

PPAR-gamma modulates allergic inflammation through up-regulation of PTEN

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ABSTRACT

The ligand-activated nuclear receptor peroxisome proliferator-activated receptor γ (PPAR γ) has been shown to regulate cell activation, differentiation, proliferation, and/or apoptosis. PPAR γ is also associated with anti-inflammatory responses. However, the signaling mechanism remains elusive. We have used a mouse model for asthma to determine the effect of PPAR γ agonists, rosiglitazone or pioglitazone, and PPAR γ on allergen-induced bronchial inflammation and airway hyperresponsiveness. Administration of PPAR γ agonists or adenovirus carrying PPAR γ cDNA (AdPPAR γ) reduced bronchial inflammation and airway hyperresponsiveness. Expression of PPAR γ was increased by ovalbumin (OVA) inhalation, and the increase was further enhanced by the administration of the PPAR γ agonists or AdPPAR γ . Levels of IL-4, IL-5, IL-13, and eosinophil cationic protein were increased after OVA inhalation, and the increased levels were significantly reduced by the administration of PPAR γ agonists or AdPPAR γ . The results also showed that the administration of PPAR γ agonists or AdPPAR γ up-regulated phosphatase and tensin homologue deleted on chromosome ten (PTEN) expression in allergen-induced asthmatic lungs. This up-regulation correlated with decreased phosphatidylinositol 3-kinase activity as measured by reduced phosphorylation of Akt. These findings demonstrate a protective role of PPAR γ in the pathogenesis of the asthma phenotype through regulation of PTEN expression.

Key words: asthma • eosinophils • phosphatidylinositol 3-kinase • signal transduction

Asthma is a chronic inflammatory disorder of the airways in which many cell types play a role (1). Eosinophil response appears to be a critical feature in asthma (2). Eosinophils play an effector role by release of proinflammatory mediators, cytotoxic mediators, and cytokines, resulting in vascular leakage, hypersecretion of mucus, smooth muscle contraction, epithelial shedding, and bronchial hyperresponsiveness. These cells are also involved in the

regulation of the airways inflammation and in the initiation of the remodeling process by the release of cytokines and growth factors.

Thiazolidinediones, such as rosiglitazone and pioglitazone, are high-affinity ligands for peroxisome proliferator-activated receptor γ (PPAR γ), a transcription factor of the nuclear hormone receptor superfamily, and are used as insulin-sensitizing drugs in type II diabetes mellitus (3). PPAR γ is originally known to regulate adipocyte differentiation and lipid metabolism (4). However, accumulating evidence indicates that PPAR γ affects cell cycle, differentiation, and apoptosis (5). In addition, PPAR γ activation down-regulates synthesis and release of immunomodulatory cytokines from various cell types (6–8). Some evidence indicates that PPAR γ may be involved in airway inflammation and remodeling in patients with asthma (9).

PPAR γ has been shown to regulate phosphatidylinositol 3-kinase (PI3K) signaling by modulating phosphatase and tensin homologue deleted on chromosome ten (PTEN) expression in inflammatory cells (10). Recently, we have demonstrated that administration of adenovirus (Ad) gene transfer vector expressing a PTEN cDNA or PI3K inhibitors reduced the inflammation and airway hyperresponsiveness in a murine model of allergic asthma (11). However, the mechanisms by which PPAR γ affects the pathogenesis of the asthma phenotype have not been clarified.

In the present study, we used a murine model of asthma to evaluate the effect of PPAR γ agonists, rosiglitazone or pioglitazone, or Ad gene transfer vector expressing a PPAR γ cDNA (AdPPAR γ) on hyperresponsiveness and inflammation of the airways and PTEN expression.

MATERIALS AND METHODS

Animals and experimental protocol

Female BALB/c mice, 8–10 weeks of age and free of murine specific pathogens, were obtained from the Korean Research Institute of Chemistry Technology (Daejeon, Korea); housed throughout the experiments in a laminar flow cabinet; and maintained on standard laboratory chow ad libitum. All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the Chonbuk National University Medical School. Mice were sensitized on days 1 and 14 by intraperitoneal injection of 20 μ g ovalbumin (OVA; Sigma-Aldrich, St. Louis, MO) emulsified in 1 mg of aluminum hydroxide (Pierce Chemical Co., Rockford, IL) in a total volume of 200 μ l, as previously described (11; [Fig. 1](#)). On days 21, 22, and 23, after the initial sensitization, the mice were challenged for 30 min with an aerosol of 3% (w/v) OVA in saline (or with saline as a control) using an ultrasonic nebulizer (NE-U12, Omron, Japan). Bronchoalveolar lavage (BAL) was performed at 72 h after the last challenge. At the time of lavage, the mice (6 mice in each group) were sacrificed with an overdose of sodium pentobarbitone (pentobarbital sodium, 100 mg/kg of body weight, administered intraperitoneally). The chest cavity was exposed to allow for expansion, after which the trachea was carefully intubated and the catheter secured with ligatures. Prewarmed 0.9% NaCl solution was slowly infused into the lungs and withdrawn. The aliquots were pooled and then kept at 4 °C. Part of each pool was then centrifuged and the supernatants were kept at –70 °C until use. Total cell numbers were counted with a hemocytometer. Smears of BAL cells were prepared with a cytospin. The smears were stained with Diff-Quik solution (Dade Diagnostics of P. R. Inc. Aguada, PR) in order to examine the cell differentials. Two independent, blinded

investigators counted the cells using a microscope. Approximately 400 cells were counted in each of four different random locations. Interinvestigator variation was <5%. The mean number from the two investigators was used to estimate the cell differentials.

Vectors

The E1/E3-deleted replication-deficient recombinant Ad was made using the AdEasy system (Quantum Biotech, Montreal, Canada) described by He et al (12). KpnI-XhoI restriction fragments from pcDNA3/wild-type PPAR γ cDNA were ligated into KpnI-XhoI-digested pShuttleCMV. To create AdLacZ, a SalI-NotI restriction fragment from pcDNA3.1/lacZ (Invitrogen, San Diego, CA) was ligated to SalI-NotI-digested pShuttleCMV. Recombination into the pAdEasy-1 viral backbone was accomplished in bacteria (*E. coli*, strain BJ5183, recA deficient) according to the manufacturer's instructions. The recombination was verified, and the adenoviral recombinant DNA was transferred to a regular strain *E. coli* (DH5 α), which generated far greater yields of DNA. Recombinant pAdEasy plasmids containing CMV-cDNA inserts were purified over Qiagen columns, and 5 μ g of PacI-digested DNA was used to transfect QBI-293A cells using the calcium phosphate method (Promega, Madison, WI). Cells were seeded at 2×10^6 cells per 150 mm culture dish 24 h prior to transfection. Lysis of transfected cells indicating adenoviral growth occurred within 4 days. Following amplification, lysates containing clonal recombinant adenovirus were prepared from 150 mm culture dishes and were purified by CsCl gradient centrifugation. Recovered virus was aliquoted and stored at -20°C in 5 mM Tris (pH 8.0) buffer containing 50 mM NaCl, 0.05% BSA, and 25% glycerol. Virus was titrated by serial dilution infection of QBI-293A cells, and plaques were counted under 0.3% agarose/10% FBS/1 $\times$ DMEM overlay.

Administration of rosiglitazone, pioglitazone, GW9662, or Ad vectors

Rosiglitazone (5 mg/kg body weight/day, SmithKline Beecham Pharmaceuticals, Worthing, UK) dissolved in distilled water or pioglitazone (10 mg/kg body weight/day, Takeda Chemical Industries Ltd, Osaka, Japan) dissolved in DMSO and diluted with 0.9% NaCl, was administered 7 times by oral gavage at 24 h intervals on days 19–25, beginning 2 days before the first challenge ([Fig. 1](#)). GW9662 (0.5 mg/kg body weight/day, Cayman, Ann Arbor, MI) dissolved in phosphate-buffered saline (PBS) was administered intratracheally two times to each animal, once on day 21 and the second time on day 25 ([Fig. 1](#)). Ad vectors were administered intratracheally (10^9 plaque-forming units) two times to each animal, once on day 21 (1 h before the first airway challenge with OVA) and the second time on day 23 (3 h after the last airway challenge with OVA) ([Fig. 1](#)).

Isolation of BAL fluid eosinophils

Eosinophils were purified as previously described (13). Briefly, BAL cells were centrifuged at $1500 \times g$ at 4°C for 10 min. The supernatant was kept at -70°C , and the pellets were resuspended in RPMI-1640 (Invitrogen Corp., San Diego, CA). The suspended cells were pooled and layered onto a discontinuous Percoll gradient, which consisted of 2 ml of 72% and 3 ml of 56% Percoll. After centrifugation at $2200 \times g$ at 4°C for 30 min, eosinophils were carefully collected from interface between the Percoll bands and washed twice with RPMI-1640. The final cell pellets were resuspended in PBS. Eosinophil purity was determined by staining

cytopsin (Shandon Scientific Ltd, Cheshire, UK) preparations with Diff-Quik. In all preparations, purity of eosinophil was 85–95%.

Measurement of cytokines

Levels of IL-4, IL-5, and IL-13 were quantified in the supernatants of BAL fluids by enzyme immunoassays according to the manufacturer's protocol (IL-4 and IL-5; Endogen, Inc., Woburn, MA, IL-13; R&D Systems, Inc., Minneapolis, MN). Sensitivities for IL-4, IL-5, and IL-13 assays were 5, 5, and 1.5 pg/ml, respectively.

Measurement of eosinophil cationic protein (ECP)

ECP was measured in the supernatants of BAL fluids. Levels of ECP were quantified by fluoroenzymeimmunoassay according to the manufacturer's protocol (UniCAP ECP; Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). Sensitivity for the assay was 2 ng/ml.

Western blot analysis

Lung tissues were homogenized in the presence of protease inhibitors to obtain extracts of proteins. Protein concentrations were determined using the Bradford reagent (Bio-Rad, Hercules, CA). Samples (30 µg of protein per lane) were loaded on a 12% SDS-PAGE gel. After electrophoresis at 120 V for 90 min, separated proteins were transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Piscataway, NJ) by the wet transfer method (250 mA, 90 min). Nonspecific sites were blocked with 5% non-fat dry milk in TBST buffer (25 mM Tris, pH 7.5; 150 mM NaCl; 0.1% Tween 20) for 1 h, and the blots were then incubated with an anti-PTEN antibody (Santa Cruz Biotechnology, Santa Cruz, CA), anti-IL-4 antibody (Serotec Ltd, Oxford, UK), anti-IL-5 antibody (Santa Cruz Biotechnology), anti-IL-13 antibody (R&D Systems, Inc. Minneapolis, MN), anti-Akt antibody (Cell Signaling Technology, Beverly, MA), or anti-phosphorylated Akt (p-Akt) antibody (Cell Signaling Technology), overnight at 4 °C. Anti-rabbit horseradish peroxidase conjugated IgG was used to detect binding of antibodies. The membranes were stripped and reblotted with anti-actin antibody (Sigma-Aldrich) to verify equal loading of protein in each lane. The binding of the specific antibodies was visualized by exposing to photographic film after treating with enhanced chemiluminescence system reagents (Amersham Pharmacia Biotech).

Determination of airway responsiveness to methacholine

Airway responsiveness was measured in mice 3 days after the last challenge in an unrestrained conscious state, as previously described (11, 14, 15). Mice were placed in a barometric plethysmographic chamber (All Medicus Co., Seoul, Korea), and baseline readings were taken and averaged for 3 min. Aerosolized methacholine in increasing concentrations (2.5 mg/ml to 50 mg/ml) were nebulized through an inlet of the main chamber for 3 min. Readings were taken and averaged for 3 min after each nebulization. Enhanced pause (Penh), calculated as: (expiratory time/relaxation time-1) × (peak expiratory flow/peak inspiratory flow), according to the manufacturers' protocol, is a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and a function of the timing of expiration. Penh was used as a measure of airway responsiveness to methacholine. Results were expressed as the percentage increase of Penh following challenge with each concentration of

methacholine, where the baseline Penh (after saline challenge) was expressed as 100%. Penh values averaged for 3 min after each nebulization were evaluated.

Isolation and primary culture of murine tracheal epithelial cells

Mice tracheal epithelial cells were isolated under sterile conditions. The trachea proximal to the bronchial bifurcation was excised, and adherent adipose tissue was removed. The trachea was opened longitudinally and cut into three pieces. The isolated tracheas were incubated in DMEM containing 0.1% protease overnight at 4 °C. Following tissue digestion, FBS (10% final) was added to the medium to deactivate enzymes, undigested fragments of tissue were removed, and tracheal epithelial cells were harvested by centrifugation at 500 rpm for 5 min. The epithelial cells were seeded onto collagen-coated 35 mm dishes for submerged culture. The growth medium DMEM/F-12 (Sigma-Aldrich) containing 10% FBS, penicillin, streptomycin, and amphotericin B was supplemented with insulin, transferrin, hydrocortisone, phosphoethanolamine, cholera toxin, ethanolamine, bovine pituitary extract, and BSA. The cells were maintained in a humidified 5% CO₂ incubator at 37°C until they adhered.

Measurement of PTEN activity

PTEN activities were measured using the PTEN malachite green assay kit according to the protocol of the manufacturer (Upstate Biotechnology Inc., Lake Placid, NY).

Measurement of PI3K enzyme activity in lung tissue

Protein extracts from lung tissue homogenates were prepared in the presence of protease inhibitors. Protein concentrations were determined using the Bradford reagent (Bio-Rad) and PI3K activity in the tissue extracts was measured by determining levels of phosphatidylinositol 3,4,5-triphosphate (PIP3) using PIP3 competition enzyme immunoassays according to the manufacturer's protocol (Echelon, Inc., Salt Lake City, UT).

Histology, immunohistochemistry, and immunocytochemistry

At 72 h after the last challenge, lungs were removed from the mice after sacrifice. Before the lungs were removed, the lungs and trachea were filled intratracheally with a fixative (0.8% formalin, 4% acetic acid) using a ligature around the trachea. Lung tissues were fixed with 10% (v/v) neutral buffered formalin. The specimens were dehydrated and embedded in paraffin. For histological examination, 4 µm sections of fixed embedded tissues were cut on a Leica model 2165 rotary microtome (Leica, Nussloch, Germany), placed on glass slides, deparaffinized, and stained sequentially with hematoxylin 2 and eosin-Y (Richard-Allan Scientific, Kalamazoo, MI). Inflammation score was graded by three independent blinded investigators. The degree of peribronchial and perivascular inflammation was evaluated on a subjective scale of 0 to 3, as described elsewhere (16). A value of 0 was adjudged when no inflammation was detectable, a value of 1 for occasional cuffing with inflammatory cells, a value of 2 for most bronchi or vessels surrounded by thin layer (one to five cells) of inflammatory cells and a value of 3 when most bronchi or vessels were surrounded by a thick layer (more than five cells) of inflammatory cells. For immunohistochemistry and immunocytochemistry of PTEN, the deparaffinized 4 µm sections, or the cytocentrifuge preparations of BAL cells, BAL eosinophils or of tracheal epithelial cells were incubated sequentially in accordance with the R.T.U. Vectastain Universal

Quick kit instruction from Vector Laboratories (Burlingame, CA). Briefly, the slides were incubated in Endo/Blocker (Biomedex, Foster City, CA) for 5 min and in pepsin solution for 4 min at 40 °C. The slides were incubated in normal horse serum for 15 min at room temperature. The slides were probed with an affinity-purified rabbit polyclonal PTEN IgG (Santa Cruz Biotechnology) overnight at 4°C and were incubated with prediluted biotinylated pan-specific IgG for 10 min. The slides were incubated in streptavidin/peroxidase complex reagent for 5 min, and then in 3-amino-9-ethylcarbazole substrate kit for 12 min. For the control, sections of lung tissue, BAL cells, or BAL eosinophils prepared from mice were treated without the primary antibody under the same conditions. After immunostaining, the slides were counterstained for 1 min with Gill's hematoxylin in 20% ethylene glycol and then mounted with Aqueous Mounting Medium (InnoGenex, San Ramon, CA), and photomicrographed (VENOX-T; Olympus, Tokyo, Japan).

Preparation of mouse lung nuclei for analysis of PPAR γ

Lungs were removed and homogenized in 8 volumes of a lysis buffer containing 1.3 M sucrose, 1.0 mM MgCl₂, and 10 mM potassium phosphate buffer, pH 7.2. The homogenate was filtered through four layers of gauze and centrifuged at 1000 $\times g$ for 15 min. The resulting pellets were carefully recovered and re-suspended in 2.4 M sucrose; 1.0 mM MgCl₂; and 10 mM potassium phosphate buffer, pH 7.2, to maintain a final 2.2 M sucrose concentration and centrifuged at 100,000 $\times g$ for 1 h. The resulting nuclear pellets were washed once with a solution containing 0.25 M sucrose; 0.5 mM MgCl₂; and 20 mM Tris-HCl, pH 7.2, and centrifuged at 1000 $\times g$ for 10 min. The pellets were solubilized with a solution containing 50 mM Tris-HCl (pH 7.2), 0.3 M sucrose, 150 mM NaCl, 2 mM EDTA, 20% glycerol, 2% Triton X-100, 2 mM PMSF, and protein inhibitor cocktails. The mixture was kept on ice for 2 h with gentle stirring and centrifuged at 12,000 $\times g$ for 30 min. The resulting supernatant was used as solubilized nuclear proteins for detection of PPAR γ .

For Western analysis, samples (30 μ g of protein per lane) were loaded on a 10% SDS-PAGE gel. After electrophoresis at 120 V for 90 min, separated proteins were transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech) by the wet transfer method (250 mA, 90 min). Nonspecific sites were blocked with 5% non-fat dry milk in TBST buffer (25 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween 20) for 1 h, and the blots were then incubated with antibody against PPAR γ (Santa Cruz Biotechnology), overnight at 4°C. Anti-rabbit horseradish peroxidase conjugated IgG was used to detect binding of antibodies. The membranes were stripped and reblotted with anti-actin antibody (Sigma-Aldrich) to verify equal loading of protein in each lane. The binding of the specific antibodies was visualized by exposing to photographic film after treating with enhanced chemiluminescence system reagents (Amersham Pharmacia Biotech).

RNA isolation and RT-PCR

Total RNA from lung tissues was isolated using a rapid extraction method (TRI-Reagent) as previously described (17). RNA was quantified by measuring absorption at 260 nm and was stored at -80°C before use. Total RNA (4 μ g) was reverse-transcribed to cDNA in a buffer containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 5 mM MgCl₂, 10 mM DTT; 0.5 μ g random hexanucleotide primers, 2.5 mM dNTP, 40 units RNase inhibitor, and 50 units/ μ l SuperScript II RT (Invitrogen, Carlsbad, CA), in a final volume of 20 μ l. This mixture was incubated for 50 min at 42°C and was finally digested with 2 units/ μ l *E. coli* RNase H for 20 min at 37°C. The

first-strand cDNAs were used for PCR amplification of IL-4, IL-5, and IL-13, or the housekeeping gene, GAPDH. PCR amplification was performed by mixing 3 μ l of the RT reaction mixture with 47 μ l of buffer containing 2.5 U of Taq DNA polymerase (Promega, Madison, WI) and 30 pmol of specific primer pairs for mouse cDNA of IL-4, IL-5, IL-13, or GAPDH, designed from published mouse gene sequences. The primers used were as follows: IL-4 (predicted length 400 bp) sense: 5'-CATCCTGCTCTTCTTTCTCGT-3', antisense: 5'-GTACTACGAGTAATCCATTG-3'; IL-5 (predicted length 201 bp) sense: 5'-GAGCACAGTGGTGAAGAGAC-3', antisense: 5'-ATGACAGGTTTGGGAATAGCATT-3'; IL-13 (predicted length 182 bp) sense: 5'-GCCAGCCCACAGTTCTACAGC-3', antisense: 5'-GTGATGTTGCTCAGCTCCTCA-3', and GAPDH (predicted length 609 bp) sense: 5'-GCCATCAACGACCCCTTCATTGAC-3', antisense: 5'-ACGGAAGGCCATGCCAGTGAGCTT-3'. PCR reactions were performed in a thermocycler (GeneAmp[®] PCR System 2400, Foster City, CA) using the following reaction conditions: After an initial incubation for 2 min at 95°C, samples were subjected to 35 cycles of 1 min at 94°C, 2 min at 55°C (IL-4, IL-5, or GAPDH) or 60°C (IL-13), and 1 min at 72°C. A final extension step at 72°C for 10 min was performed. The RT-PCR products were electrophoretically fractionated on 2% agarose gels stained with ethidium bromide. DNA bands were visualized after exposure to UV light.

Cytosolic and nuclear protein extractions for analysis of NF- κ B p65

Lungs were removed and homogenized in 2 volumes of buffer A (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10% glycerol, 0.5 mM DTT, 5 mM MgCl₂, and 1 mM PMSF) containing protease inhibitor cocktails. The homogenates were centrifuged at 1000 $\times g$ for 15 min at 4°C. The supernatant fraction was incubated on ice for 10 min and centrifuged at 100,000 $\times g$ for 1 h at 4°C to obtain cytosolic protein extracts. The pellets were washed twice in buffer A and resuspended in buffer B (1.3 M sucrose, 1.0 mM MgCl₂, and 10 mM potassium phosphate buffer, pH 6.8) and pelleted at 1000 $\times g$ for 15 min. The pellets were suspended in buffer B with a final sucrose concentration of 2.2 M and centrifuged at 100,000 $\times g$ for 1 h. The resulting pellets were washed once with a solution containing 0.25 M sucrose, 0.5 mM MgCl₂, and 20 mM Tris-HCl, pH 7.2, and centrifuged at 1,000 $\times g$ for 10 min. The pellets were solubilized with a solution containing 50 mM Tris-HCl (pH 7.2), 0.3 M sucrose, 150 mM NaCl, 2 mM EDTA, 20% glycerol, 2% Triton X-100, 2 mM PMSF, and protease inhibitor cocktails. The mixture was kept on ice for 1 h with gentle stirring and centrifuged at 12,000 $\times g$ for 30 min. The resulting supernatant was used as soluble nuclear proteins.

For Western analysis, samples (30 μ g of protein per lane) were loaded on a 10% SDS-PAGE gel. After electrophoresis at 120 V for 90 min, separated proteins were transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech) by the wet transfer method (250 mA, 90 min). Nonspecific sites were blocked with 5% non-fat dry milk in TBST buffer (25 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween 20) for 1 h, and the blots were then incubated with antibody against NF- κ B p65 (Upstate Biotechnology Inc.) overnight at 4°C. Anti-rabbit horseradish peroxidase conjugated IgG was used to detect binding of the antibody. The binding of the specific antibody was visualized by exposing to photographic film after treating with enhanced chemiluminescence system reagents (Amersham Pharmacia Biotech).

Densitometric analyses and statistics

All immunoreactive and phosphorylation signals were analyzed by densitometric scanning (LAS-3000; Fuji Film, Tokyo, Japan). Data were expressed as mean $\pm$ SEM. Statistical comparisons were performed using one-way ANOVA followed by the Fisher's test. Significant differences between groups were determined using the unpaired Student's *t*-test. Statistical significance was set at $P < 0.05$.

RESULTS

Effect of rosiglitazone, pioglitazone, or AdPPAR γ on cellular changes in BAL fluids

Numbers of total cells and eosinophils were increased significantly at 72 h after OVA inhalation compared with levels after saline inhalation ([Fig. 2](#)). The increased numbers of eosinophils in BAL fluids at 3 days after OVA inhalation were significantly reduced by the administration of rosiglitazone, pioglitazone, or AdPPAR γ . The inhibitory effect of rosiglitazone treatment on numbers of eosinophils in BAL fluids was abrogated when a PPAR γ antagonist GW9662 was administered concomitantly with the agonist. These results indicate that rosiglitazone was mainly acting through PPAR γ in this model.

Effect of rosiglitazone, pioglitazone, or AdPPAR γ on pathological changes of OVA-induced asthma

Histologic analyses revealed typical pathologic features of asthma in the OVA-exposed mice. Numerous inflammatory cells, including eosinophils, infiltrated around the bronchioles, the airway epithelium was thickened, and mucus and debris had accumulated in the lumen of bronchioles ([Fig. 3B](#)) compared with the control ([Fig. 3A](#)). Mice treated with rosiglitazone ([Fig. 3C](#)), pioglitazone ([Fig. 3D](#)), AdPPAR γ ([Fig. 3E](#)), showed marked reductions in the thickening of airway epithelium, in the infiltration of inflammatory cells in the peribronchiolar region, in the number of inflammatory cells, and in the amount of debris in the airway lumen. In contrast, no significant changes were observed in AdLacZ-treated mice ([Fig. 3F](#)).

The scores of peribronchial, perivascular, and total lung inflammation were increased significantly at 72 h after OVA inhalation compared with scores after saline inhalation ([Fig. 3G](#)). The increased peribronchial, perivascular, and total lung inflammation after OVA inhalation were significantly decreased by the administration of rosiglitazone, pioglitazone, or AdPPAR γ . These results suggest that rosiglitazone, pioglitazone, and AdPPAR γ inhibit antigen-induced inflammation in the lungs, including the influx of eosinophils.

Effect of rosiglitazone, pioglitazone, or AdPPAR γ on IL-4, IL-5, IL-13, and ECP expression

Western blot analysis revealed that IL-4, IL-5, and IL-13 protein levels in lung tissues were increased significantly at 72 h after OVA inhalation compared with the levels after saline inhalation. The increased IL-4, IL-5, and IL-13 levels at 72 h after OVA inhalation were significantly reduced by the administration of rosiglitazone, pioglitazone, or AdPPAR γ . Consistent with the results obtained from the Western blot analysis, enzyme immunoassays revealed that levels of IL-4, IL-5, and IL-13 in BAL fluids were also increased significantly at 72 h after OVA inhalation compared with the levels after saline inhalation. The increased IL-4, IL-5, and IL-13 levels at 72 h after OVA inhalation were significantly reduced by the administration

of rosiglitazone, pioglitazone, or AdPPAR γ . In addition, RT PCR analysis revealed that increased IL-4, IL-5, and IL-13 mRNA expression in lung tissues was increased significantly at 72 h after OVA inhalation compared with the levels after saline inhalation. The increased IL-4, IL-5, and IL-13 mRNA expression in lung tissues at 72 h after OVA inhalation was decreased by the administration of rosiglitazone, pioglitazone, or AdPPAR γ ([Fig. 4](#)).

The increased ECP levels in the BAL fluids at 72 h after OVA inhalation were also decreased significantly by the administration of rosiglitazone, pioglitazone, or AdPPAR γ ([Fig. 5](#)). These results indicate that rosiglitazone, pioglitazone, and AdPPAR γ attenuate the antigen-induced release of soluble mediators of inflammation into the lungs.

Effect of rosiglitazone, pioglitazone, or AdPPAR γ on airway hyperresponsiveness

Airway responsiveness was assessed as a percent increase of Penh in response to increasing doses of methacholine. In OVA-sensitized and -challenged mice, the dose-response curve of percent Penh shifted to the left compared with that of control mice ([Fig. 6](#)). In addition, the percent Penh produced by methacholine administration (at doses from 2.5 mg/ml to 50 mg/ml) increased significantly in the OVA-sensitized and -challenged mice compared with the controls. OVA-sensitized and -challenged mice treated with rosiglitazone, pioglitazone, and AdPPAR γ showed a dose-response curve of percent Penh that shifted to the right compared with that of untreated mice or OVA-sensitized and -challenged mice treated with AdLacZ. Thus, these results indicate that rosiglitazone, pioglitazone, or AdPPAR γ treatment reduces OVA-induced airway hyperresponsiveness. The inhibitory effect of rosiglitazone treatment on airway hyperresponsiveness was abrogated when GW9662 was administered concomitantly with the agonist.

PPAR γ agonists and AdPPAR γ increase PPAR γ levels in lung tissues of OVA-sensitized and -challenged mice

Western blot analysis revealed that PPAR γ levels were increased significantly at 72 h after OVA inhalation compared with the levels after saline inhalation ([Fig. 7](#)). The increased PPAR γ levels in lung tissues at 72 h after OVA inhalation were increased further by the administration of rosiglitazone, pioglitazone, or AdPPAR γ .

PPAR γ agonists and AdPPAR γ increase PTEN protein levels and PTEN activities in lung tissues of OVA-sensitized and -challenged

Western blot analysis revealed that PTEN protein levels were decreased significantly at 72 h after OVA inhalation compared with the levels after saline inhalation ([Fig. 8A, B](#)). The administration of rosiglitazone, pioglitazone, or AdPPAR γ resulted in elevation of PTEN protein levels that were similar to the control levels. Consistent with these results, PTEN enzyme assays revealed that PTEN activity was decreased significantly at 72 h after OVA inhalation compared with the levels after saline inhalation ([Fig. 8C](#)). The decreased PTEN activity at 72 h after OVA inhalation was increased by the administration of rosiglitazone, pioglitazone, or AdPPAR γ .

Localization of immunoreactive PTEN in lung tissues, tracheal epithelial cells, BAL fluids, and BAL fluid eosinophils of OVA-induced asthma

Immunoreactive PTEN localized in epithelial layers around the bronchioles of control mice ([Fig. 9A](#)). This immunoreactive PTEN disappeared in allergen-induced asthmatic lungs ([Fig. 9B](#)). Interestingly, intratracheal administration of AdPPAR γ restored the PTEN expression in allergen-induced asthmatic mice lungs ([Fig. 9C](#)), whereas AdLacZ did not ([Fig. 9D](#)).

The expression of PTEN in lung epithelial cells after isolation and primary culture of murine tracheal epithelial cells was observed. Immunocytologic analysis showed localization of immunoreactive PTEN in the tracheal epithelial cells from the control mice ([Fig. 9E](#)). However, immunoreactive PTEN was markedly reduced in tracheal epithelial cells from OVA-exposed mice ([Fig. 9F](#)). Intratracheal administration of AdPPAR γ restored the PTEN expression in tracheal epithelial cells from the allergen-induced asthmatic mice ([Fig. 9G](#)), but AdLacZ did not ([Fig. 9H](#)).

Immunocytologic analysis of BAL fluids showed localization of PTEN in the BAL cells from the control mice ([Fig. 9I](#)). However, immunoreactive PTEN was markedly reduced in precipitated cells from OVA-exposed mice ([Fig. 9J](#)). Intratracheal administration of AdPPAR γ restored the PTEN expression in BAL cells from the allergen-induced asthmatic mice ([Fig. 9K](#)), whereas AdLacZ did not ([Fig. 9L](#)).

In addition, the expression of PTEN in BAL fluid eosinophils isolated by Percoll gradients was also examined by means of immunocytochemistry. Immunoreactive PTEN was markedly reduced in eosinophils from OVA-exposed mice ([Fig. 9M](#)). Intratracheal administration of AdPPAR γ restored the immunoreactive PTEN expression in BAL fluid eosinophils from the allergen-induced asthmatic mice ([Fig. 9N](#)), and additional AdLacZ treatment did not affect immunoreactivity in OVA-exposed mice ([Fig. 9O](#)).

Determination of Akt phosphorylation and PI3K activity

To support the contention that the effects of rosiglitazone, pioglitazone, or AdPPAR γ on allergen-induced bronchial inflammation and airway hyperresponsiveness were specifically directed through the PI3K pathway, we used Western blotting to determine Akt phosphorylation. Levels of p-Akt protein in the lung tissues were increased at 72 h after OVA inhalation compared with levels in the control mice ([Fig. 10](#)). However, no significant changes in Akt protein levels were observed in any of the groups tested. The increased p-Akt but not Akt protein levels in lung tissues at 72 h after OVA inhalation, were significantly reduced by the administration of rosiglitazone, pioglitazone, or AdPPAR γ .

PI3K activities in the BAL fluids were increased at 72 h after OVA inhalation compared with levels in the control mice ([Fig. 11](#)). The increased PI3K activities in the BAL fluids at 72 h after OVA inhalation were significantly reduced by the administration of rosiglitazone, pioglitazone, or AdPPAR γ .

Effect of rosiglitazone, pioglitazone, or AdPPAR γ on NF- κ B p65 protein levels in lung tissues of OVA-sensitized and -challenged mice

Western blot analysis revealed that levels of NF- κ B p65 protein in nuclear protein extracts of lung tissues were increased at 72 h after OVA inhalation compared with the levels in the control mice (Fig. 12). The increased NF- κ B p65 levels in nuclear protein extracts of lung tissues at 72 h after OVA inhalation were decreased by the administration of rosiglitazone, pioglitazone, or AdPPAR γ . However, levels of NF- κ B p65 protein in cytosolic protein extracts of lung tissues were decreased at 72 h after OVA inhalation compared with the levels in the control mice (Fig. 12). The decreased NF- κ B p65 levels in cytosolic protein extracts of lung tissues at 72 h after OVA inhalation were increased by the administration of rosiglitazone, pioglitazone, or AdPPAR γ .

DISCUSSION

Recent studies have indicated that PPAR γ plays an important role in anti-inflammatory responses (5–8) and that PPAR γ signaling is associated with regulation of PTEN expression (10). It is also known that up-regulation of PTEN expression reduces asthmatic pathogenesis (11). However, interrelationship between these proteins in regulation of anti-inflammatory function has not been examined with an in vivo asthma model. Our present study with OVA-induced murine model of asthma has revealed that activation of PPAR γ with the agonists enhances expression of PPAR γ and PTEN, resulting in reduction of eosinophilic inflammation and airway hyperresponsiveness. We have also found that overexpression of PPAR γ by administration of AdPPAR γ increases PTEN expression while decreasing the levels of ECP and various cytokines (IL-4, IL-5, and IL-13), which are induced by OVA-inhalation. These findings suggest that PPAR γ uses PTEN to modulate asthmatic responses.

Studies have demonstrated that activation of PPAR γ reduces expression of various cytokines, airway hyperresponsiveness, and activation of eosinophils, which are increased by induction of asthma (18–19). Consistent with these observations, our results have shown that administration the PPAR γ agonists or AdPPAR γ substantially inhibits expression of cytokines (IL-4, IL-5, and IL-13), airway hyperresponsiveness, and eosinophilic inflammation. However, these results are in contradiction to the findings reported by Hammad and colleagues (20). They have shown that rosiglitazone fails to reduce eosinophilic airway inflammation when it was administered by oral gavage. The reasons of the discrepancy might be due to the administration methods (duration and amounts) of each compound (30 min before OVA aerosol versus 7 times by oral gavage at 24 h intervals on days 19–25, beginning 2 days before the first challenge). Moreover, induction of asthma through OVA-challenge increased expression of PPAR γ itself and administration of the agonists further increased the receptor expression. Up-regulation of PPAR γ expression is also observed in human asthmatic airways (9). Overexpression of PPAR γ by administration of AdPPAR γ resulted in reduction of all asthmatic features. These findings indicate that PPAR γ is associated with anti-inflammatory responses in asthma.

A number of studies have demonstrated that inflammatory mediators attract and activate eosinophils via signal transduction pathways involving PI3K (21–25). PTEN functions primarily as a lipid phosphatase, which regulates crucial signal transduction pathways mediated by PIP3 (26). PTEN has been implicated in regulating cell survival signaling through the PI3K/Akt pathway. PTEN opposes the action of PI3K by dephosphorylating the signal lipid PIP3. PIP3,

produced by PI3K following activation by receptor tyrosine kinases, activated Ras or G proteins, leads to the stimulation of several downstream targets, including the serine/threonine protein kinase Akt (27). We have previously demonstrated that PI3K activity is up-regulated, while PTEN expression is reduced in allergen-induced model of asthma (11). Consistent with these previous findings, in the present OVA-induced model of asthma, PTEN protein level is substantially reduced while Akt phosphorylation and PI3K activity are increased. Administration of PPAR γ agonists or AdPPAR γ results in increase of PTEN expression up to the level of the control. Supporting the observations, Akt phosphorylation and PI3K activity increased by the induction of asthma with OVA is significantly reduced by the administration of the PPAR γ agonists or AdPPAR γ .

Previous reports have demonstrated that NF- κ B plays a critical role in immune and inflammatory responses, including asthma (28–33). Activation of NF- κ B in allergic airway inflammation has also been observed (34, 35). Determination of NF- κ B protein level in nuclear extracts has revealed that this protein level is substantially increased in our present OVA-induced model of asthma, suggesting that NF- κ B is activated. The administration of rosiglitazone, pioglitazone, or AdPPAR γ results in significant reduction of nuclear NF- κ B level. These results indicate that PPAR γ agonists or AdPPAR γ inhibit NF- κ B activity by preventing translocation of this transcription factor into nucleus. Inhibitory κ B (I κ B) has 5–7 conserved domains known as ankyrin repeats, each consisting of around 30 amino acids, forming a unit that can interact with NF- κ B subunits, thus masking the nuclear localization signals and preventing activation of NF- κ B and its translocation to the nucleus (30, 36). Activation of Akt has been shown to enhance the degradation of the I κ Bs and to cooperate with other factors to induce NF- κ B-mediated activation of NF- κ B-responsive model promoters (37). Therefore, we suggest that PPAR γ agonists or AdPPAR γ inhibit NF- κ B signal transduction pathway by decreasing NF- κ B binding activity to the promoter regions of a large variety of genes, which are considered to be involved in airway inflammation in asthma through the regulation of PI3K/Akt pathway.

The signaling mechanism by which stimulation of PPAR γ with the agonists regulates PTEN expression as well as Akt phosphorylation remains to be elucidated. However, our results are in agreement with the observation that anti-inflammatory action of the PPAR γ agonists is mediated via up-regulation of PTEN (10). Mounting evidence indicates that many inflammatory mediators attract and activate eosinophils via signal transduction pathways involving PI3K (11, 21–25). Studies have shown that administration of PI3K inhibitors such as wortmannin suppresses generation of various inflammatory mediators in eosinophils (21–25). Recently, we have also demonstrated that administration of AdPTEN cDNA or PI3K inhibitor significantly attenuates bronchial inflammation and airway hyperresponsiveness (11). Collectively, these findings suggest a protective role of PPAR γ in the pathogenesis of the asthma phenotype through regulation of PTEN expression.

ACKNOWLEDGMENT

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Fig. 1

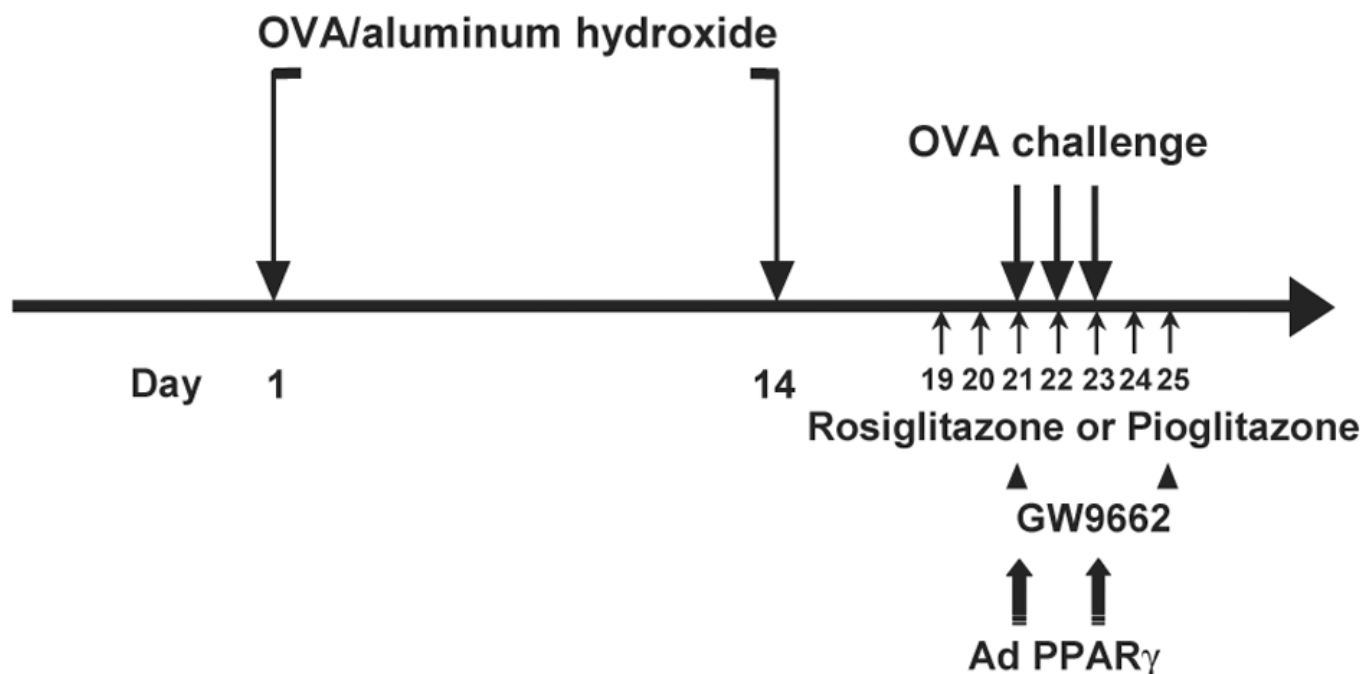


Figure 1. Schematic diagram of the experimental protocol. Mice were sensitized on days 1 and 14 by intraperitoneal injection of OVA emulsified in 1 mg of aluminum hydroxide. On days 21, 22, and 23 after the initial sensitization, the mice were challenged for 30 min with an aerosol of 3% (w/v) OVA in saline (or with saline as a control) using an ultrasonic nebulizer. In the case of treatment with rosiglitazone or pioglitazone, oral gavage was given 7 times at 24-h intervals on days 19–25, beginning 2 days before the first challenge. In the case of treatment with GW9662, GW9662 was administered intratracheally two times to each animal, once on day 21 and the second time on day 25. Ad vector was administered intratracheally 2 times to each animal, once on day 21 (1 h before the first airway challenge with OVA) and the second time on day 23 (3 h after the last airway challenge with OVA).

Fig. 2

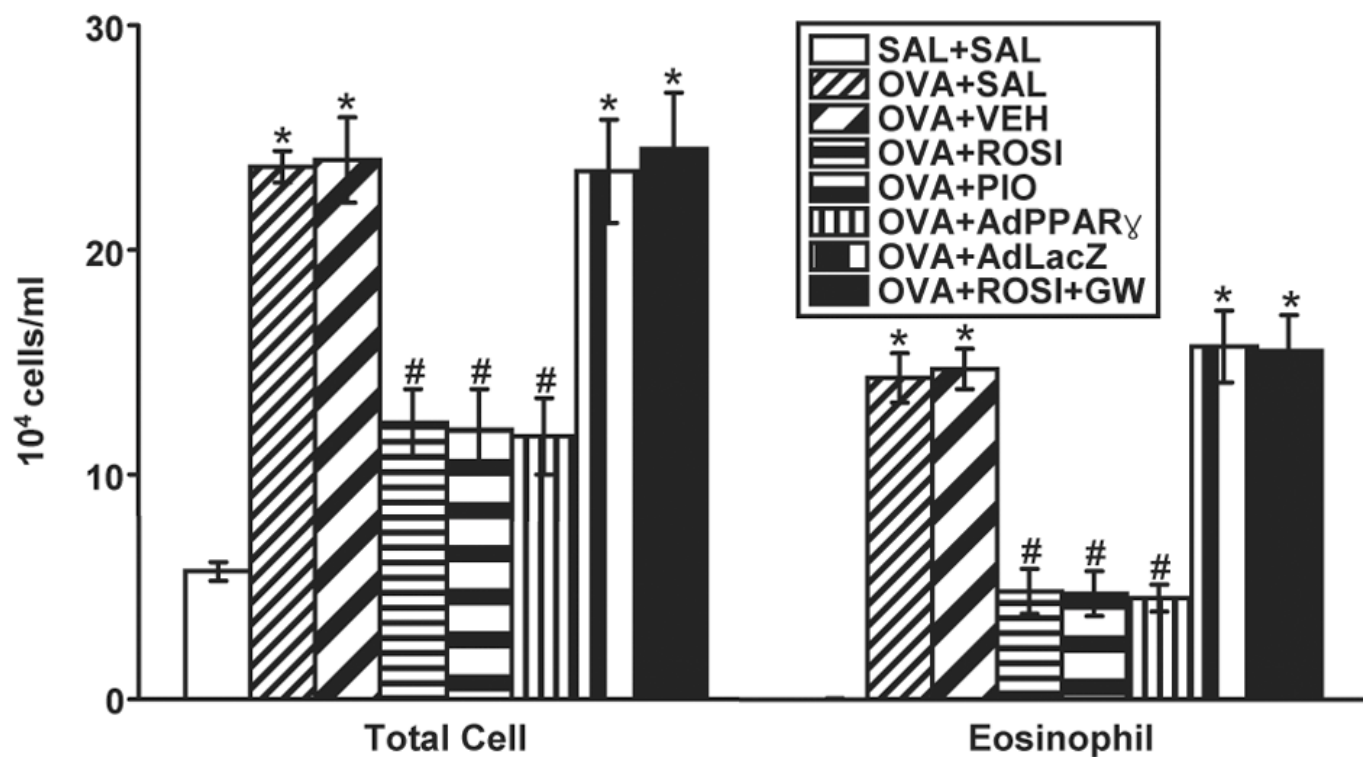


Figure 2. Effect of rosiglitazone, pioglitazone, GW9662 plus rosiglitazone, or AdPPAR γ on total cells and eosinophils in BAL fluids of OVA-sensitized and -challenged mice. The numbers of total cells and eosinophils of BAL from saline-inhaled mice administered saline (SAL+SAL), OVA-inhaled mice administered saline (OVA+SAL), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered rosiglitazone (OVA+ROSI), OVA-inhaled mice administered pioglitazone (OVA+PIO), OVA-inhaled mice administered AdPPAR γ (OVA+AdPPAR γ), OVA-inhaled mice administered AdLacZ (OVA+AdLacZ), and OVA-inhaled mice administered GW9662 plus rosiglitazone (OVA+ROSI+GW) were counted at 72 h after the last challenge. Bars represent mean $\pm$ SEM from six independent experiments. * P < 0.05 versus SAL + SAL; # P < 0.05 versus OVA+SAL.

Fig. 3

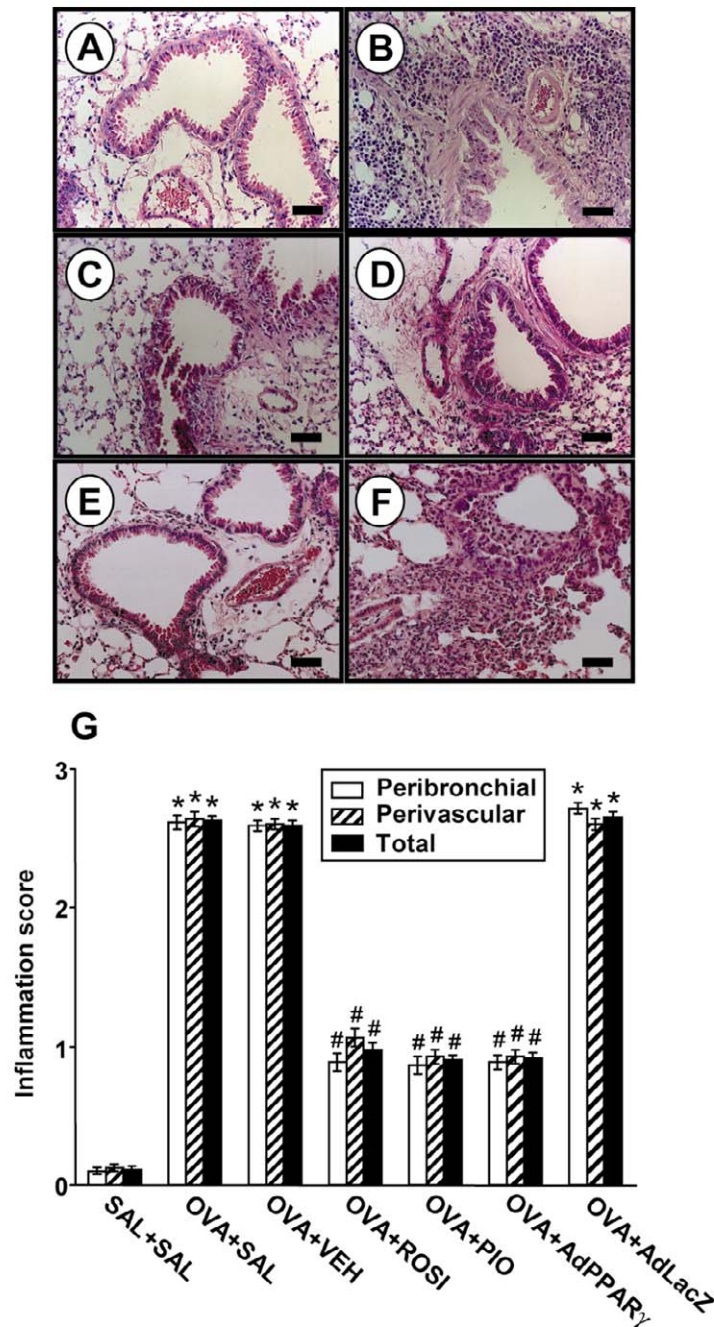


Figure 3. Effect of rosiglitazone, pioglitazone, or AdPPAR γ in lung tissues of OVA-sensitized and -challenged mice. Representative hematoxylin and eosin-stained sections of the lungs. Sampling was performed at 72 h after the last challenge in saline-inhaled mice administered saline (A), OVA-inhaled mice administered saline (B), OVA-inhaled mice administered rosiglitazone (C), OVA-inhaled mice administered pioglitazone (D), OVA-inhaled mice administered AdPPAR γ (E), and OVA-inhaled mice administered AdLacZ (F). Bars indicate scale of 50 μ m. Peribronchial and perivascular lung inflammation were measured at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+Sal), OVA-inhaled mice administered saline (OVA+Sal), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered rosiglitazone (OVA+ROSI), OVA-inhaled mice administered pioglitazone (OVA+PIO), OVA-inhaled mice administered AdPPAR γ (OVA+AdPPAR γ), and OVA-inhaled mice administered AdLacZ (OVA+AdLacZ) (G). Bars represent mean $\pm$ SEM from 6 independent experiments. * $P < 0.05$ versus SAL+Sal; # $P < 0.05$ versus OVA+Sal.

Fig. 4

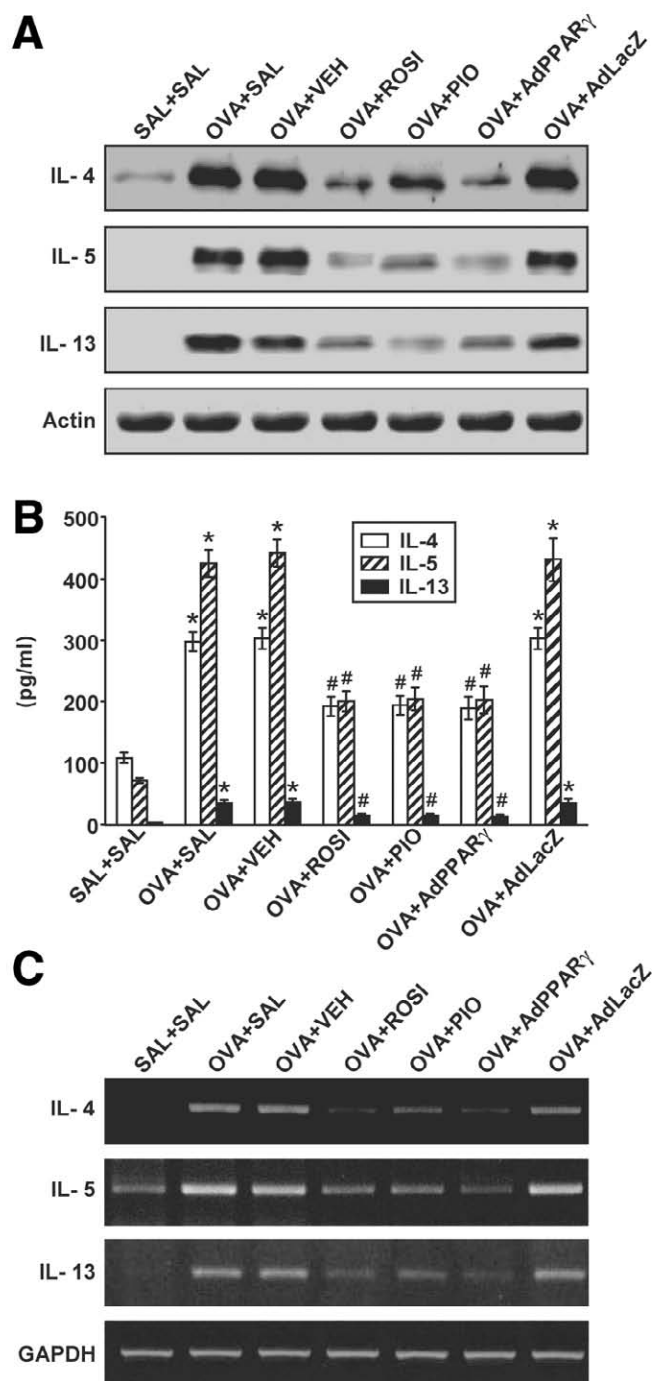


Figure 4. Effect of rosiglitazone, pioglitazone, or AdPPAR γ on IL-4, IL-5, and IL-13 expression in lung tissues and in BAL fluids of OVA-sensitized and -challenged mice. Western blotting of IL-4, IL-5, and IL-13 (**A**). Enzyme immunoassay of IL-4, IL-5, and IL-13 (**B**). RT-PCR of IL-4, IL-5, and IL-13 (**C**). Sampling was performed at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+Sal), OVA-inhaled mice administered saline (OVA+Sal), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered rosiglitazone (OVA+ROSI), OVA-inhaled mice administered pioglitazone (OVA+PIO), OVA-inhaled mice administered AdPPAR γ (OVA+AdPPAR γ), and OVA-inhaled mice administered AdLacZ (OVA+AdLacZ). Bars represent mean $\pm$ SEM from 6 independent experiments. * $P < 0.05$ versus SAL + SAL; # $P < 0.05$ versus OVA+Sal.

Fig. 5

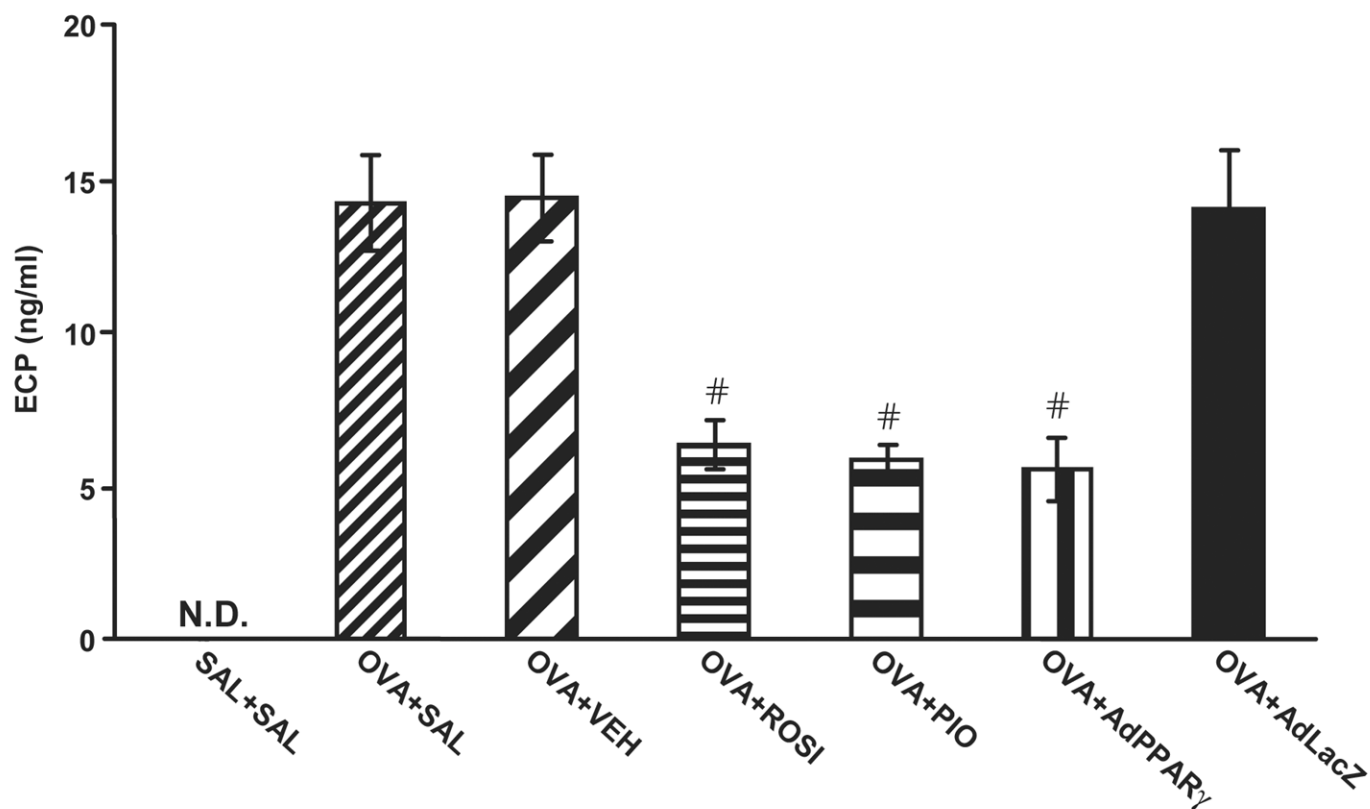


Figure 5. Effect of rosiglitazone, pioglitazone, or AdPPAR γ on ECP levels in BAL fluids of OVA-sensitized and -challenged mice. Enzyme immunoassay of ECP. Sampling was performed at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+SAL), OVA-inhaled mice administered saline (OVA+SAL), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered rosiglitazone (OVA+ROSI), OVA-inhaled mice administered pioglitazone (OVA+PIO), OVA-inhaled mice administered AdPPAR γ (OVA+AdPPAR γ), and OVA-inhaled mice administered AdLacZ (OVA+AdLacZ). Bars represent mean $\pm$ SEM from 6 independent experiments. N.D., not detected. # $P < 0.05$ versus OVA+SAL.

Fig. 6

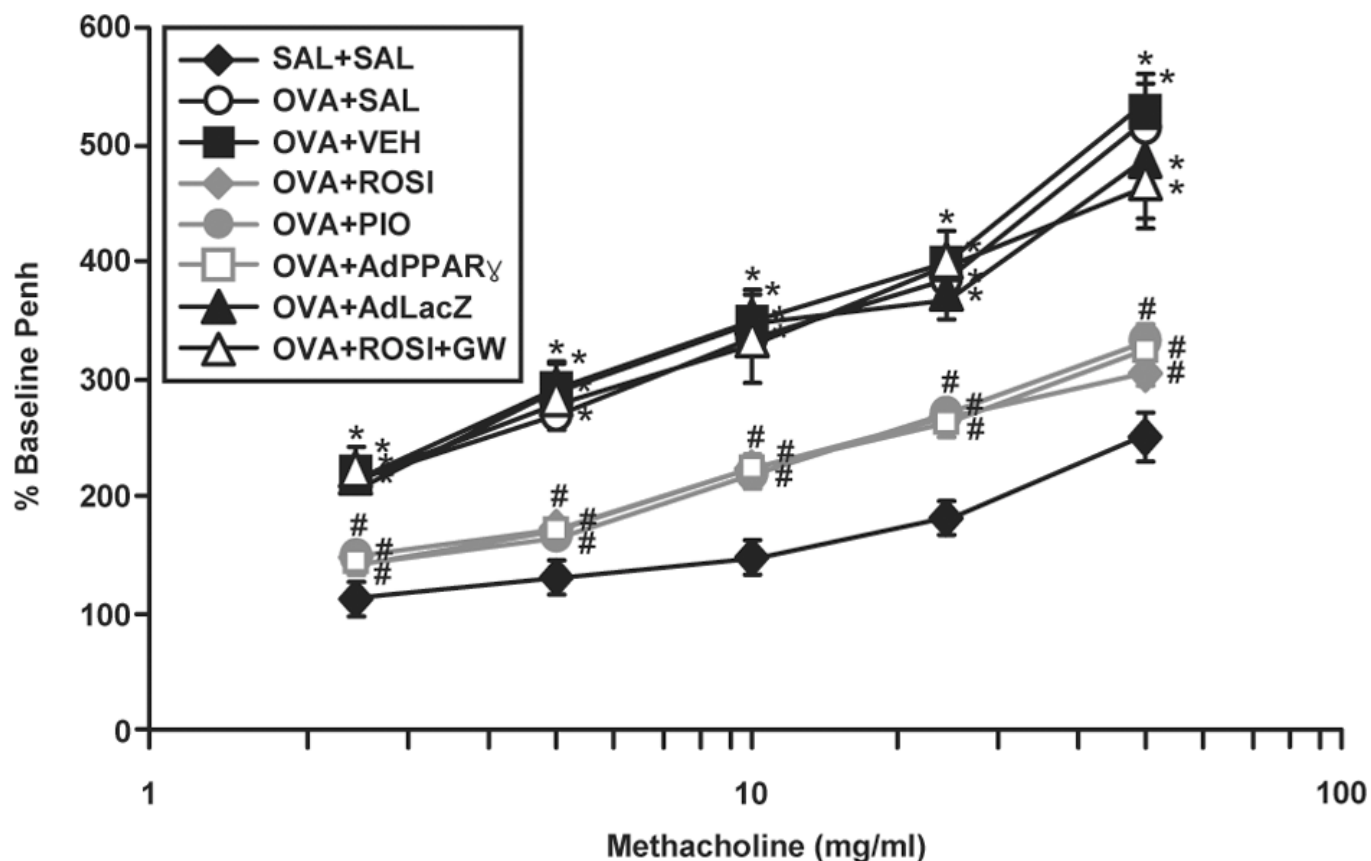


Figure 6. Effect of rosiglitazone, pioglitazone, GW9662 plus rosiglitazone, or AdPPAR γ on airway responsiveness in OVA-sensitized and -challenged mice. Airway responsiveness was measured at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+SAL), OVA-inhaled mice administered saline (OVA+SAL), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered rosiglitazone (OVA+ROSI), OVA-inhaled mice administered pioglitazone (OVA+PIO), OVA-inhaled mice administered AdPPAR γ (OVA+AdPPAR γ), OVA-inhaled mice administered AdLacZ (OVA+AdLacZ), and OVA-inhaled mice administered GW9662 plus rosiglitazone (OVA+ROSI+GW). Airway responsiveness to aerosolized methacholine was measured in unrestrained, conscious mice. Mice were placed in the main chamber of a barometric plethysmograph and nebulized first with saline and then with increasing doses (2.5 to 50 mg/ml) of methacholine for 3 min for each nebulization. Readings of breathing parameters were taken for 3 min after each nebulization, during which Penh values were determined. Data represent mean $\pm$ SEM from 6 independent experiments. * P < 0.05 versus SAL+SAL; # P < 0.05 versus OVA+SAL.

Fig. 7

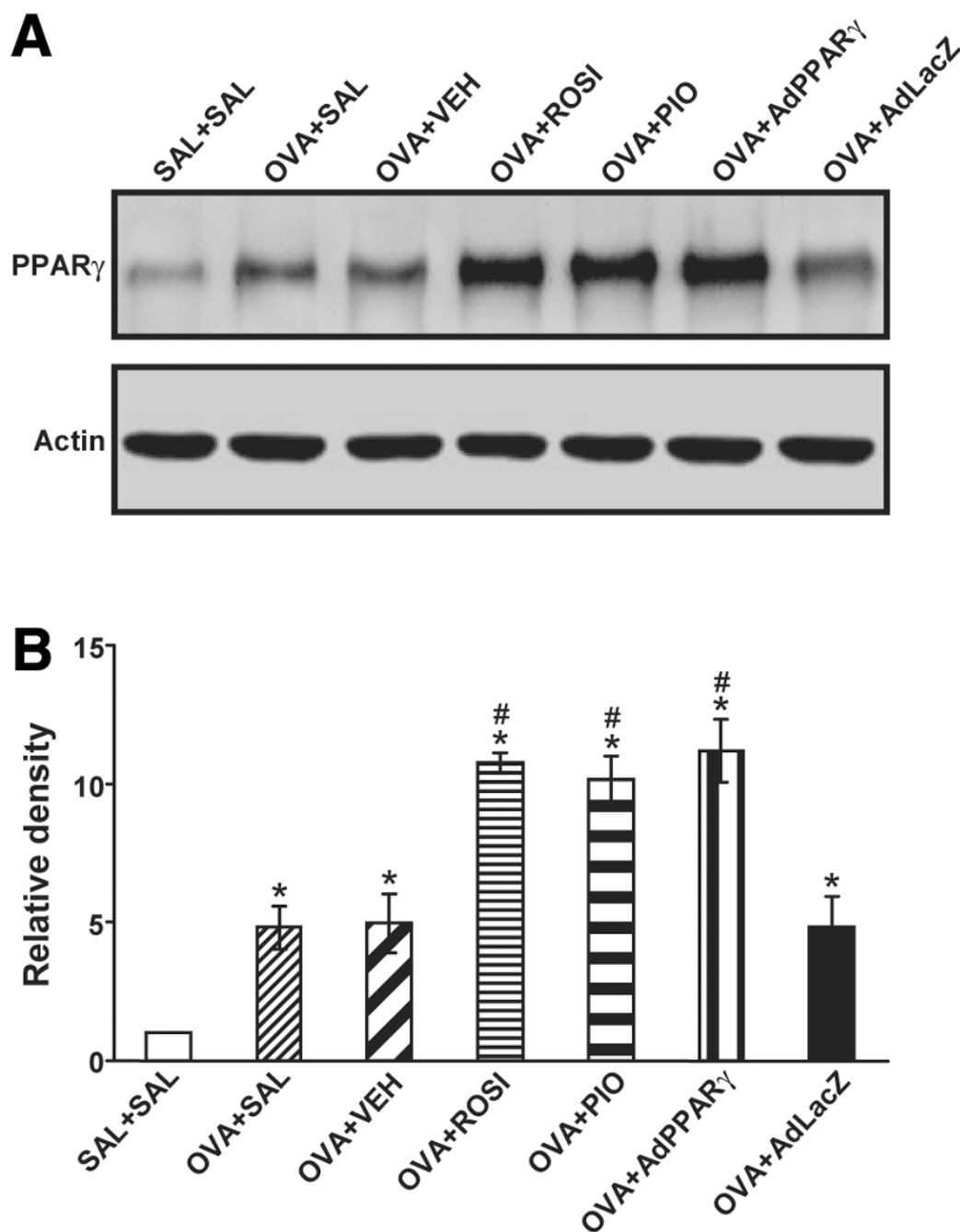


Figure 7. Effect of rosiglitazone, pioglitazone, or AdPPAR γ on PPAR γ protein expression in lung tissues of OVA-sensitized and -challenged mice. Western blotting of PPAR γ (A). PPAR γ protein expression was measured at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+Sal), OVA-inhaled mice administered saline (OVA+Sal), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered rosiglitazone (OVA+ROSI), OVA-inhaled mice administered pioglitazone (OVA+PIO), OVA-inhaled mice administered AdPPAR γ (OVA+AdPPAR γ), and OVA-inhaled mice administered AdLacZ (OVA+AdLacZ). Densitometric analyses are presented as the relative ratio of PPAR γ to actin (B). The relative ratio of PPAR γ in the lung tissues of SAL+Sal is arbitrarily presented as 1. Data represent mean $\pm$ SEM from six independent experiments. * P < 0.05 versus SAL+Sal; # P < 0.05 versus OVA+Sal.

Fig. 8

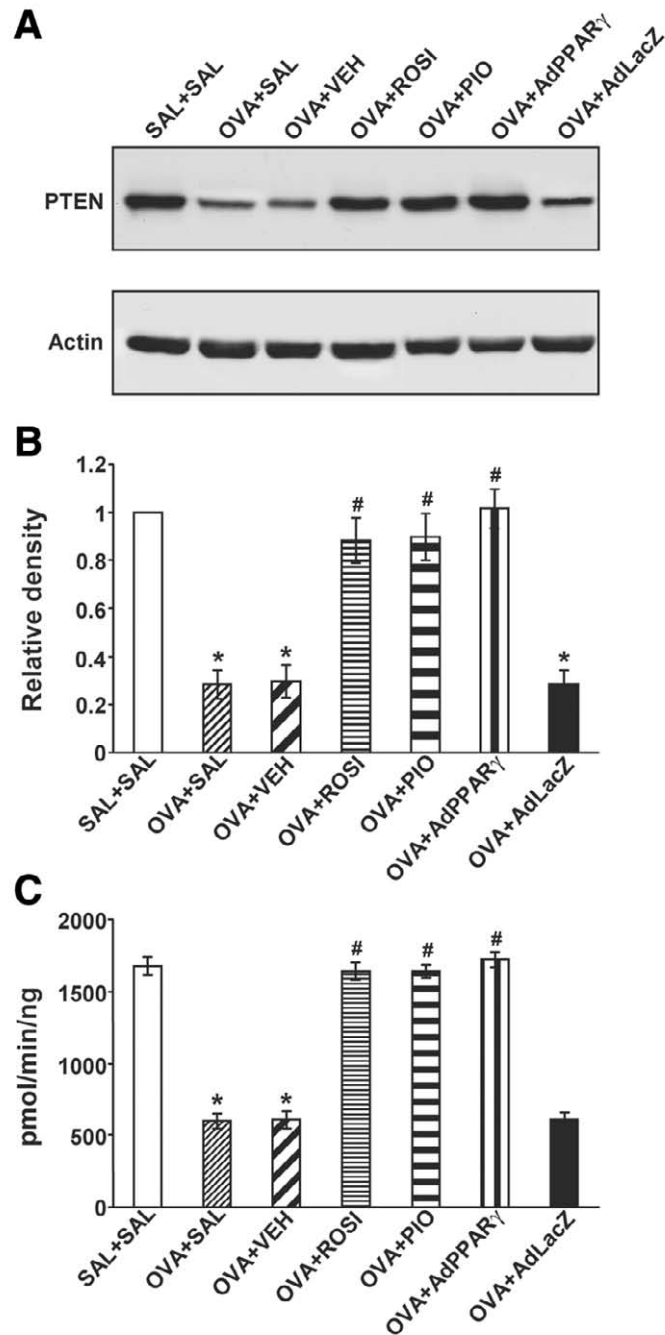


Figure 8. Effect of rosiglitazone, pioglitazone, or AdPPAR γ on PTEN protein and PTEN activity in lung tissues of OVA-sensitized and -challenged mice. Western blotting of PTEN (A). PTEN protein expression was measured at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+Sal), OVA-inhaled mice administered saline (OVA+Sal), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered rosiglitazone (OVA+ROSI), OVA-inhaled mice administered pioglitazone (OVA+PIO), OVA-inhaled mice administered AdPPAR γ (OVA+AdPPAR γ), and OVA-inhaled mice administered AdLacZ (OVA+AdLacZ). Densitometric analyses are presented as the relative ratio of PTEN to actin (B). The relative ratio of PTEN in the lung tissues of SAL+Sal is arbitrarily presented as 1. PTEN activity was measured in lung tissues from sensitized mice challenged with OVA or with saline (C). Data represent mean $\pm$ SEM from six independent experiments. * $P < 0.05$ versus SAL+Sal; # $P < 0.05$ versus OVA+Sal.

Fig. 9

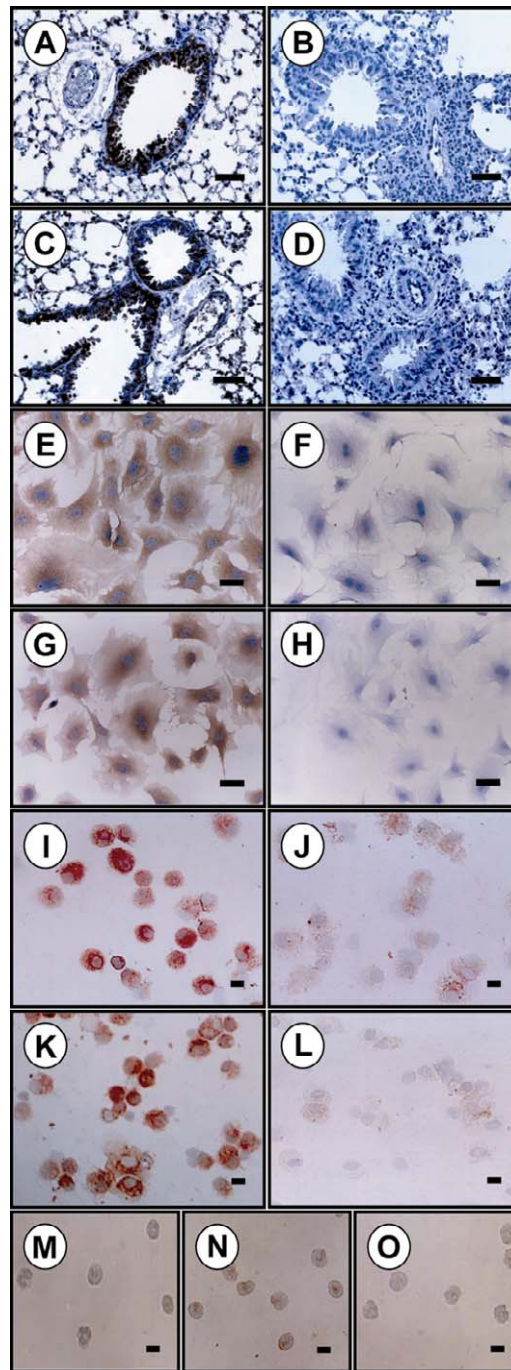


Figure 9. Localization of immunoreactive PTEN in lung tissues, tracheal epithelial cells, BAL fluids, and BAL fluid eosinophils of OVA-sensitized and -challenged mice. Sampling was performed at 72 h after the last challenge in lung tissues, tracheal epithelial cells, and BAL fluids from sensitized mice challenged with saline (**A**, **E**, and **I**), from sensitized mice challenged with OVA (**B**, **F**, **J**, and **M**), from OVA-inhaled mice administered AdPPAR γ (**C**, **G**, **K**, and **N**), and from OVA-inhaled mice administered AdLacZ (**D**, **H**, **L**, and **O**). Representative light microscopy shows PTEN-positive cells in the bronchioles (**A**, **B**, **C**, and **D**; the dark brown color indicates PTEN-positive cells), tracheal epithelial cells (**E**, **F**, **G**, and **H**; the dark brown color indicates PTEN-positive cells), in the BAL fluids (**I**, **J**, **K**, and **L**; the brown color indicates PTEN-positive cells), and in the BAL fluid eosinophils (**M**, **N**, and **O**; the dark brown color indicates PTEN-positive cells). Bars indicate scale of 50 μ m (**A**, **B**, **C**, **D**, **E**, **F**, **G**, and **H**) or 10 μ m (**I**, **J**, **K**, **L**, **M**, **N**, and **O**).

Fig. 10

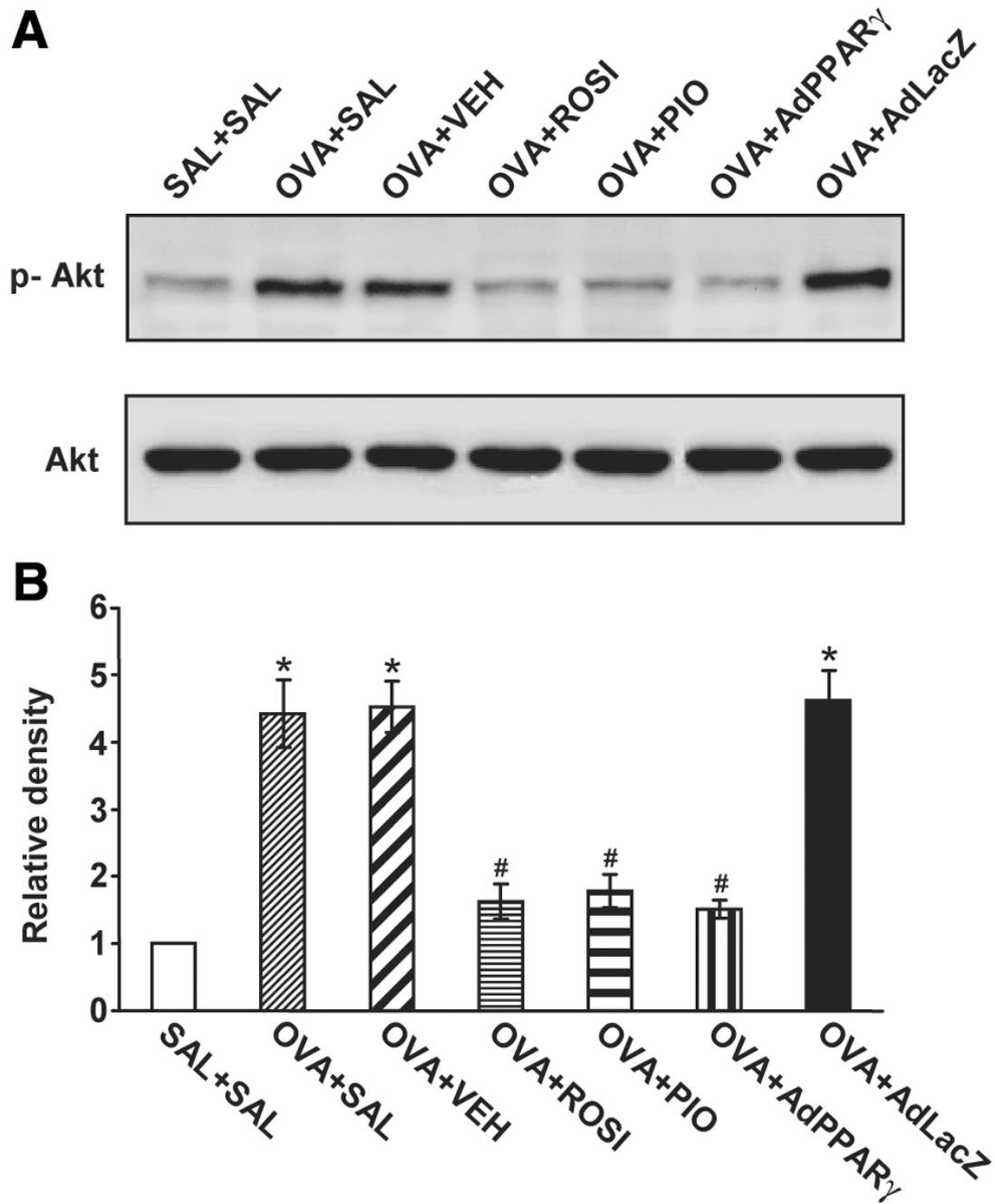


Figure 10. Effect of rosiglitazone, pioglitazone, or AdPPAR γ on p-Akt and Akt protein expression in lung tissues of OVA-sensitized and -challenged mice. Western blotting of p-Akt and Akt (A). p-Akt and Akt protein expression were measured at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+Sal), OVA-inhaled mice administered saline (OVA+Sal), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered rosiglitazone (OVA+ROSI), OVA-inhaled mice administered pioglitazone (OVA+PIO), OVA-inhaled mice administered AdPPAR γ (OVA+AdPPAR γ), and OVA-inhaled mice administered AdLacZ (OVA+AdLacZ). Densitometric analyses are presented as the relative ratio of p-Akt to Akt (B). The relative ratio of p-Akt in the lung tissues of SAL+Sal is arbitrarily presented as 1. Data represent mean $\pm$ SEM from six independent experiments. * $P < 0.05$ versus SAL+Sal; # $P < 0.05$ versus OVA+Sal.

Fig. 11

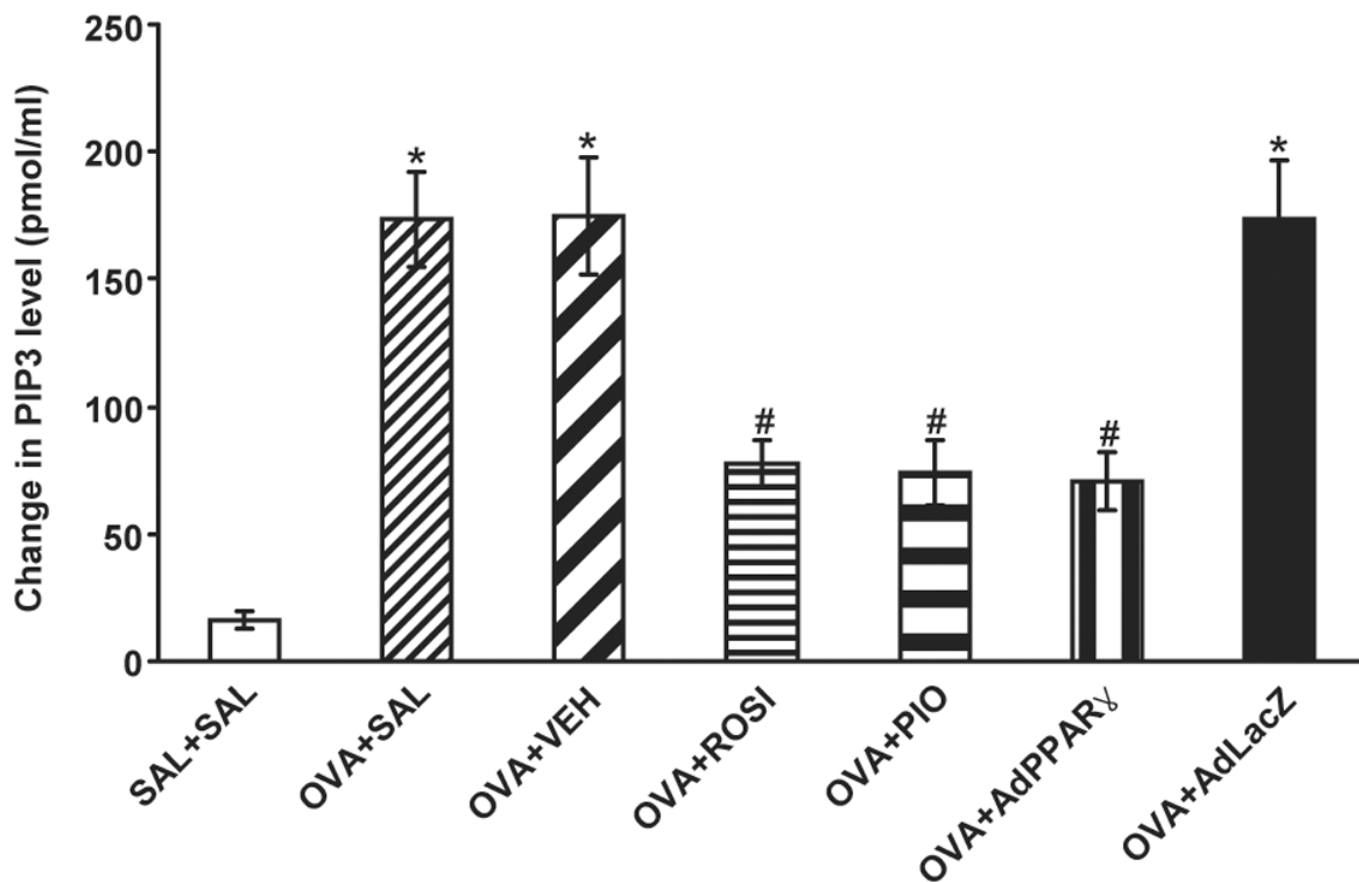


Figure 11. Effect of rosiglitazone, pioglitazone, or AdPPAR γ on PI3K activities in lung tissue extracts of OVA-sensitized and -challenged mice. PIP3 generation by PI3K in lung tissue extracts was measured at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+SAL), OVA-inhaled mice administered saline (OVA+SAL), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered rosiglitazone (OVA+ROSI), OVA-inhaled mice administered pioglitazone (OVA+PIO), OVA-inhaled mice administered AdPPAR γ (OVA+AdPPAR γ), and OVA-inhaled mice administered AdLacZ (OVA+AdLacZ). Bars represent mean $\pm$ SEM from six independent experiments. * P < 0.05 versus SAL + SAL; # P < 0.05 versus OVA + SAL.

Fig. 12

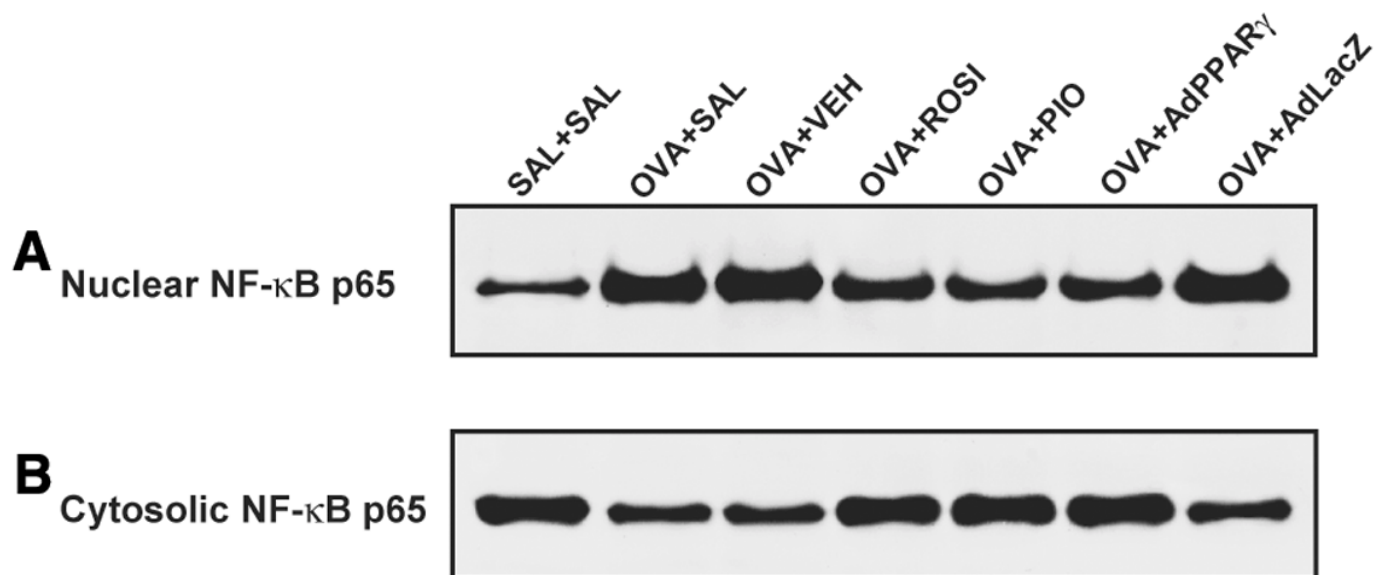


Figure 12. Effect of rosiglitazone, pioglitazone, or AdPPAR γ on NF-κB p65 protein level in nuclear and cytosolic protein extracts of lung tissues of OVA-sensitized and -challenged mice. **A)** NF-κB p65 protein level in nuclear protein extracts of lung tissues. **B)** NF-κB p65 protein level in cytosolic protein extracts of lung tissues. NF-κB protein levels were measured at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+SAL), OVA-inhaled mice administered saline (OVA+SAL), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered rosiglitazone (OVA+ROSI), OVA-inhaled mice administered pioglitazone (OVA+PIO) OVA-inhaled mice administered AdPPAR γ (OVA+AdPPAR γ), and OVA-inhaled mice administered AdLacZ (OVA+AdLacZ). Results were similar in six independent experiments.