



Suppression of allergen-induced airway inflammation and immune response by the peroxisome proliferator-activated receptor- α agonist fenofibrate

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Received 7 August 2007; received in revised form 20 November 2007; accepted 21 November 2007

Abstract

In the present study, we have assessed the effect of the peroxisome proliferator-activated receptor- α (PPAR α) agonist fenofibrate on allergen-induced airway inflammation and immune response. C57BL/6 or PPAR α knock-out (PPAR $\alpha^{-/-}$) mice were sensitized with ovalbumin and challenged with ovalbumin alone or with ovalbumin+lipopolysaccharides. Fenofibrate was administered to allergen-exposed animals during challenge only or from the day prior to sensitization to the end of challenge. Inflammation and immune response were assessed by determining cell counts and cytokine levels in bronchoalveolar lavage fluids, expression of the transcription factors Gata-3 and T-bet in lung tissue and ovalbumin-specific IgE and IgG2a in serum. Treatment with fenofibrate (0.15–15 mg/day) during allergen challenge dose-dependently reduced airway inflammatory cell infiltrate induced by ovalbumin in C57BL/6 mice. Reduction reached 74.3% ($P<0.001$) in animals treated with 15 mg/day of the PPAR α agonist, whereas this treatment failed to suppress cell infiltrate induced by allergen in PPAR $\alpha^{-/-}$ mice. In addition, when administered from the day prior to sensitization to the end of challenge, fenofibrate (15 mg/day) triggered switching of the immune response to allergen towards a Th1 profile, as evidenced by an increase in IgG2a levels, a reduction in IL(interleukin)-4 and IL-5 together with an increase in interferon- γ , and a decrease in Gata-3/T-bet expression ratio. Upon challenge with ovalbumin+lipopolysaccharides, sensitized mice developed a severe inflammatory response characterized by infiltration of eosinophils, neutrophils, lymphocytes and macrophages and by increased release of IL-4, IL-5, tumor necrosis factor- α , macrophage-inflammatory protein-2 and monocyte chemoattractant protein-1. Administration of fenofibrate during allergen challenge dramatically reduced all responses. In conclusion, our data clearly demonstrate that fenofibrate exhibits an anti-inflammatory activity in allergic asthma, including in severe conditions, and that the PPAR α agonist is also capable of switching the immune response to allergen towards a Th1 profile when given from the day prior to sensitization.

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Keywords: Fenofibrate; PPAR α ; Inflammation; Allergy; Asthma

1. Introduction

Allergen-induced airway inflammation that characterizes asthma involves infiltration of eosinophils in the lung and is associated with elevated allergen-specific immunoglobulin (Ig) E in serum (Barnes, 1996). These features result from an aberrant T helper 2 (Th2) immune response to allergen that leads to the recruitment of CD4⁺ Th2 lymphocytes in the lung. Th2 cells then produce the Th2 cytokines interleukin-4 (IL-4) that switches B cells towards IgE synthesis and IL-5 that promotes eosinophil infiltration in the airways (Barnes et al.,

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1998; Larche et al., 2003). Th2 cell differentiation is driven by the transcription factor Gata-3, which controls expression of Th2 cytokines and is suppressed by the Th1 promoting transcription factor T-bet (Rengarajan et al., 2000).

Fibrates, including fenofibrate, are ligands for the peroxisome proliferator-activated receptor- α (PPAR α), a transcription factor that activates expression of a variety of genes implicated in the transport and catabolism of fatty acids (Desvergne and Wahli, 1999; Francis et al., 2003). This pathway largely explains the normolipidemic action of fibrates. PPAR α was first identified for its role in the regulation of lipid metabolism, but observation that mice deficient in the nuclear receptor displayed a prolonged ear-swelling in response to the pro-inflammatory mediator leukotriene B4 led to the discovery that PPAR α controls also inflammation (Devchand et al., 1996). In consistency with this observation, PPAR α activators, including fenofibrate, were reported to exhibit an anti-inflammatory activity in animal models of inflammatory diseases, including atherosclerosis, colitis, contact dermatitis and rheumatoid arthritis (Duez et al., 2002; Okamoto et al., 2005; Sheu et al., 2002; Tanaka et al., 2001). In addition, administration of fenofibrate in patients with atherosclerosis resulted in decreased serum levels of the pro-inflammatory cytokines IL-6 and tumor necrosis factor- α (TNF- α) (Madej et al., 1998; Staels et al., 1998).

Allergic asthma represents a major health problem worldwide. In this lung disease, inflammation contributes to airway dysfunction and tissue remodeling and is a main target for efficient therapies. Glucocorticoids are nowadays the most potent anti-inflammatory drug in asthma, but they exhibit some major side effects and have poor activity in patients with severe forms of the disease (Barnes, 2006; Tillie-Leblond et al., 1999). Therefore, there is a need for the development of alternative or new therapies to treat inflammation associated with asthma (Barnes, 2006; Belvisi et al., 2004). In a recent study, we reported that fenofibrate dose-dependently suppressed airway inflammation induced by lipopolysaccharides (LPS) in mouse lung, suggesting that the PPAR α agonist may have a therapeutic potential in airway inflammatory diseases (Becker et al., 2006; Delayre-Orthez et al., 2005).

The aim of the present study was to assess the effect of fenofibrate on allergen-induced airway inflammation and immune response. This work was carried out in a murine model of ovalbumin-sensitization and challenge characterized by airway eosinophilia and allergen-specific IgE, as well as in a model of severe allergic asthma triggered by challenge of sensitized mice with ovalbumin+LPS (Delayre-Orthez et al., 2004).

2. Materials and methods

2.1. Animals

Nine-week-old male C57BL/6 mice were purchased from Charles River Laboratories (Saint-Germain-sur-l'Arbresle, France). Male wild-type (PPAR $\alpha^{+/+}$) and homozygous PPAR α knockout (PPAR $\alpha^{-/-}$) mice were expanded from

breeding pairs and used at the age of 9 weeks (Lee et al., 1995). Animals were maintained under controlled environmental conditions with a 12 h/12 h light/dark cycle according to the EU guide for use of laboratory animals. Food (UAR-alimentation, Villemoisson, France) and tap water were available *ad libitum*. Animal experimentation was conducted with the approval of the government body that regulates animal research in France.

2.2. Ovalbumin-sensitization and challenge

Mice were sensitized and challenged with ovalbumin (chicken egg albumin, Grade V, Sigma Chemical, St Louis, USA), as previously described (Delayre-Orthez et al., 2004). Briefly, animals were sensitized on days 0 and 7 by i.p. injection of 50 μ g ovalbumin adsorbed on aluminum hydroxide (Sigma Chemical) in saline. Control animals received i.p. injections of aluminum hydroxide alone. Mice were challenged on days 18, 19, 20 and 21 by intranasal instillations of 10 μ g ovalbumin in saline, or of saline alone for controls. Intranasal administrations (12.5 μ l per nostril) were carried out under light anaesthesia (50 mg/kg ketamine (Imalgene, Merial, Lyon, France) and 3.33 mg/kg xylazine (Rompun, Bayer, Puteaux, France) given i.p.).

2.3. Challenge of sensitized mice with allergen and LPS

Sensitized mice (see above) were challenged with ovalbumin+LPS, as previously described (Delayre-Orthez et al., 2004). Briefly, 25 μ l of saline containing ovalbumin (10 μ g) and LPS (100 ng, *Escherichia coli* O55:B5, Sigma Chemical) were administered once daily on days 18, 19, 20 and 21 by intranasal instillation.

2.4. Mouse treatment with fenofibrate

Fenofibrate (Sigma Chemical) suspended in 1% carboxymethylcellulose (low viscosity, Sigma Chemical) in water was administered once daily p.o.. In one set of experiments, animals were treated during allergen challenge (10-day treatment from day 12 to day 21 of the protocol of ovalbumin-sensitization and challenge). Duration of treatment was selected from a previous study showing protection against LPS-induced airway inflammation (Delayre-Orthez et al., 2005). In another set of experiments, fenofibrate was given from the day prior to the first sensitization to the last day of challenge (23-day treatment). Control animals received administrations of vehicle.

2.5. Collection of fluids and tissues

Eighteen to twenty-four hours after the last allergen challenge, mice were anaesthetized by i.p. injection of ketamine (150 mg/kg) and xylazine (10 mg/kg). Blood was drawn from the abdominal vein for serum determination of ovalbumin-specific IgE. Sera were collected by centrifugation (3000 g for 15 min at 4 °C) and stored at -20 °C until use. After blood collection, the trachea was cannulated to perform

bronchoalveolar lavages. Lungs were lavaged by 10 instillations of 0.5 ml ice-cold saline supplemented with 2.6 mM EDTA (saline-EDTA). Bronchoalveolar lavage fluids recovered from the two first instillations were centrifuged (160 g for 5 min at 4 °C). The resulting supernatant was stored at –20 °C until cytokine measurements. After collection of bronchoalveolar lavage fluids, lungs were perfused through the pulmonary artery with ice-cold phosphate-buffered saline, collected, frozen in liquid nitrogen and stored at –80 °C until RNA extraction.

2.6. Immunoglobulin assay

Ovalbumin-specific IgE and IgG2a serum levels were determined by enzyme-linked immunosorbent assay. Microtiter plates were coated overnight at 4 °C with ovalbumin (2 µg/well in 0.1 M NaHCO₃, pH 8.3) and blocked for 2 h at 37 °C with 1% bovine serum albumin in phosphate buffered saline. Serum samples or pooled serum from ovalbumin-sensitized animals (internal standard) were then incubated overnight at 4 °C. After three washings with phosphate-buffered saline containing 0.05% Tween-20, a biotinylated anti-mouse IgE or IgG2a antibody (clones R35-118 and R19-15, respectively ; PharMingen, San Diego, CA) was added to the wells and incubated for 1 h at room temperature. The plates were then washed and incubated with an extravidin-horseradish peroxidase conjugate (Sigma Chemical) for 30 min at room temperature. After final washings, the horseradish peroxidase substrate, tetramethylbenzidine (PharMingen) was added and coloration was allowed to develop for 15–20 min. The reaction was stopped by addition of 0.5 M H₂SO₄ and absorbance intensity was read at 450 nm. Serum levels of ovalbumin-specific Igs were expressed as arbitrary units (a.u.) relative to the absorbance of the internal standard.

2.7. Determination of total and differential cell counts in bronchoalveolar lavage fluids

Bronchoalveolar lavage fluids were centrifuged (160 g for 5 min at 4 °C) to pellet cells and erythrocytes were lysed by hypotonic shock. Cells were then resuspended in 500 µl ice-cold saline-EDTA and total cell counts were determined using a hemocytometer (Neubauer's chamber). Differential cell counts were assessed on cytologic preparations obtained by cytocentrifugation (Cytospin 3, Shandon Ltd, Runcorn, Cheshire, UK) of 200 µl of diluted bronchoalveolar lavage fluid (250,000 cells/ml in ice-cold saline-EDTA). Slides were stained with Diff-Quik (Dade Behring AG, Düringen, Switzerland) and determinations were performed by counting at least 400 cells for each preparation. Eosinophil, neutrophil, lymphocyte and macrophage numbers were expressed as absolute numbers from total cell counts.

2.8. Cytokine and chemokine assay

IL-4, IL-5, TNF-α, interferon-γ (IFN-γ), macrophage-inflammatory protein-2 (MIP-2) and monocyte chemoattractant

protein-1 (MCP-1) were quantified in bronchoalveolar lavage fluids using capture enzyme-linked immunosorbent assay kits according to instructions provided by the manufacturer (BD PharMingen, Le Pont-de-Claix, France for IL-4, IL-5, IFN-γ and TNF-α ; R&D Systems Europe, Lille, France for MIP-2 and MCP-1).

2.9. Isolation of total RNA and reverse transcription

Total RNA was prepared from lung tissue using Tri-Reagent® (1 ml/100 mg of tissue, Molecular Research Center Inc., Cincinnati, OH). RNA pellets were suspended in nuclease-free water. Concentration of each preparation was determined spectrophotometrically (GeneQuant spectrophotometer, Pharmacia, Freiburg, Germany). Reverse transcription mixtures consisted of 25 µl of 1X reverse transcriptase buffer containing 1 mM of each dNTP, 1 µg random hexamers, 40 U RNasin, 200 U RNase H(–)–Moloney leukemia virus reverse transcriptase (all reagents from Promega, Charbonnières, France) and 5 µg of denaturated total RNA. Reverse transcription reaction was allowed to proceed for 60 min at 37 °C, inactivated by heating (99 °C for 5 min) and cooled on ice. cDNAs were stored at –20 °C until use.

2.10. Quantitative polymerase chain reaction

Quantitative polymerase chain reaction of the Th1 and Th2 specific transcription factors, T-box expressed in T cells (T-bet) and Gata-binding protein 3 (Gata-3), respectively, was performed by real-time fluorescent polymerase chain reaction (LightCycler®, Roche Diagnostics, Meylan, France). Reactions were conducted in a 20 µl final volume containing 200 nM of each specific sense and anti-sense primers, 3 mM MgCl₂, 2 µl of appropriately diluted cDNA (standard or sample) and 2 µl of LightCycler FastStart DNA master SYBR Green I mix (Roche Diagnostics). After denaturation at 95 °C for 8 min, mixtures were proceeded through cycles of denaturation (15 s at 95 °C), annealing [10 s at 63 °C (Gata-3), 62 °C (T-bet) or 56 °C (GADPH, housekeeping gene)], and extension [72 °C for 19 s (Gata-3) or 10 s (T-bet and GADPH)]. Specificity of amplification was confirmed by melting curve analysis and sequencing. Primer sets used for amplification were as follows (5'-3'): Gata-3 sense: TCAGCCACACCCATTACCACCT; Gata-3 antisense: CGGGTCTGGATGCCTTCTTTCTTCA; T-bet sense: AATCGACAACAACCCCTTTG; T-bet antisense: AACTGTGTTCCCGAGGTGTC; GAPDH sense: GTGAACGGATTGGCCG; GAPDH antisense: CCATCTTCCAGGAGCGA. Standard samples of cDNAs for quantification of polymerase chain reaction products were prepared by polymerase chain reaction, separated by electrophoresis on agarose gels, purified using the QIAEX II gel extraction kit (Qiagen, Courtaboeuf, France) and quantified spectrophotometrically.

2.11. Statistical analysis of the data

Data are presented as means±SEM. Statistical differences were analyzed from raw data by analysis of variance followed

by unpaired two-tailed Student's *t*-test for comparison of single pairs of groups or Bonferonni's or Dunnett's test for multiple comparisons. Data were considered significantly different when $P < 0.05$.

3. Results

3.1. Fenofibrate reduced allergen-induced inflammatory cell infiltration in ovalbumin-sensitized and challenged mice

Bronchoalveolar lavage fluids from control C57BL/6 mice (vehicle-exposed) contained few cells differentiated exclusively as macrophages (Table 1). Ovalbumin-sensitized and challenged animals exhibited a significant increase in total cell number when compared to control mice (Table 1). This increase resulted from a marked eosinophilia and a significant enhancement in the number of macrophages. The effect of fenofibrate on allergen-induced inflammatory cell infiltration was assessed in animals treated with the PPAR α agonist during allergen challenge only. Dose (0.15 to 15 mg/day) and duration of the treatment (10-day) were selected from our previous study showing protection against LPS-induced airway inflammation (Delayre-Orthez et al., 2005). As shown on Fig. 1, mouse treatment with fenofibrate dose-dependently suppressed allergen-induced inflammatory cell infiltration. Reduction in total cell, eosinophil and macrophage number reached 74.3% ($P < 0.001$), 81.4% ($P < 0.001$) and 57.9% ($P < 0.001$), respectively in animals exposed to 15 mg/day of the PPAR α agonist (Fig. 1).

3.2. PPAR α mediated the anti-inflammatory activity of fenofibrate

The role of PPAR α in the anti-inflammatory activity of fenofibrate was assessed using PPAR α ^{-/-} mice. Upon ovalbumin-sensitization and challenge, animals invalidated for PPAR α displayed a greater inflammatory cell infiltration when compared to PPAR α ^{+/-} mice, confirming that PPAR α exhibits

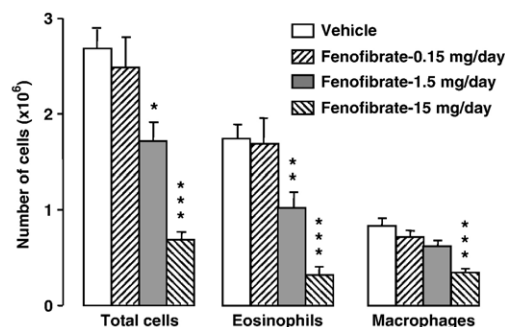


Fig. 1. Total and differential cell counts in bronchoalveolar lavage fluids from allergen-sensitized and challenged C57BL/6 mice treated with increasing doses of fenofibrate (0.15 to 15 mg/day) or with its vehicle during allergen challenge. Data are mean $\pm$ SEM of $n = 7$ –19 animals. Statistically significant differences at (*) $P < 0.05$, (**) $P < 0.01$ and (***) $P < 0.001$ when compared to vehicle-treated animals.

an anti-inflammatory activity in the lung (Table 2). Fenofibrate (15 mg/day) given during allergen challenge suppressed ovalbumin-induced inflammatory cell infiltration in PPAR α ^{+/-} mice (Table 3). By contrast, it failed to modify cell influx triggered by allergen in PPAR α ^{-/-} animals, demonstrating that PPAR α mediates the anti-inflammatory activity of fenofibrate (Table 3).

3.3. Fenofibrate modulated orientation of the immune response to allergen

In our model of allergen-sensitization and challenge, invalidation of PPAR α resulted in changes in the orientation of the immune response induced by allergen. Indeed, upon sensitization and challenge with ovalbumin, PPAR α ^{-/-} mice exhibited an increase in ovalbumin-specific IgE associated with a profound decrease in ovalbumin-specific IgG2a in serum when compared to PPAR α ^{+/-} mice (Table 2). These changes were associated with increased levels of the Th2 cytokines IL-4 and IL-5 and decreased production of the Th1 cytokine IFN- γ in bronchoalveolar lavage fluids (Table 2). As well, mice invalidated for PPAR α displayed a 1.5-fold increase in Gata-3/T-bet expression ratio in lung tissue (Table 2). All together,

Table 1

Number of inflammatory cells and level of cytokines in bronchoalveolar lavage fluids from control mice (vehicle), sensitized mice challenged with allergen only (ovalbumin) or sensitized mice challenged with allergen in the presence of LPS (ovalbumin+LPS)

		Vehicle	Ovalbumin	Ovalbumin+LPS
Number of cells ($\times 10^5$)	Total cells	1.95 $\pm$ 0.16	17.33 $\pm$ 2.80 ^a	43.79 $\pm$ 6.58 ^{a,b}
	Lymphocytes	0.00 $\pm$ 0.00	0.10 $\pm$ 0.03	0.95 $\pm$ 0.28 ^{a,b}
	Neutrophils	0.01 $\pm$ 0.00	0.14 $\pm$ 0.05	4.19 $\pm$ 0.75 ^{a,b}
	Eosinophils	0.00 $\pm$ 0.00	10.68 $\pm$ 2.29 ^a	25.92 $\pm$ 5.45 ^{a,b}
	Macrophages	1.94 $\pm$ 0.16	6.41 $\pm$ 0.53 ^a	12.73 $\pm$ 0.92 ^{a,b}
Cytokines (pg/mL)	IL-4	33.7 $\pm$ 5.8	31.9 $\pm$ 3.0	128.0 $\pm$ 12.0 ^{a,b}
	IL-5	37.8 $\pm$ 4.5	54.0 $\pm$ 9.9	83.0 $\pm$ 5.4 ^{a,b}
	IFN- γ	48.3 $\pm$ 7.1	17.2 $\pm$ 3.8 ^a	16.4 $\pm$ 3.1 ^a
	TNF- α	33.3 $\pm$ 1.8	68.1 $\pm$ 11.9 ^a	132.4 $\pm$ 12.7 ^{a,b}
	MIP-2	23.4 $\pm$ 2.6	24.8 $\pm$ 2.2	39.8 $\pm$ 10.9
	MCP-1	1.2 $\pm$ 0.8	8.7 $\pm$ 1.8	63.9 $\pm$ 27.2 ^{a,b}

Data are mean $\pm$ SEM of $n = 6$ –8 animals. Statistically significant differences at $P < 0.05$ when compared to vehicle (a) or ovalbumin (b).

Table 2

Allergen-induced responses in ovalbumin-sensitized and challenged PPAR α ^{+/-} and PPAR α ^{-/-} mice

Mice		PPAR α ^{+/-}	PPAR α ^{-/-}
Number of cells ($\times 10^5$)	Total cells	8.14 $\pm$ 1.10	13.5 $\pm$ 2.16 ^a
	Lymphocytes	0.02 $\pm$ 0.01	0.05 $\pm$ 0.03
	Neutrophils	0.06 $\pm$ 0.02	0.07 $\pm$ 0.02
	Eosinophils	3.93 $\pm$ 0.62	8.32 $\pm$ 1.73 ^a
	Macrophages	4.14 $\pm$ 0.50	6.25 $\pm$ 1.77
Ovalbumin-specific Ig (a.u.)	IgE	15.1 $\pm$ 6.6	93.0 $\pm$ 55.3
	IgG2a	77.0 $\pm$ 22.8	12.2 $\pm$ 4.4 ^a
Cytokines (pg/mL)	IL-4	101.1 $\pm$ 57.5	137.6 $\pm$ 77.8
	IL-5	151.2 $\pm$ 70.7	268.4 $\pm$ 111.5
	IFN- γ	104.5 $\pm$ 17.5	53.6 $\pm$ 10.6 ^a
	Gata-3/T-bet	72.0 $\pm$ 9.3	111.3 $\pm$ 26.5

Data are mean $\pm$ SEM of $n = 7$ –8 animals. Statistically significant differences at ^a $P < 0.05$ when compared to PPAR α ^{+/-} mice.

Table 3

Number of inflammatory cells in bronchoalveolar lavage fluids from allergen-sensitized and challenged PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ mice treated with fenofibrate or its vehicle

Group	Number of cells ($\times 10^5$)				
	Total	Lymphocytes	Neutrophils	Eosinophils	Macrophages
PPAR $\alpha^{+/+}$ -vehicle	11.57 $\pm$ 1.47	0.00 $\pm$ 0.00	0.01 $\pm$ 0.01	7.16 $\pm$ 1.70	4.40 $\pm$ 0.35
PPAR $\alpha^{+/+}$ -fenofibrate	4.38 $\pm$ 0.44 ^b	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.12 $\pm$ 0.04 ^b	4.25 $\pm$ 0.42
PPAR $\alpha^{-/-}$ -vehicle	21.50 $\pm$ 3.92 ^a	0.03 $\pm$ 0.03	0.07 $\pm$ 0.04	14.69 $\pm$ 3.36 ^a	6.71 $\pm$ 1.02 ^a
PPAR $\alpha^{-/-}$ -fenofibrate	23.23 $\pm$ 5.34 ^a	0.00 $\pm$ 0.00	0.20 $\pm$ 0.15	14.35 $\pm$ 4.41 ^a	8.68 $\pm$ 1.01 ^a

Data are mean $\pm$ SEM of n=6 animals. Statistically significant differences at ^a $P<0.05$ and ^b $P<0.01$ when compared to PPAR $\alpha^{+/+}$ mice treated with vehicle.

these data suggested that PPAR α controlled orientation of the immune response to allergen. We therefore investigated the effect of fenofibrate (15 mg/day) on this response in C57BL/6 mice. To do so, two durations of treatment were investigated, a 10-day duration that covered the challenge period only and a 23-day duration that lasted from the day prior sensitization to the last day of challenge to fully mimic PPAR α invalidation experiment. Animals treated with fenofibrate during challenge only (10-day) showed no significant modifications in ovalbumin-specific IgE or IgG2a (Fig. 2). They displayed a significant reduction in levels of IL-5 (42.9%, $P<0.05$) in bronchoalveolar lavage fluids, but exhibited no significant changes in other cytokines (Fig. 3). As well, Gata-3/T-bet expression ratio in lung tissue remained unchanged (Fig. 4). By contrast, animals treated with fenofibrate during sensitization and challenge (23-day) exhibited a marked increase in serum levels of ovalbumin-specific IgG2a (3.5-fold, $P<0.05$), a significant reduction in levels of IL-4 (52.8%, $P<0.05$) and IL-5 (53.6%, $P<0.05$) together with an increase in IFN- γ levels (1.5-fold, $P<0.05$) and a 61.1 % reduction ($P<0.001$) in Gata-3/T-bet ratio (Figs. 2, 3 and 4).

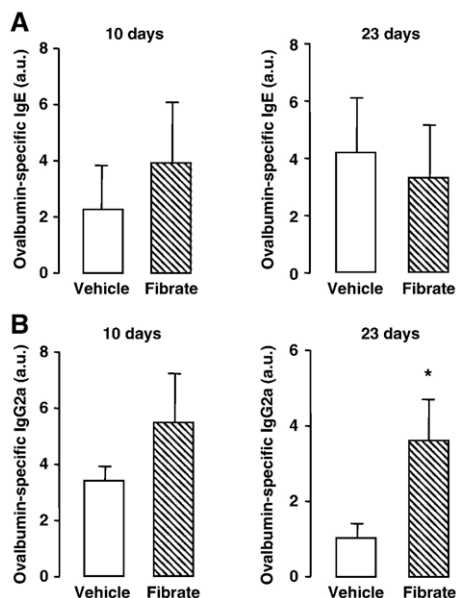


Fig. 2. Levels of ovalbumin-specific IgE (A) and IgG2a (B) in serum from allergen-sensitized and challenged C57BL/6 mice treated with fenofibrate (15 mg/day) or its vehicle for 10 or 23 days. Data are mean $\pm$ SEM of n=8–12 animals. Statistically significant differences at (*) $P<0.05$ when compared to vehicle-treated animals.

3.4. Effect of fenofibrate in sensitized mice exposed to LPS during allergen challenge

Sensitized C57BL/6 mice exposed to LPS during allergen challenge exhibited an increased number of cells in bronchoalveolar lavage fluids when compared to animals challenged with ovalbumin alone (Table 1). This greater infiltrate resulted from an increased influx of eosinophils and macrophages, as well as from the presence of a significant number of lymphocytes and neutrophils that were not observed in animals challenged with ovalbumin only (Table 1). As well, mice challenged with ovalbumin+LPS displayed increased levels of IL-4, IL-5, TNF- α , MIP-2 and MCP-1 in bronchoalveolar lavage fluids when compared to mice challenged with ovalbumin alone (Table 1). Fenofibrate (15 mg/day during

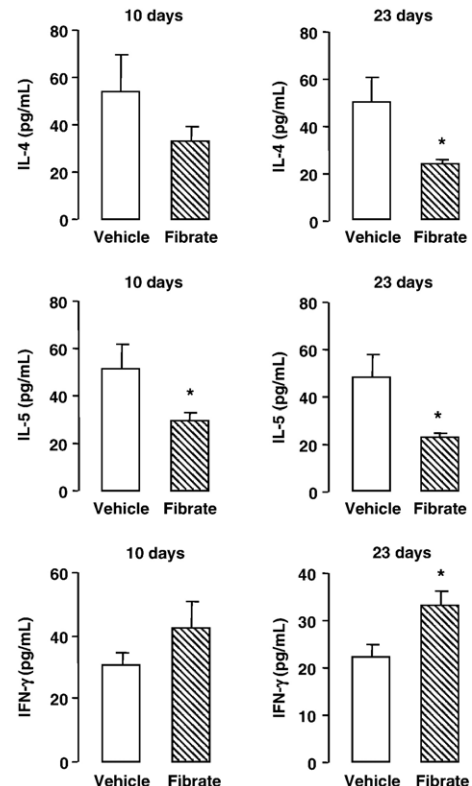


Fig. 3. Th2 and Th1 cytokine levels in bronchoalveolar lavage fluids from allergen-sensitized and challenged C57BL/6 mice treated with fenofibrate (15 mg/day) or its vehicle for 10 or 23 days. Data are mean $\pm$ SEM of n=8–12 animals. Statistically significant differences at (*) $P<0.05$ when compared to vehicle-treated animals.

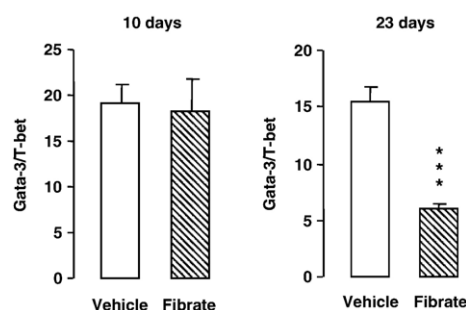


Fig. 4. Gata-3/T-bet expression ratio in lung tissue from allergen-sensitized and challenged C57BL/6 mice treated with fenofibrate (15 mg/day) or its vehicle for 10 or 23 days. Data are mean $\pm$ SEM of $n=6-8$ animals. Statistically significant differences at (***) $P<0.001$ when compared to vehicle-treated animals.

allergen challenge) markedly reduced the inflammatory cell infiltration evoked by ovalbumin+LPS in sensitized mice (Fig. 5). Reduction concerned all cell types, reaching 92.7% ($P<0.01$), 91.6% ($P<0.001$), 91.7% ($P<0.01$) and 71.0% ($P<0.001$), for lymphocytes, neutrophils, eosinophils and macrophages, respectively. Fenofibrate suppressed also IL-4, IL-5, TNF- α , MIP-2 and MCP-1 production in bronchoalveolar lavage fluids by 79.8% ($P<0.001$), 54.5% ($P<0.01$), 63.6% ($P<0.001$), 55.2% ($P<0.05$) and 83.4% (NS), respectively (Fig. 5).

4. Discussion

In the present study, we have assessed the effect of fenofibrate on allergen-induced airway inflammation and immune response. Our results showed that fenofibrate decreases airway inflammation induced by allergen and switches the immune response to allergen from Th2 to Th1 profile. Th1 switching of the immune response occurs when fenofibrate is administered from the day prior to sensitization to the end of challenge.

Fenofibrate was reported to exhibit an anti-inflammatory activity in animal models of inflammatory diseases (Duez et al., 2002; Tanaka et al., 2001). In a previous study, we showed that the agonist reduced airway inflammation induced by LPS in the mouse, as evidenced by decreased airway neutrophil and macrophage infiltration and reduced release of chemoattractants and metalloproteinases (Delayre-Orthez et al., 2005). In the present work, we found that fenofibrate suppressed also airway inflammatory cell infiltration induced by allergen, resulting in decreased number of eosinophils and macrophages. Furthermore, we demonstrated that this effect is mediated by the nuclear receptor PPAR α . Indeed, whereas fenofibrate suppressed inflammatory cell infiltrate triggered by allergen in PPAR $\alpha^{+/+}$ mice, it failed to modify allergen-induced cell influx in PPAR $\alpha^{-/-}$ animals. In agreement with previous studies (Trifilieff et al., 2003; Woerly et al., 2003), these data suggest that the PPAR α activator may exhibit an anti-inflammatory activity in asthma. PPAR α is expressed in eosinophils and its activation by the agonist WY14653 was reported to inhibit eosinophil chemotaxis in vitro (Woerly et al., 2003). Fenofibrate may thus reduce airway eosinophilia associated with asthma by

directly regulating these cells. However, down-regulation of the production of eosinophilic chemoattractants by infiltrated or resident cells may also be involved. Indeed, PPAR α is expressed in T lymphocytes (Jones et al., 2002), macrophages (Chinetti et al., 1998), airway epithelial cells (Trifilieff et al., 2003) and bronchial smooth muscle cells (Patel et al., 2003), which are potent sources of eosinophil chemotactic factors. Furthermore, PPAR α has been shown to inhibit the transactivation of NF- κ B and AP-1 (Delerive et al., 1999), which play a critical role in asthma by controlling the expression of numerous pro-inflammatory cytokines and chemokines (Barnes and Adcock, 1997). In agreement with this hypothesis, we found that levels of the eosinophil chemoattractant IL-5 were decreased in bronchoalveolar lavage fluids from fenofibrate-treated mice when compared to vehicle-exposed animals. Similar changes were also observed for TNF- α , which is known to enhance eosinophil survival, to induce expression of eosinophil chemoattractants and adhesion molecules and to mediate eosinophil recruitment in allergen-challenged mice. Our data therefore add to previous studies by showing that PPAR α activators, including fenofibrate may exhibit an anti-inflammatory activity in asthma by blocking several mediator pathways promoting eosinophil sequestration in the lung.

Inflammation in asthma results from the development of an aberrant Th2 immune response to allergen. We therefore assessed in the present study the effect of fenofibrate on this

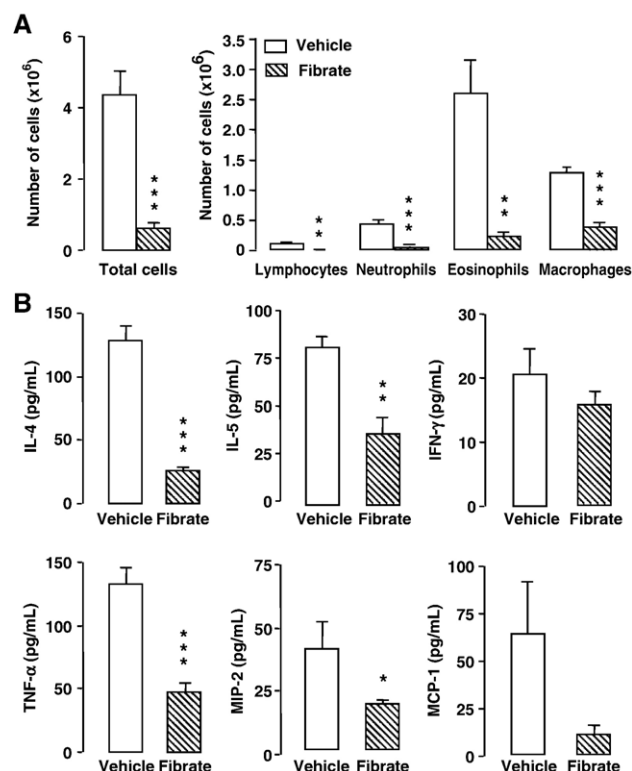


Fig. 5. Total and differential cell counts (A) and cytokine levels (B) in bronchoalveolar lavage fluids from sensitized C57BL/6 mice challenged with ovalbumin+LPS and treated with fenofibrate (15 mg/day) or its vehicle for 10 days. Data are mean $\pm$ SEM of $n=6-8$ animals. Statistically significant differences at (*) $P<0.05$, (**) $P<0.01$ and (***) $P<0.001$ when compared to vehicle-treated animals.

response. We found that mice treated with fenofibrate during allergen sensitization and challenge displayed increased levels of allergen-specific IgG2a in serum. As well, these animals exhibited a change in Th2/Th1 cytokine profile in bronchoalveolar lavage fluids that was characterized by a decrease in IL-4 and IL-5 associated with an increase in IFN- γ . Taken altogether, these data suggest that fenofibrate may suppress allergen-induced airway inflammation by switching orientation of the immune response to allergen towards a Th1 profile. In consistency with this hypothesis, we observed that increased inflammatory cell infiltration in ovalbumin-sensitized and challenged PPAR $\alpha^{-/-}$ mice was associated with an increased Th2 and a decreased Th1 immune response. Differentiation of Th2 cells is driven by the transcription factor Gata-3, whereas expansion of Th1 cells is promoted by T-bet (Rengarajan et al., 2000). Furthermore, relative predominance of the two key regulators of Th cell differentiation determines Th2/Th1 fate. We thus assessed in the present study Gata-3/T-bet expression ratio in lung tissue from mice treated with fenofibrate. We found that changes in Th2/Th1 cytokine profile observed in animals treated with fenofibrate were associated with consistent modifications in Gata-3/T-bet expression ratio. Indeed, treatment with fenofibrate during allergen sensitization and challenge caused a profound decrease in Gata-3/T-bet ratio. Likewise, ovalbumin-sensitized and challenged PPAR $\alpha^{-/-}$ animals exhibited an increased Gata-3/T-bet ratio when compared to PPAR $\alpha^{+/+}$ mice. These results suggest that fenofibrate may modulate orientation of the immune response to allergen through changes in the relative predominance of Gata-3 and T-bet. Whereas mice treated with fenofibrate during ovalbumin-sensitization and challenge displayed changes in orientation of the immune response to allergen, animals treated with fenofibrate during challenge only did not exhibit this effect. Indeed, no significant modification in ovalbumin-specific IgE or IgG2a, Th2/Th1 cytokine balance or Gata-3/T-bet ratio was observed in mice treated with fenofibrate for 10 days. These data suggest that switching of the immune response to allergen by fenofibrate occurs before expansion of Th cells.

To further characterize the anti-inflammatory potential of fenofibrate in asthma, we assessed the effect of the drug in animals sensitized to ovalbumin and challenged with ovalbumin+LPS. Upon challenge with ovalbumin+LPS, sensitized mice developed an aggravated airway inflammatory response that involved a cell infiltrate composed of eosinophils, neutrophils, lymphocytes and macrophages and a plethora of cytokines and chemokines, including TNF- α , MIP-2 and MCP-1. This exaggerated inflammatory pattern mimics the inflammatory response encountered in patients with severe asthma. Indeed, about 5% of patients with asthma have severe disease characterized by a mixed eosinophil and neutrophil infiltrate and an intense production of pro-inflammatory mediators (Tillie-Leblond et al., 1999). These patients that have a limited sensitivity to typical treatments represent a small fraction of asthmatics but account for more than half of the health care spending on asthma (Barnes, 2006). Evaluating the effect of fenofibrate in our mouse model of severe asthma, our results

showed that the agonist is effective in reducing infiltration of all inflammatory cell types, as well as production of all mediators assessed. This data suggest that fenofibrate could be valuable in treating patients with severe asthma.

In conclusion, our data clearly demonstrate that fenofibrate inhibits allergen-induced airway inflammation, including in an exacerbated form, and is also able to switch the immune response to allergen towards a Th1 profile. The PPAR α agonist may thus be a valuable alternative to the use of glucocorticoids in allergic asthmatics, particularly in patients suffering of a severe form of the disease.

Acknowledgments

The PPAR $\alpha^{-/-}$ mice used in this study were a kind gift of Dr F. Gonzalez at the NHCI in Bethesda. We thank F. Picard for his experimental contribution. This work was supported by the Centre National de la Recherche Scientifique, Institut National pour la Santé et la Recherche Médicale and Université Louis Pasteur. We thank the Agence de l'Environnement et de la Maitrise de l'Energie, the Société de Pneumologie de Langue Française and the Région Alsace for supporting C. Delayre-Orthez and J. Becker.

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