



Rosiglitazone, a peroxisome proliferator-activated receptor- γ agonist, attenuates airway inflammation by inhibiting the proliferation of effector T cells in a murine model of neutrophilic asthma

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ABSTRACT

An imbalanced Th17-mediated immune response contributes substantially to neutrophilic asthma. Studies have also demonstrated that peroxisome proliferator-activated receptor- γ (PPAR γ) plays a critical role in inflammatory disease. However, the effect of PPAR γ on airway inflammation in neutrophilic asthma remains unclear.

In the current study, we evaluated the potential therapeutic role of rosiglitazone (RSG) in a new mouse model of asthma characterised by increased neutrophils rather than eosinophils. A co-culture system of DCs with CD4⁺ naïve T cells was established to evaluate the effects of RSG on T cell differentiation.

After challenge with OVA, mice developed the typical pathophysiological features of asthma, including an increased number of neutrophils in the BALF and the up-regulation of IL-17. The numbers of Th17 cells and Th2 cells were also greatly elevated in the lungs. The administration of rosiglitazone reduced the pathophysiological features of asthma and decreased the up-regulated inflammatory mediators and cytokines. Furthermore, the cell viability in the co-culture system was markedly reduced by RSG. T-bet, Gata-3 and ROR γ t mRNA were down-regulated by RSG. These findings suggest that PPAR γ is critical for airway inflammation during neutrophilic asthma, possibly due to its effect on Th cell proliferation and differentiation.

These findings suggest that the therapeutic effect of rosiglitazone in neutrophilic asthma is partially due to the role of the PPAR γ pathway in regulating T cell proliferation and differentiation.

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1. Introduction

CD4⁺ T lymphocytes (CD4⁺ T) play a major role in the pathogenesis of asthma. A number of studies have indicated that an enhanced Th2-mediated immune response is the primary reason for the clinical symptoms of asthma, particularly eosinophil infiltration [1–3]. Recently, the Global Initiative for Asthma (GINA) classified bronchial asthma into four phenotypes according to the granulocyte pattern of sputum cells [4]. The Th2 response results in the sensitisation to an inhaled antigen, leading to high levels of allergen-specific IgE and eosinophilic airway inflammation in

eosinophilic asthma (EA) [2]. Several other studies demonstrated that neutrophils, not eosinophils, are the major inflammatory cell type in asthma patients with steroid-persistent or acute exacerbation [5]. IL-17A, a marker of Th17 cells, plays an essential role in regulating the maturation, recruitment and chemotaxis of neutrophils [6–8]. Accumulating evidence suggested that asthma is a highly heterogeneous disease. Thus, research into the role of neutrophils in asthma is required to identify additional options for the treatment of neutrophilic airway inflammation during asthma.

Peroxisome proliferators activated receptors (PPARs) belong to the nuclear receptor superfamily [9]. Four different subtypes have been identified: PPAR α , PPAR β/δ and PPAR γ . They are ubiquitously expressed through the whole lung tissues. PPARs are present in the cytosol and nucleus and perform functions in both gene-dependent and -independent mechanisms [10,11]. Rosiglitazone is a notable synthetic high-affinity PPAR γ agonist. GW9662 is a non-thiazolidinedione PPAR γ agonist and can block rosiglitazone binding [12,13]. PPAR γ ligands have recently been implicated as the targets of cellular inflammatory and immune responses [14,15].

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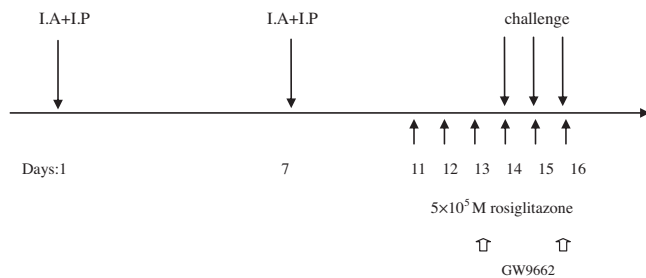


Fig. 1. Experimental protocol. The mice were divided into four groups. PBS: Mice administered vehicle PBS during sensitisation as a negative control. OVA-LPS: Mice were anaesthetised with 4% chloral hydrate and sensitised on days 1 and 7 by I.A. instillation of 100 μ g OVA emulsified in 50 μ l of PBS containing 0.1 μ g of LPS and by I.P. injection of 100 μ g of OVA emulsified in 100 μ l of aluminium hydroxide. On days 13, 14 and 15, the mice were challenged for 60 min with an aerosol of 1% OVA in PBS using a PARI ultrasonic nebuliser. OVA-LPS + RSG: Rosiglitazone was administered by nebulisation for 30 min on days 11–16. OVA-LPS + RSG + GW: GW9662 was administered intratracheally on days 13 and 16.

Given its anti-inflammatory and immunomodulatory properties, PPAR γ has been used in the treatment of inflammatory diseases, including asthma [11].

Dendritic cells (DCs) are generally considered to be the most powerful APCs and major regulators of the innate and adaptive immune responses, and these cells play an important role in maintaining self-tolerance. Myeloid dendritic cells (mDCs) are required for the induction of eosinophilic asthma inflammation [2]. Plasmacytoid DCs (pDCs) are thought to contribute to immune tolerance by promoting the development of regulatory T (Treg) cells [16]. The aim of our study was to evaluate the therapeutic value of rosiglitazone in a model of neutrophilic asthma. We utilised a DC and naïve CD4⁺ T cell co-culture system to determine the potential therapeutic mechanism of rosiglitazone.

2. Materials and methods

2.1. Animals and experimental protocol

Female BALB/c mice, 6–8 wks of age and free of murine-specific pathogens, were obtained from the Chongqing Medical University. The mice were housed throughout the experiments in a laminar flow cabinet, and all experiments were conducted in accordance with the Institutional Animal Care and Use Committee at the National Institute of Environmental Health Sciences. The neutrophilic asthma murine model was established using an intratracheal LPS protocol. The mice were sensitised on days 1 and 7 by intraperitoneal injection (I.P.) with 100 μ g of ovalbumin (Sigma, endotoxin concentration <1 EU/mg OVA) complexed in 50% aluminium hydroxide (Pierce) in a total volume of 200 μ l. Meanwhile, the mice were instilled intratracheally (I.A.) with 100 μ g of ovalbumin containing 0.1 μ g of LPS (Sigma, prepared from *Escherichia coli* 0111:B4) with PBS as a vehicle in a total volume of 50 μ l, as described previously [17,18]. On days 13, 14, and 15 after the initial sensitisation, the mice were challenged for 60 min with an aerosol of 1% (w/v) OVA in PBS (or with PBS as a control) using an ultrasonic nebuliser (Pari-Master). For our experiments, 5×10^5 M rosiglitazone (Qbiogene) dissolved in PBS was administered 6 times at 24-h intervals for 60 min on days 11–16, beginning 3 days before the first nebulisation challenge [19]. A selective antagonist of PPAR γ , GW9662, was dissolved in PBS and administered intratracheally on days 13 and 16 to each animal (Fig. 1). The negative control group was administered PBS using the same technique and volume.

2.2. Determination of airway responsiveness

The airway responsiveness was determined after the administration of increasing doses of methacholine (Sigma) using a computer-controlled small animal ventilator (Emka). Individual peak responses were determined at each dose per mouse. The results are represented as the fold increases in R (cm H₂O s/ml) above baseline and were calculated as follows: $[R(\text{response}) - R(\text{baseline})]/R(\text{baseline})$ [18].

2.3. Measurement of serum OVA-specific IgE

Mice were sacrificed at 48 h after the last challenge by injection of an overdose of chloral hydrate (400 mg/kg body weight, administered i.p.). A blood sample was taken from the retroocular plexus using a glass capillary and was centrifuged. The serum was snap-frozen in liquid nitrogen and stored at -70°C for OVA-specific IgE measurements using a mouse OVA-sIgE ELISA kit (R&D Systems).

2.4. Airway inflammation evaluation

BALF was performed as described previously [20]. Briefly, the total cell numbers were determined using a haemocytometer. Smears of BALF cells were stained with Gimsa to determine the differential cell counts. The levels of IFN- γ , IL-5, IL-17, IL-8 and TNF- α in the BALF were determined by enzyme immunoassays according to the manufacturer's protocols (R&D Systems).

2.5. Histology

The lungs were fixed in 10% formalin and embedded in paraffin. Longitudinal sections 4 mm thick were cut from the left lobe, placed on glass slides, deparaffinised, and stained sequentially with H&E (Richard-Allan Scientific).

2.6. Immunohistochemistry

Deparaffinised and PBS-washed sections were incubated with 3% H₂O₂ in PBS for 15 min to block endogenous peroxidases, and the background non-specific binding was reduced by incubating with 1% BSA in PBS for 60 min at room temperature. The sections were incubated with antibodies against IL-17 (Abcam, 1:200) overnight at 4°C . After washing, the sections were incubated with biotin-conjugated anti-rabbit IgG (Jackson ImmunoResearch, 50:1 H+L) for 50 min at room temperature. The biotinylated reagents were detected with ABC complex horseradish peroxidase (HRP) (Dako), and the sections were counterstained with haematoxylin.

2.7. Quantitative real time-PCR

Total RNA was isolated from the lung tissues according to the manufacturer's protocol (TAKALA). Quantitative RT-PCR (qPCR) analysis was performed using the Fast-Start Universal SYBR Green-Master (ROX; Roche). The relative mRNA levels of each sample were calculated according to the manufacturer's protocol. The relative gene expression levels were calculated using the comparative Ct ($\Delta\Delta\text{Ct}$) method with β -actin as a reference gene. The sequences of the primers are shown in Table 1.

2.8. Mixed lymphocytes reaction

DCs were generated from the bone marrow cells of BALB/c mice according to a previously described protocol [21]. Briefly, bone marrow cells were obtained from the femurs and iliac bones of mice and placed in DC culture medium (DC-CM; RPMI 1640 containing

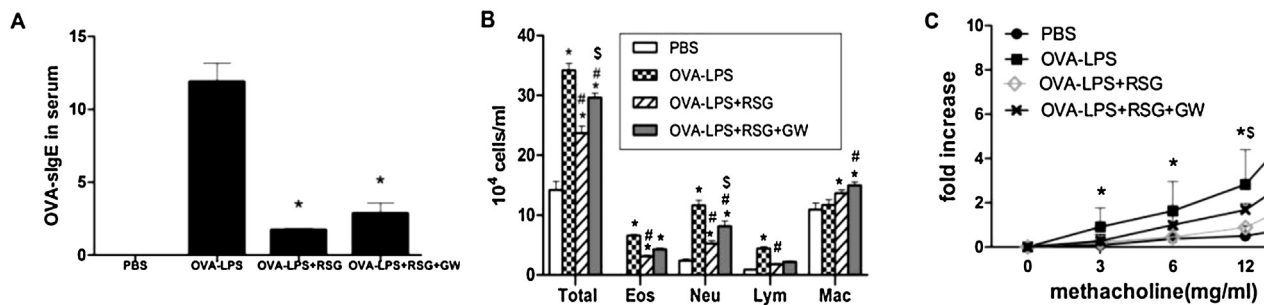


Fig. 2. In vivo treatment with rosiglitazone significantly suppressed the development of neutrophilic asthma. Mice were treated with vehicle PBS, OVA-LPS, OVA-LPS + RSG, or OVA-LPS + RSG + GW. (A) An ELISA kit was used to determine the OVA-sIgE levels in the serum at 48 h after the last challenge. (B) Comparison of rosiglitazone with or without GW9662 on cellular changes in the BALF. The BALF was performed at 48 h post-challenge. Giemsa-stained BALF cell sedimentation was observed under an oil microscope. (C) Effect on airway hyperresponsiveness tested at 48 h after the last challenge with 1% OVA. * $p < 0.05$ vs. PBS; # $p < 0.05$ vs. OVA-LPS; \$ $p < 0.05$ vs. OVA-LPS + RSG.

Table 1
Relevant gene names and sequences.

	Sense	Antisense
β -actin	5'-gaaatcgtgcgtgacatcaaa-3'	5'-tgtagtgttcattggtccacag-3'
T-bet	5'-caaccagcaccagacagat-3'	5'-accaagaccacatccacaac-3'
GATA-3	5'-ccaggcaagatgagaaagagt-3'	5'-ataggcggtatggtggaatg-3'
ROR γ t	5'-ttcagtattgtgtggagtgttc-3'	5'-aaaagactgtgtgtgtgtgtg-3'
PPAR γ	5'-atgccattctggccacacactt-3'	5'-cccttgcatccttcacaagcatg-3'

10% heat-inactivated foetal calf serum [FCS], 50 mM 2-ME, 2 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin (GIBCO), 10 ng/ml recombinant mouse GM-CSF, and 10 ng/ml recombinant mouse IL-4 (R&D Systems). On day 6, the DCs were purified using CD11c⁺ microbeads (Miltenyi Biotec). The purified cells were pulsed with OVA (200 mg/ml) and LPS (2 μ g/ml) for 24 h and washed three times with PBS. Then, the pulsed DCs were incubated with rosiglitazone with or without GW9662 for 24 h. As a control, DCs were also cultured in PBS. The cells were collected after 8 days. The levels of PPAR γ mRNA were determined by qPCR, and Western blotting was used to detect the PPAR γ protein. CD4⁺ naïve T cells were generated from spleen cells of C57BL/6 mice and purified using microbeads (Miltenyi Biotec). Splenic T cells and DCs that had been treated for 8 days were co-cultured for 72 h. On day 3, the cells were collected and purified with 35% Ficoll for total T cell enumeration and qPCR.

2.9. Western blot analysis

The PPAR γ expression levels in lungs were analysed by Western blot, as described previously [20]. The blots were then incubated with an anti-PPAR γ Ab (Abcam).

2.10. Statistical analysis

All of the data from the quantitative assays were expressed as the mean $\pm$ SEM. Statistical analyses were performed using the independent-samples *t*-test or one-way ANOVA. Differences were considered statistically significant when $p < 0.05$. All of the statistical analyses were performed using GraphPad5.0 software.

3. Results

3.1. Effect of rosiglitazone with or without GW9662 on serum OVA-specific IgE levels, cellular changes in BALF and airway hyperresponsiveness

After OVA sensitisation and LPS stimulation, the levels of OVA-sIgE in the serum were up-regulated and notable hyperresponsiveness was observed. In contrast, mice treated with

rosiglitazone showed decreased specific IgE levels in the serum and improved lung tissues. This protective effect could be weakened by the PPAR γ antagonist GW9662 (Fig. 2A and C).

The number of total BALF cells obtained from the PBS control group was 14.27×10^4 cells, including only a small number eosinophils and few neutrophils (2.39×10^4). However, the numbers of total lung cells (34.2×10^4), neutrophils (9.26×10^4) and eosinophils (6.57×10^4) in the BALF of the OVA-LPS sensitisation group were significantly higher. The total cellular count was significantly reduced in the RSG-treated mice (23.68×10^4), and RSG also reduced the absolute numbers of neutrophils (5.3×10^4) and eosinophils (3.09×10^4) (Fig. 2B).

3.2. Rosiglitazone weakened the function of T cells and reduced the secretion of T cell-specific cytokines and inflammatory mediators

To investigate the immune response mediated by effector T cells, the levels of IFN- γ , IL-5 and IL-17 in the BALF were determined using ELISA kits. In our asthma model, the levels of IL-17, the main cytokine that characterises Th17 cells, and IL-5, which is predominantly secreted by Th2 cells, were both significantly increased. However, rosiglitazone markedly suppressed their production (Fig. 3). To clarify the correlation between neutrophils and inflammation, we tested the levels of the inflammatory factors IL-8 and TNF- α . The results showed that only the OVA-LPS group had a high level of IL-8. The other groups had levels below the limit of detection for the measurement kit. Similar trends were observed for TNF- α (Fig. 3).

3.3. Rosiglitazone suppresses airway inflammation and the expression of IL-17 in lung tissues

To investigate airway inflammation directly, we performed histological analyses. To determine the distribution of IL-17, we incubated sections with an IL-17 antibody. In the OVA-LPS-induced asthma model, numerous inflammatory cells, primarily neutrophils and eosinophils, were observed around the bronchioles and vessels. Mucus and debris had accumulated in the lumens of the bronchioles, and there was an increase in IL-17-positive cells (Fig. 4B) compared with the control (Fig. 4A). Mice treated with rosiglitazone (Fig. 4C) showed marked reductions in the infiltration of inflammatory cells in the peribronchiolar and perivascular regions, and these mice also had less mucus and no mucus plugs in the lumen. The anti-inflammatory effect was inhibited by GW9662, and mice that had received GW9662 showed inflammatory cells surrounding the airway and vasculature (Fig. 4D). The IL-17 levels and distribution were closely associated with neutrophil infiltration.

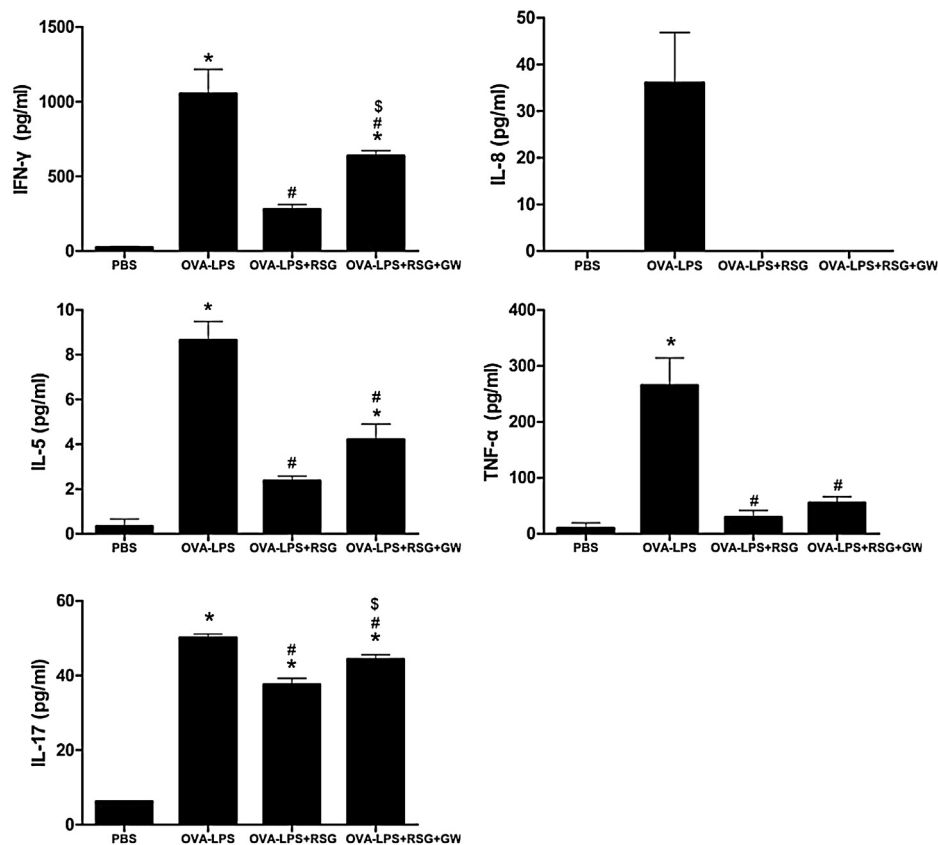


Fig. 3. Effect of rosiglitazone with or without GW9662 on the levels of cytokines and inflammatory factors in BALF, as determined using enzyme immunoassay kits. BALF was performed at 48 h post-challenge. Data represent the mean $\pm$ SEM from six mice per group. * $p < 0.05$ vs. PBS; # $p < 0.05$ vs. OVA-LPS; \$ $p < 0.05$ vs. OVA-LPS + RSG.

3.4. Rosiglitazone inhibited T cells mediated immune response by up-regulation PPAR γ expression in lung tissues

To investigate the link between the effector T cell and the immune response, the expression levels of nuclear transcription factors were tested by qPCR. T-bet is a nuclear transcription factor that is active in Th1 cells; there were no significant differences in T-bet between the experimental groups. GATA-3 and ROR γ t are nuclear transcription factors that are specific for Th2 and Th17 cells, respectively. Our data suggested that mice sensitised with OVA and LPS produced redundant Th2 and Th17 cells (Fig. 5A). We examined the gene and protein expression of PPAR γ . Its changing trend is inversely proportional to the degree of inflammation and T cell proliferation. Rosiglitazone is an agonist of PPAR γ and can significantly increase its expression (Fig. 5B and C). These results indicate that rosiglitazone may affect T cell proliferation and differentiation.

3.5. Effectiveness of rosiglitazone with or without GW9662 on T cells differentiation in vitro

To investigate whether rosiglitazone affects the number and function of T cells, a DC and CD4⁺ naïve T cell co-cultured system was established. The extent of T cell proliferation and activation was determined by counting the total number of cells and quantifying the mRNA expression of T cell nucleus transcription factors. DCs treated with OVA and LPS could stimulate the proliferation of T cells. When OVA-LPS-treated DCs were administered rosiglitazone, the proliferation of Th1, Th2 and Th17 effector cells was reduced. GW9662 antagonises rosiglitazone, and the naïve T cells in each group treated with GW9662 demonstrated a restored ability to proliferate and differentiate (Fig. 6A and B). Rosiglitazone up-regulated the expression of PPAR γ in the nucleus and decreased the proliferation and activation of T cells (Fig. 6C and D).

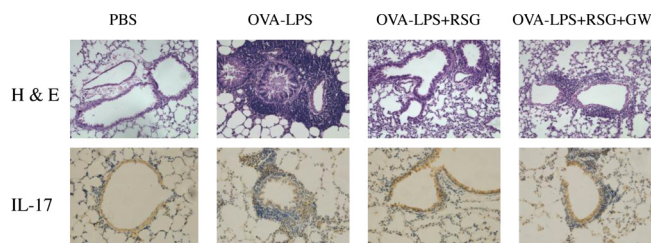


Fig. 4. Effect of rosiglitazone with or without GW9662 on lung pathology and expression of IL-17 in lung tissues. Representative sections with bars indicate a scale of 20 μ m (200 $\times$). The tan color indicates IL-17-positive staining. (A) PBS mice administered the drug vehicle, (B) OVA-LPS mice administered the drug vehicle, (C) OVA-LPS mice administered rosiglitazone, (D) and OVA-LPS mice administered rosiglitazone plus GW9662.

4. Discussion

Th2 cells are known to be crucial for eosinophilic asthma. However, in non-eosinophilic asthma, Th17 cells, not Th2 cells, mediate the immune response that plays an important role in the disease. R.H. Wilson et al. showed that IL-17 and Th2 responses alone were sufficient to confer AHR [18]. Exposure to a low dosage of LPS enhanced the Th17-mediated response, resulting in the release of IL-17 and other cytokines and neutrophil infiltration. Furthermore, LPS used as an adjuvant in asthmatic mice weakened Th2 cell function [22]. A recent study showed that the overexpression of IL-5 and IL-17A mRNA in sputum was more common in uncontrolled asthmatics than in patients with controlled asthma [23]. In the current study, classical pathological alterations with an over-activated

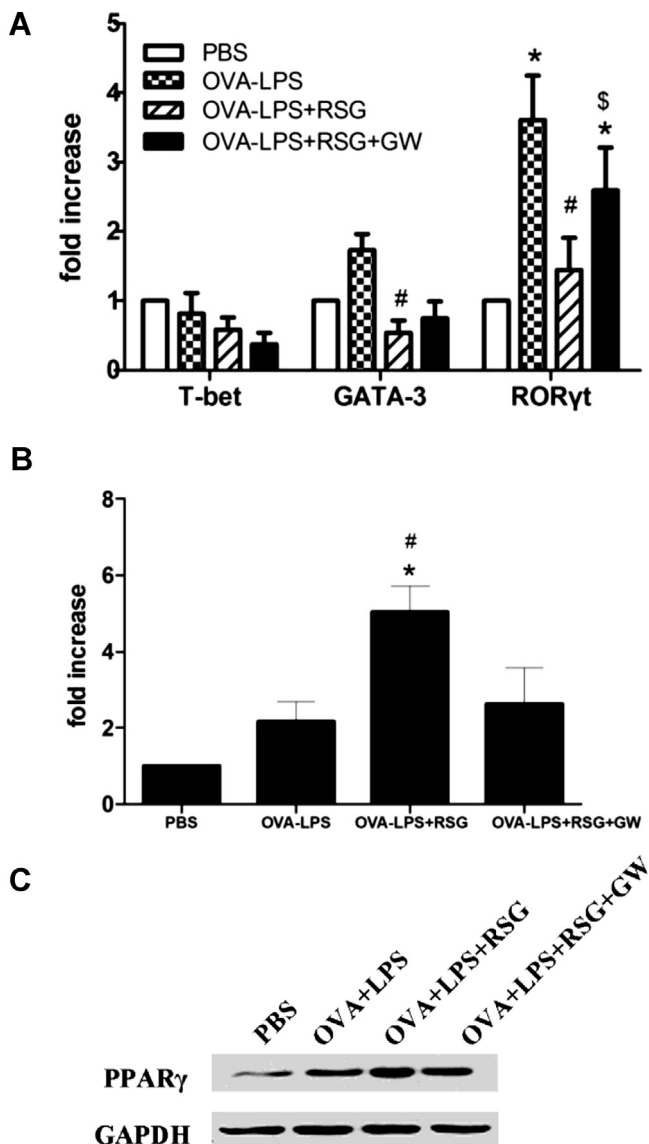


Fig. 5. (A) Effective of rosiglitazone with or without GW9662 on T cell proliferation in lung tissues. The qPCR was used to measure the expression levels of nuclear transcription factors in effector T cells. (B) Effects of rosiglitazone with or without GW9662 on the mRNA levels of PPAR γ . (C) Effects of rosiglitazone with or without GW9662 on PPAR γ protein levels. Data represent the mean $\pm$ SEM from 6 mice per group. * $p < 0.05$ vs. PBS; # $p < 0.05$ vs. OVA-LPS; \$ $p < 0.05$ vs. OVA-LPS + RSG.

Th17 immune response and up-regulated IL-5 were found in mice after OVA sensitisation and LPS stimulation; this was the initial attempt to establish an animal model of neutrophilic asthma.

We administered the atomised PPAR γ agonist rosiglitazone 3 days before the first challenge, and this treatment played a protective role against asthma in mice. To our knowledge, this study is the first to demonstrate the beneficial effects of rosiglitazone in a Th17-mediated neutrophilic mouse model of allergic airway inflammation and further extends the rationale for the evaluation of this drug in human studies. The total numbers of inflammatory cells in the BALF and neutrophil percentage were significantly reduced by treatment with RSG. The T cell cytokines IFN- γ , IL-5, and IL-17 were markedly decreased, and the neutrophil-associated inflammatory mediators TNF- α and IL-8 showed similar changes. The significant reduction in AHR is strong evidence that the airway inflammation was alleviated. The low expression of ROR γ t and decreased IL-17-positive cells infiltration indicated that Th17 cell expansion was impaired. Thus, rosiglitazone had a comprehensive

and significant anti-inflammatory effect in our model of neutrophilic asthma. T cell nuclear transcription factors were detected in the lung tissues with qPCR. The mRNA levels of T-bet, GATA-3 and ROR γ t were all decreased after treatment with rosiglitazone. We speculate that rosiglitazone attenuated inflammatory cell infiltration by regulating the differentiation of naïve T cells into effector T cells.

The role of Th2 cells in asthma is well-defined. IL-5, a pleiotropic Th2 cytokine, contributes to many features of asthma, particularly eosinophil differentiation, recruitment, activation, and survival [24]. Some approaches, such as disrupting the IL-5 gene or treatment with an IL-5 monoclonal antibody, suppress the production of IL-5 and effectively inhibit eosinophilia and AHR in mice [25,26]. A multicentre trial at 81 research centres in 13 countries showed that mepolizumab was an effective and well-tolerated IL-5 monoclonal antibody that reduced the risk of asthma exacerbations in patients with severe eosinophilic asthma [27]. In our study, IL-5 production in the BALF and eosinophilia were both suppressed. One possible explanation for this observation is that RSG reduced the early expansion of Th2 cells.

It is also possible that the Th2-mediated immune response was attenuated by LPS exposure. In a house dust mite-induced allergic asthma model, LPS inhibited eosinophil recruitment into the lungs and mucus production in the airways in a dose-dependent manner by inducing the release of IL-17, IL-33, IFN- γ and TNF- α while suppressing the production of Th2 cytokines [22]. Interestingly, R.H. Wilson also observed that the inhalation of LPS can delay the accumulation of Treg cells in the lungs [18]. Thus, it appears that LPS plays a major role in both T cell proliferation and differentiation; however, further research is required to elucidate the possible mechanisms.

Accumulating evidence has suggested that increased IL-17 induces the secretion of pro-inflammatory cytokines, such as TNF- α and IL-6, and chemokines, such as the CXCL family members, that are closely associated with neutrophil infiltration [28–30]. Elevated sputum IL-17A and IL-8 mRNA levels indicate that the neutrophilic inflammation in mild to moderate-to-severe asthmatics is strongly linked to the infiltration of Th17 cells in asthmatic airways [31]. Clinical studies revealed that acute exacerbation correlated with increased IL-17 levels, and values above 20 pg/ml are an independent risk factor for severe asthma [32,33]. In our study, mice treated with rosiglitazone showed a significantly decreased number of neutrophils and lower IL-17 levels in the BALF. Decreased airway neutrophilia were also indicated by low levels of TNF- α and IL-8. Immunohistochemically, IL-17-producing CD4 $^{+}$ T cells were distinctly absent around the bronchioles and vasculature in the RSG-treated mice.

To further confirm that rosiglitazone inhibits the differentiation of naïve T cells, we performed in vitro co-culture experiments. Dendritic cells (DCs) are the most effective antigen presented cells and play a significant role in polarising naïve T cells to become Th1, Th2 or Th17 effector cells; DCs are necessary for T cell-mediated immunity to clear pathogens [2,34,35]. As a target for therapy, the regulation of DC function can affect the type of immune response as well as its intensity. PPARs are present in the cytosol and nucleus and function through gene-dependent and -independent mechanisms [35]. Our data showed that PPAR γ in DCs is expressed mainly in the nucleus after treatment with rosiglitazone. As a result, the ability of DCs to promote CD4 $^{+}$ naïve T cell proliferation and differentiation is restrained. The expression levels of T-bet, GATA-3 and ROR γ t mRNA was decreased, indicating that the transcription process was suppressed. It is likely that the DCs failed to provide adequate stimulus signals for T cells. Our next experiments will identify the necessary co-stimulatory molecules on the surface of DCs determine the mechanisms of this phenomenon.

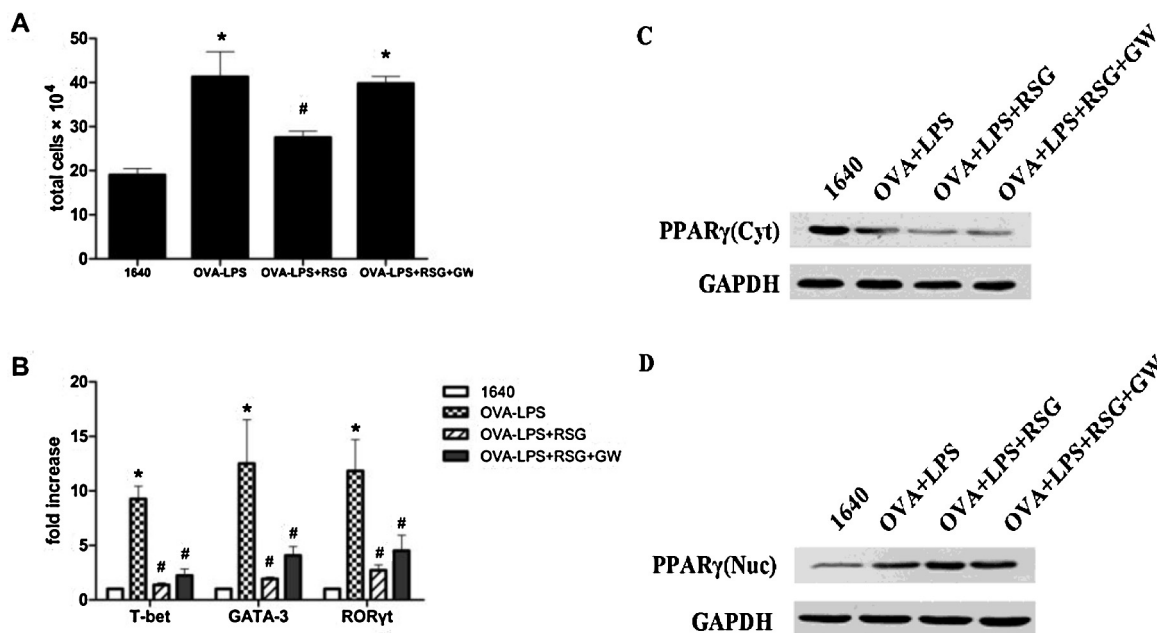


Fig. 6. (A) Total cells of the co-culture system with DCs administered 1640 as a control, DCs administered OVA-LPS, OVA-LPS-DCs administered rosiglitazone, and OVA-LPS-DC administered GW9662 plus rosiglitazone. (B) Effects of rosiglitazone with or without GW9662 on T cell proliferation and differentiation in the co-culture system. (C and D) Western blotting of the PPAR γ protein in nuclear (Nuc) and cytosolic (Cyt) extracts from DCs. The experiment was repeated 5 times. * $p < 0.05$ vs. 1640; # $p < 0.05$ vs. OVA-LPS.

It is worth noting that there are some differences between our model and neutrophilic asthmatics. First, chronic allergen exposure in patients leads to chronic allergic asthma, whereas the twice-repeated sensitisation in our protocol causes an acute response. Second, neutrophilic inflammation is largely present in non-atopic asthma. Sudden-onset fatal asthma patients have an almost complete absence of eosinophils. Third, in contrast with patients who have obvious AHR even when they are symptom-free, the mice exhibited only transient methacholine-induced AHR following allergen exposure. Due to the heterogeneous characteristics of the disease itself, differences in the animal model species and the disunity of each model's established protocol, it is difficult to characterise the inflammation type of asthma model according to clinical method. However, observations from our model support many existing paradigms, and our model can be applied to test new treatments for neutrophilic asthma. To construct a model that can more fully represent the clinical manifestations of neutrophilic asthma, more attention and hard work are required.

In general, the activation of PPAR has a definite protective effect in mice with Th-17 mediated neutrophilic asthma. Amazingly, it also reduces the Th2 immune response level. The reduction of inflammatory cells in the BALF, reduced AHR and decreased concentration of serum OVA-sIgE are closely related to the decreased Th2 and Th17 immune response strength. These benefits are primarily derived from a decrease in the number of effector T cells due to regulation of DC function via the PPAR γ pathway.

Conflict of interest

The authors have no conflicts of interest of any kind to declare in the materials or services referred to in this article.

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