests that ciglitazone does not affect the ability of T cells to proliferate in the absence of further ciglitazone exposure (data not shown). Ciglitazone treatment has no effect on antigen-specific IgE production in vivo. Immunization with OVA plus alum results in significant increases in IgE, although the role of IgE in the development of experimental asthma is controversial (17). We found no difference in antigen-specific IgE in the serum of mice with allergic

asthma with or without the two treatments with ciglitazone. All mice immunized with OVA plus alum had significantly increased OVA-specific IgE compared with unsensitized control mice (data not shown).

PPAR γ agonists reduce AHR in mice with allergic asthma. A major physiological effect of allergic asthma is the development of AHR (14). AHR is an acute response in the asthmatic patient and the development of elevated AHR requires immediate treatment. We therefore wanted to determine the effect of PPAR γ agonists on the development of AHR in this murine model of allergic asthma. For these experiments, we used the high affinity ligand for PPAR γ , GW1929 (2). Similar groups of mice were fed diets containing GW1929 ($0.12 \mu\text{M}$) or control diets without this PPAR γ analog. The mice were immunized and challenged as described above. Twenty-four hours after the final challenge, mice were analyzed for AHR using a Buxco whole-body plethysmograph (21). Our data indicated that oral GW1929 at this concentration did not affect AHR in the mice. The experiments were repeated using intraperitoneal delivery of GW1929 (5 mg/kg) that led to reductions in AHR, however, these differences did not reach significance (data not shown). We reasoned that intranasal exposure of mice with allergic asthma may be more efficacious in affecting the acute symptoms of this disease. Mice were immunized with OVA or alum alone, and on each day of the intranasal OVA challenge, mice were exposed to GW1929 (5 mg/kg), followed 4 h later by the intranasal OVA challenge. Twenty-four hours after the final intranasal challenge, AHR was determined. The results showed that intranasal treatment with GW1929 resulted in significantly reduced AHR. In addition, the histopathology scores of the lungs from the GW1929-treated mice showed reduced airway inflammation. We tested the effect of GW1929 on a second model of experimental asthma, the OVA-specific TCR transgenic mice, OT-II (22). These mice carry a TCR specific for an epitope within OVA, and up to 90% of their T cells carry this transgene and are reactive against OVA. The asthma that develops in this model is accelerated since there is a high proportion of antigen-specific T cells. Intranasal exposure of OVA 4 times, once a day, was sufficient to induce airway inflammation and AHR in these mice. OT-II TCR transgenic mice were exposed to OVA intranasally, and the mice were evaluated 24 h following the final exposure for the development of AHR. The data indicated that 4-d intranasal exposure of these OVA-specific TCR transgenic mice induced AHR. However, intranasal exposure of GW1929 only 4 h before OVA exposure resulted in inhibition of AHR. In contrast, similar treatments with the PPAR γ antagonist [GW9662, (2)] did not lead to the inhibition of AHR. Altogether, these data

Table 1

Effects of PPAR γ agonists and antagonists on allergic asthma in mice

Compound	Route of administration	Dose	AHR	Effect on cytokines
Ciglitazone	Oral (feeding)	$0.12 \mu\text{M}$	ND	Reduced
GW1929	Oral (feeding)	$0.12 \mu\text{M}$	No effect	ND
GW1929	Intraperitoneally	5 mg/kg	Reduced but not significant	ND
GW1929	Intranasal	5 mg/kg	Significant reduction	ND
GW9662	Intranasal	5 mg/kg	No effect	ND

ND, not done.

indicate that agonists of PPAR γ may be efficacious in treating the acute symptoms of allergic asthma including AHR (Table 1).

DISCUSSION

In this report, we discuss data indicating that agonistic ligands for PPAR γ reduce airway inflammation, as well as AHR, in a murine model of allergic asthma. Our data suggest that ligands for PPAR γ may be efficacious in treating allergic asthma (18). Similar findings have been reported by other investigators (6,23,24). PPAR γ has been shown to be expressed in several relevant target cells and tissues where ligands could potentially affect the development of airway inflammation and AHR. This includes T cells, dendritic cells, macrophages, eosinophils and airway epithelia, and smooth muscle (3–6,23–25).

Benayoun et al. (26) reported that PPAR γ protein is expressed in human subjects who were asthmatic and found elevated expression in the bronchial submucosa, the airway epithelium, and smooth muscle cells compared with control subjects. Similarly, Patel et al. (27) reported that human airway smooth muscle cells expressed PPAR γ , and that exposure of these cells to PPAR γ ligands could inhibit their secretion of granulocyte macrophage colony stimulating factor as well as granulocyte colony stimulating factor. Others have reported that ligands for PPAR γ can affect migration and immunogenicity of dendritic cells, eosinophils, the response of airway epithelial cells, in addition to their well-known effect on macrophage activation (4–6,24). Furthermore, our data (18), that of Cunard et al. (10), and Clark et al. (3) support a role for PPAR γ in regulating cytokine secretion by T cells. However, it is not clear what the targets of PPAR γ ligands are in the inflamed lung. Dombrowicz et al. (6,25) have suggested that eosinophils are the main target, as PPAR γ ligands affect the ability of eosinophils to migrate in response to chemokine signals, thus affecting allergic airway responses. However, a role for eosinophils in regulating AHR is controversial, with data supporting as well as negating a role for these cells in mouse models (28,29). Similarly, anti-IL-5 trials in humans, which target eosinophils, have not been as successful as predicted, although this may be due to inadequate depletion of eosinophils in the airways (30). Eosinophils may be important in regulating long-term lung remodeling in this disease, although the effect of PPAR γ ligands on this was not examined by Woerly et al. (6,28,30).

It has also been suggested that dendritic cells are the targets for PPAR γ ligands in modulating allergic asthma, in that pretreatment with PPAR γ analog rosiglitazone reduced the ability of transferred dendritic cells to induce the proliferation of T cells and subsequent development of AHR, and that PPAR γ analogs affect the immunogenicity of dendritic cells (4,23). Finally, the expression of PPAR γ in lung epithelia as well as airway smooth muscle, and the effects of PPAR γ ligands on these cells suggest that these cells may also be targets of PPAR γ ligands (25–27,31). Indeed, in most of the studies analyzing the role of PPAR γ ligand on AHR, including our studies, intranasal delivery of these ligands exhibited the best inhibition of AHR (6,25).

It is thus likely that PPAR γ analogs target dendritic cells, T cells, and lung epithelia to affect airway inflammation and AHR. Indeed, in our studies, continuous treatment with ciglitazone was able to prevent airway inflammation and subsequent cytokine production by in vitro restimulated T cells. In contrast, late ciglitazone treatment (during airway challenge) led to reduction in airway inflammation, but not mucous production, and had no effect on cytokine production by in

vitro rechallenged T cells. These data would support the idea that PPAR γ ligands affect priming of T cells to reduce their subsequent effector function. This could happen at the T cell level, or at the level of dendritic cells, as has been suggested (4,23). Careful studies will have to be performed to decipher the cellular target of these compounds. In addition, PPAR γ analogs inhibit the AHR response, which is an acute response in the asthmatic lung, which suggests that signal transduction through the PPAR γ may have a direct and immediate effect on effector cell functions in the inflamed lung. Regardless, these and other studies provide strong support for a role for PPAR γ in regulating airway inflammation and AHR, and suggest that agonistic compounds may represent treatments for human asthma.

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