



Contents lists available at SciVerse ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimmPPAR- γ agonists, mainly 15d-PGJ₂, reduce eosinophil recruitment following allergen challengeThais S. Farnesi-de-Assunção^{a,b}, Claudiney F. Alves^b, Vanessa Carregaro^c, Jhony R. de Oliveira^b, Carlos A.T. da Silva^{a,b}, Alessandra B. Cherain^b, Fernando Q. Cunha^d, Marcelo H. Napimoga^{b,e,*}^a Laboratory of Immunology, Federal University of Triângulo Mineiro, Uberaba, MG, Brazil^b Laboratory of Biopathology and Molecular Biology, University of Uberaba, Uberaba, MG, Brazil^c Department of Immunology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil^d Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil^e Laboratory of Immunology and Molecular Biology, São Leopoldo Mandic Institute and Research Center, Campinas, SP, Brazil

ARTICLE INFO

Article history:

Received 19 August 2011

Accepted 29 November 2011

Available online xxx

Keywords:

PPAR- γ 15d-PGJ₂

Eosinophils

Inflammation

ABSTRACT

We evaluate the immunomodulation of Peroxisome proliferator-activated receptor- γ (PPAR- γ) agonists 15d-PGJ₂ and rosiglitazone (RGZ) in a model of chronic eosinophilia. 15d-PGJ₂ and RGZ significantly reduce eosinophil migration into the peritoneal cavity and down-regulate the eosinopoiesis. The synthesis of IL-5 was decreased after the treatment with 15d-PGJ₂ and RGZ corroborating with the eosinophil migration inhibition. However, IgE was decreased only after the administration of 15d-PGJ₂ in part due to B-cell inhibition. We also observed a decrease in the synthesis of IL-33, IL-17 and IL-23, suggesting that besides the modulation of Th2 pattern, there is a modulation via IL-23 and IL-17 suggesting a role of these cytokines in the eosinophil recruitment. In fact IL-17^{-/-} mice failed to develop an eosinophilic response. Altogether, the results showed that PPAR- γ agonists mainly 15d-PGJ₂, have therapeutic efficacy in eosinophil-induced diseases with an alternative mechanism of control, via IL-23/IL-17 and IL-33.

© 2011 Elsevier Inc. All rights reserved.

1. Introduction

Peroxisome proliferator-activated receptor- γ (PPAR- γ) is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors. Both natural and synthetic ligands have been reported for PPAR- γ . Naturally occurring compounds that have been reported to bind PPAR- γ include a number of fatty acids and eicosanoid derivatives such as 9- or 13-hydroxyoctadecenoic acid and prostaglandin derivative 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂) [1]. Prostanoids play a critical role in inflammation and for many years, cyclooxygenases (COX) enzymes and their products have been considered mainly pro-inflammatory agents [2], however, it has been proposed a role of COX-2 in inflammatory resolution. A late peak of PGD₂ production and its product, the 15d-PGJ₂ were observed together with an exacerbating effect of NSAIDs at late stages of the inflammatory response [3]. Besides, the most widely used synthetic agonists of PPAR- γ are members of a class of antidiabetic agents known as thiazolidinediones (TZDs), including rosiglitazone, troglitazone, and pioglitazone.

A number of *in vivo* studies have reported anti-inflammatory effects of the PPAR- γ agonists in different animal models [4–9] as well as its potential useful to control eosinophil-mediated inflammatory diseases [10,11], since it was demonstrated that eosinophils express PPAR- γ and the stimulation with a synthetic agonist for PPAR- γ attenuated the factor-induced eosinophil survival and chemotaxis [12,13]. Besides, administration of PPAR- γ agonists had beneficial effects on pathological conditions, including airway hyper-responsiveness and lung eosinophilia in a murine model of asthma [14] as well as negatively regulates allergic rhinitis in mice [15]. Also, eosinophils cultured with therapeutic concentrations of theophylline and dexamethasone markedly enhanced both mRNA and protein levels of PPAR- γ , suggesting that the increase in PPAR- γ expression on eosinophils may play a role in the anti-inflammatory effects of both drugs.

The initial event in an asthma-like disease is the interaction between dendritic cells (DC) and T cells, leading to the generation of Th2 cells including IL-5 an important cytokine to induce the eosinophil recruitment [16]. Another Th2-related cytokine is the IL-33, which can act directly on Th2 cells increasing the secretion of Th2 cytokines such as IL-5 and IL-13 [17], as well as can also act as a chemo-attractant for Th2 cells [18]. Asthma-like disease has long been characterized as a Th2-based immune response, however, studies suggest that this disease is a heterogeneous disorder with distinct types of inflammatory processes, including

* Corresponding author. Address: Laboratory of Immunology and Molecular Biology, São Leopoldo Mandic Institute and Research Center, R. José Rocha Junqueira, 13 Campinas, SP, CEP. 13045-755, Brazil. Fax: +55 19 3211 3712.

E-mail addresses: napimogamh@yahoo.com, marcelo.napimoga@gmail.com (M.H. Napimoga).

IL-17-secreting (Th17) cells [19]. IL-23, an important cytokine to amplify the Th17 cells, has been demonstrated to be an important immunoregulatory factor during experimental asthma. Indeed, excess generation of IL-23 [20] or transgenic over-expression of IL-23 receptor [21] can exacerbate allergen-induced inflammation through increased Th2-cell and eosinophil activity and/or recruitment.

It is noteworthy that the PPAR- γ agonists, bind to the PPAR- γ /RXR heterodimer that competes for recruitment of the co-activators CREB binding protein (CBP) and p-300 [1]. The limited availability of these co-activators inhibits the activation of the transcription factors activator protein-1 (AP-1), NF- κ B, and consequently inhibits pro-inflammatory gene expression. However, 15d-PGJ₂ may also exert PPAR- γ -independent anti-inflammatory effects through direct modification of cysteine residues of the inhibitor-KB kinase and NF- κ B subunits, and direct inhibition of the extracellular regulated kinase (ERK1/2) [22]. Thus, the use of 15d-PGJ₂ as a pharmacological tool may be more effective than the use of other PPAR- γ agonists. Besides, there are few data regarding the use of natural agonist 15d-PGJ₂ as a therapeutic drug choice to control eosinophilic inflammation.

Therefore, in the present study, we used a model of chronic eosinophilic inflammation induced in mice by subcutaneous egg white implants (EWI) to evaluate the immunomodulatory effect of the PPAR- γ agonists. This model involves surgical implantation of a piece of heat-coagulated hen egg white, which is followed by chronic, intense eosinophilic inflammation at the implant site, accompanied by a large increase in bone-marrow eosinophilopoiesis lasting several weeks [23].

2. Material and methods

2.1. Animals

Male C57BL/6 wild-type and IL-17 deficient (IL-17^{-/-}) mice weighing 20–25 g were obtained from the Faculty of Medicine of Ribeirão Preto (University of São Paulo, São Paulo, Brazil). The animals were kept in appropriate cages in a temperature-controlled room, with a 12 h dark/light cycle. Free access to water and food was provided, and an acclimatization period of about 7 days in the laboratory was used prior to the experiment. All animals were manipulated in accordance with the Guiding Principles in The Care and Use of Animals, approved by the Council of the American Physiologic Society. This animal study was deemed to be ethical according to the Brazilian Guidelines (Resolution 11794/2008) and was approved by the Animal Ethics Committee of the University of Uberaba (# 049/2009).

2.2. Egg white implant

Dehydrated hen egg white was prepared as previously described [23], by dissolving it in distilled water, to a 100 g/l final concentration, and dispensing 45 μ l per well in a 96-well plate (4.5 mg/well). Heat coagulation was carried out on the plate in a microwave oven (90 s at 900 Hz). Pellets were recovered and dehydrated in absolute ethanol for 48 h. The dehydrated pellets were allowed to dry at room temperature for 24 h and stored at room temperature until use. Mice were anaesthetized with tribromoethanol (250 mg/kg, i.p.) and a dorsal incision was made after local trichotomy. Rehydrated egg white pellets were implanted subcutaneously through the dorsal incision, and the skin was sutured. Control (sham-implanted) mice were submitted to the surgery, but received no implant. After 15 days from surgery, different groups of animals were challenged with ovalbumin (10 μ g; OVA) or PBS (phosphate buffer solution, pH 7.2) by intraperitoneal injection.

Animals were euthanized at 0, 24, 48 or 72 h after the challenge with ovalbumin. The current conditions used (challenge with 10 μ g per cavity and 15 days after implant perform the challenge with ovalbumin), was chosen based on a previous study [23].

2.3. Drugs and treatments

To evaluate the effect of 15d-PGJ₂ (100, 300 or 1000 μ g/kg; Sigma-Aldrich, USA) or rosiglitazone (1, 3 or 10 mg/kg, RGZ; Avandia®, Glaxo-SmithKline, USA) on eosinophil recruitment, mice were injected with the indicated doses of each drug 30 min before challenge and every 12 h (for 15d-PGJ₂) or after 24 h (for RGZ) of intraperitoneal challenge with OVA. Cell migration was evaluated 48 h after challenge (based on the time course curve, Fig. 1). 15d-PGJ₂ was dissolved in sterile PBS and injected subcutaneously (s.c.) in a final volume of 0.2 ml as previously described [5]. RGZ was dissolved in sterile PBS and administered by oral gavage in a final volume of 0.2 ml as previously described [8].

2.4. Blood sample collection and IgE quantification

At 48 h after the challenge, blood was drawn by puncturing the orbital plexus and centrifuged. Serum was frozen and stored at -70°C for IgE measurements by ELISA using protocols supplied by the manufacturer (BD Biosciences). After all standard procedures, the optical density (O.D.) was measured at 450 nm. Results were expressed as pg/mL based on the standard curves.

2.5. Leukocyte and eosinophil counts from peritoneal lavage or bone-marrow

Forty-eight hours after challenge, the mice were euthanized, and the peritoneal cavity washed for 3 min with 3 mL of PBS containing 1 mM EDTA, and the solution was recovered. Also, bone-marrow cells were obtained by flushing the two femurs of mice with RPMI-1640 medium (LGC Biotechnology, São Paulo, Brazil) containing 10% fetal calf serum (Invitrogen) and penicillin/streptomycin (Invitrogen). Total counts were performed in a Newbauer chamber and the frequency of cells stained for eosinophil peroxidase (EPO) was determined in cytocentrifuge (Cientec, Piracicaba, SP, Brazil) smears of freshly harvested peritoneal lavage or bone-marrow cells. Data are reported as means $\pm$ standard deviations of the number of eosinophils per cavity, and each count was carried out at least twice, with differences of less than 10%.

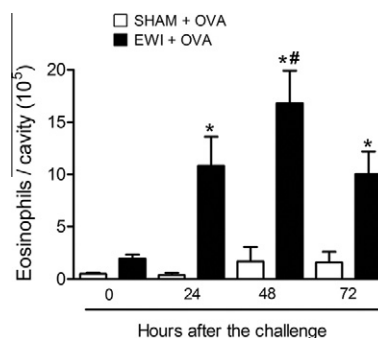


Fig. 1. Time course of eosinophil migration following challenge with OVA. Mice submitted or not (Sham) to a subcutaneous implant of heat-coagulated egg white (EWI) were challenged with OVA (10 μ g/cavity) 15 days thereafter. The eosinophils migration was determined in the peritoneal exudates 24, 48 and 72 h after challenge. Data are the mean $\pm$ SD and are representative of 6 mice/group. * $P < 0.05$ compared to 24 and 72 h EWI-challenged. ** $P < 0.01$ compared with Sham controls.

2.6. Cytokine measurements (ELISA)

The IL-5, IL-17, IL-23 and IL-33 levels in the peritoneal exudates from OVA-challenged mice were detected by ELISA using protocols supplied by the manufacturer (R&D Systems, Minneapolis, USA). After all standard procedures, the optical density (O.D.) was measured at 490 nm. Results are expressed as pg/mL of each cytokine, based on the standard curves.

2.7. B cell proliferation

To assess the influence of 15d-PGJ₂ treatment in B cell proliferation, the spleens from naïve mice were harvested and washed twice with PBS separately. Tissues were minced and the cells were filtered through a cell strainer and centrifuged at 500g at 4 °C for 10 min. The cell pellet was re-suspended in RPMI-1640 medium (LGC Biotechnology) to a concentration of 2.5×10^6 cells/ml and it was added in 24-well microtitre plates. Plated cells were treated with 15d-PGJ₂ (5.0 µM) or vehicle for 1 h and, then, stimulated with LPS (2400 ng/ml) or medium for 24 h. Afterwards, samples obtained from above mentioned culture were suspended and incubated for 30 min at 4 °C in PBS containing 2% of bovine serum albumin (PBS-S) and FcγIR block mAb (CD16/CD32) to avoid nonspecific background staining. After the blocking step, B cells were identified by characteristic size (FSC) and granularity (SSC) combined with two-color analysis. Briefly, the lymphocytes were identified as CD19⁺ using specific antibody conjugate with PE (BD Biosciences PharMingen, San Diego, CA, USA). The isotype controls used were rat IgG2b (BD Biosciences PharMingen). After staining, cells were fixed with 1% paraformaldehyde and analyzed by flow cytometry (FACScan™ and CELLQuest™ software; BD Biosciences PharMingen).

2.8. Statistical analysis

The means from different treatments were compared using ANOVA. When statistically significant differences were identified, individual comparisons were subsequently made using Bonferroni's *t*-test for unpaired values. Statistical significance was set at $P < 0.05$.

3. Results

We first carried out an experiment to evaluate the time course of eosinophil migration following challenge with the standardized dose (10 µg/cavity) of OVA. As shown in Fig. 1, significantly increased eosinophil numbers were observed in the peritoneal cavity of EWI mice, relative to Sham controls 24, 48 and 72 h after challenge ($p < 0.01$ in all cases). Moreover, at 48 h after the challenge in the EWI mice, an increased eosinophil migration was observed, statistically significant in comparison to 24 and 72 h ($p < 0.05$), demonstrating that at 48 h the eosinophil migration to the peritoneal cavity reached the peak. Therefore, the time point of 48 h was chosen as the observation time for the subsequent experiments.

To evaluate the pharmacological potential of PPAR-γ agonists to inhibit eosinophil migration induced by ovalbumin, EWI mice were treated, before ovalbumin challenge, with 15d-PGJ₂ (100, 300 and 1000 µg/kg) or rosiglitazone (1, 3 and 10 mg/kg). As demonstrated in the Fig. 2A, the sham mice challenge with PBS or OVA did not induce an eosinophilic recruitment to the peritoneal cavity. The same pattern was observed in the PBS-challenge EWI mice. All three groups were used as control. On the other hand, the OVA-challenge EWI mice had a statistical increase ($p < 0.05$) in the eosinophil migration at 48 h.

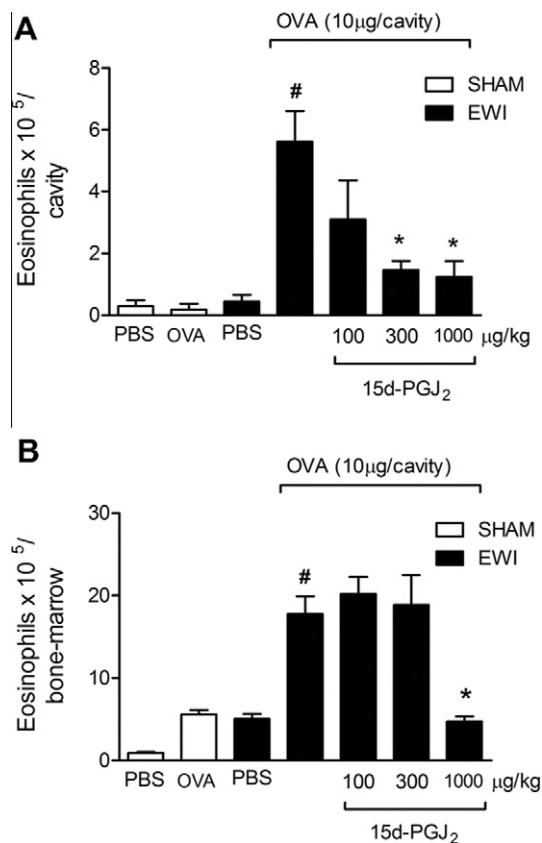


Fig. 2. 15d-PGJ₂ decrease the eosinophil migration and the eosinopoiesis. Mice submitted or not (Sham) to a subcutaneous implant of heat-coagulated egg white (EWI) were challenged with OVA (10 µg/cavity) or PBS. EWI groups were treated, 30 min before ovalbumin challenge, with 15d-PGJ₂ (100, 300 or 1000 µg/kg) each 12 h. The eosinophils were harvested from the peritoneal cavity (A) or bone-marrow (B) from OVA-challenged mice treated with different doses of 15d-PGJ₂ 48 h after challenge. [#] $P < 0.05$ compared with control groups. ^{*} $P < 0.05$ compared with OVA-challenge mice without treatment. Data are the mean $\pm$ SD and are representative of 6 mice/group.

The treatment with the natural PPAR-γ agonist 15d-PGJ₂ 100, 300 or 1000 µg/kg decreased in a dose-dependent manner ($p < 0.05$) the eosinophil migration to the peritoneal cavity in mice, induced by intraperitoneal injection of OVA determined 48 h later. Moreover, we also evaluate the effect of 15d-PGJ₂ in bone-marrow eosinopoiesis in EWI recipients, as well as sham-implanted mice. OVA-challenge EWI mice had a statistical increase ($p < 0.05$) in the eosinopoiesis at 48 h, and only the dose of 1000 µg/kg of 15d-PGJ₂ statistically decrease the eosinopoiesis on bone-marrow ($p < 0.05$) as demonstrated in Fig. 2B.

We further analyzed the potential role of the synthetic PPAR-γ agonist RGZ to inhibit eosinophil migration to the peritoneal cavity. As demonstrated in the Fig. 3A, OVA-challenge EWI mice had a statistical increase ($p < 0.05$) in the eosinophil migration to the peritoneal cavity at 48 h. The oral administration of RGZ 1, 3 and 10 mg/kg statistically decreased the eosinophil migration ($p < 0.05$). We did not observe a dose-dependent pattern, although the dose of 10 mg/kg demonstrated a slight better efficacy. Regarding the effect of RGZ on bone-marrow eosinopoiesis in EWI recipients we demonstrated that both doses of 3 and 10 mg/kg did statistically ($p < 0.05$) reduce the eosinopoiesis (Fig. 3B).

Next, we analyzed the pattern of some cytokines in the OVA-sensitize mice treated with both PPAR-γ agonists. IL-5 (Fig. 4A) and IL-33 (Fig. 4B) levels in the OVA-challenge EWI mice was statistically higher ($p < 0.05$) than Sham mice. Both RGZ and 15d-PGJ₂ inhibited the release of IL-5 and IL-33 in a dose-dependent fashion,

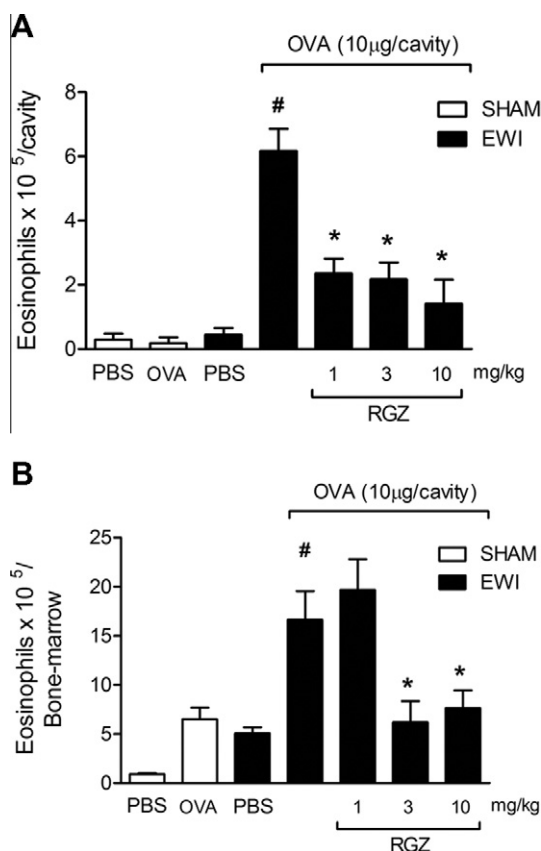


Fig. 3. Rosiglitazone (RGZ) decrease the eosinophil migration and the eosinopoiesis. Mice submitted or not (Sham) to a subcutaneous implant of heat-coagulated egg white (EWI) were challenged with OVA (10 μ g/cavity) or PBS. EWI groups were treated, 30 min before ovalbumin challenge, with rosiglitazone (RGZ) (1, 3 or 10 mg/kg) every 24 h. The eosinophils were harvested from the peritoneal cavity (A) or bone-marrow (B) from OVA-challenged mice treated with different doses of RGZ 48 h after challenge. [#] $P < 0.05$ compared with control groups. ^{*} $P < 0.05$ compared with OVA-challenge mice without treatment. Data are the mean $\pm$ SD and are representative of 6 mice/group.

but the statistical significant doses were only 10 mg/kg of RGZ and 1000 μ g/kg of 15d-PGJ₂ for both cytokines. Furthermore, IL-17 (Fig. 4C) and IL-23 (Fig. 4D) levels in the OVA-challenge EWI mice was statistically increased ($p < 0.05$) than Sham mice. Both RGZ and 15d-PGJ₂ inhibited the release of IL-17 in a dose-dependent fashion, but the statistical significant doses were 3 and 10 mg/kg of RGZ and 300 and 1000 μ g/kg of 15d-PGJ₂ (Fig. 4C). Regarding the IL-23 the doses of 3 and 10 mg/kg of RGZ and 1000 μ g/kg of 15d-PGJ₂ showed statistical significant decrease (Fig. 4D).

To confirm the importance of the IL-17 in the recruitment of eosinophils, we used a set of C57/B6 IL-17^{-/-} mice. As observed in the Fig. 5A, the EWI-challenge mice had a significant increase in the eosinophil influx in contrast to EWI-PBS challenge ($p < 0.05$). Interestingly, the EWI-challenge IL-17^{-/-} mice did not show an intense eosinophil migration after the OVA-challenge being the same as PBS-challenge mice (Fig. 5A, white bars). The same pattern was observed in the eosinopoiesis by analyzing the number of eosinophils from the bone-marrow (Fig. 5B).

Serum levels of IgE in the OVA-challenge EWI mice group were significantly higher than that in the control groups. Interestingly, the administration of RGZ (all doses tested) did not statistically reduce the serum levels of IgE (Fig. 6A). However, at the dose of 1000 μ g/kg of 15d-PGJ₂ it was observed a statistical decrease ($p < 0.05$) in the levels of IgE (Fig. 6A). Next, we tested the effect of the 15d-PGJ₂ in an *in vitro* B cell proliferation in the presence

of LPS. As demonstrated in Fig. 6B, it was possible to observe a significant inhibition of the B cell proliferation when incubated with 5 μ M of 15d-PGJ₂.

4. Discussion

In the present study we partially elucidated the mechanism underlying the role of PPAR- γ agonists-mediated suppression of eosinophils recruitment toward inflammatory stimuli using a mouse asthma-like model, in which eosinophils recruitment is a key event. Furthermore, we also showed that this decreased eosinophil recruitment is associated with PPAR- γ agonists-mediated suppression of several cytokines including the cytokines that acts selectively on the eosinophil lineage, promoting eosinopoiesis, migration, activation and survival of the eosinophils. Moreover, IgE was also diminished only in the 15d-PGJ₂-treated animals in part due to B-cell inhibition. It has been demonstrated that human eosinophils express PPAR- γ , and stimulation of eosinophils with a synthetic PPAR- γ agonist inhibit the factor-induced eosinophil activation, such as degranulation, survival, and chemotaxis [12,13]. Furthermore, administration of PPAR- γ agonists had beneficial effects on pathological conditions, including airway hyperresponsiveness and lung eosinophilia in a murine model of asthma [14] as well as negatively regulates allergic rhinitis in mice [15]. Thus, pharmacological PPAR- γ agonists may be a new therapeutic modality for the treatment of allergic diseases including asthma by negatively modulating eosinophil functions [24].

In the present study we demonstrated that PPAR- γ agonists 15d-PGJ₂ and rosiglitazone are able to reduce the eosinophil influx after the OVA-challenge and this effect is in part due to the decrease of eosinopoiesis and the inhibition of some important cytokines involved in this process. Furthermore, there is few data regarding the potential use of the natural agonist 15d-PGJ₂ in the allergic (asthma-like) model, herein, we demonstrated their important and potent effect. The 15d-PGJ₂ dose used in this study may be considered somehow high, however, it has been estimated that >50% of the 15d-PGJ₂ added exogenously to a biological system binds to albumin which limits its action but also did not induce any known side effect [25].

PPAR- γ is also expressed in smooth muscle cells, myofibroblasts, endothelial cells of the pulmonary vasculature, and inflammatory cells such as alveolar macrophages, neutrophils, eosinophils, lymphocytes, and mast cells [10,26]. Interestingly, PPAR- γ expression is increased in bronchial submucosa, airway epithelium, and smooth muscle cells of asthmatics when compared with healthy subjects [27] as well as is involved in airway inflammation and remodeling in asthma [28,29]. Thus, the up-regulation of PPAR- γ expression in asthma may represents a self-regulatory mechanism for preventing airway inflammatory and remodeling. In this way, the use of PPAR- γ agonist drugs may be an interesting approach, as reinforced by our results in which both 15d-PGJ₂ and rosiglitazone decreased the eosinophil migration and eosinopoiesis after OVA-challenge.

Previous studies have shown that activation of PPAR- γ reduces the expression of various cytokines, airway hyperresponsiveness, and activation of eosinophils, which are increased in asthma, suggesting the therapeutic potential of PPAR- γ agonists for asthma [14]. Upon allergen challenge, naive CD4⁺ T cells differentiate into a prevailing Th2 effectors phenotype, and these cells predominantly secrete interleukin-4 (IL-4), IL-5, and IL-13 promoting the recruitment of eosinophils, mast cells and lymphocytes, hyperplasia of smooth muscle, and airway hyperresponsiveness, which are frequently associated with increased serum IgE concentration [30]. As observed in the present study, the production of the Th2 cytokines (IL-5 and IL-33) was reduced in both treated animals (15d-

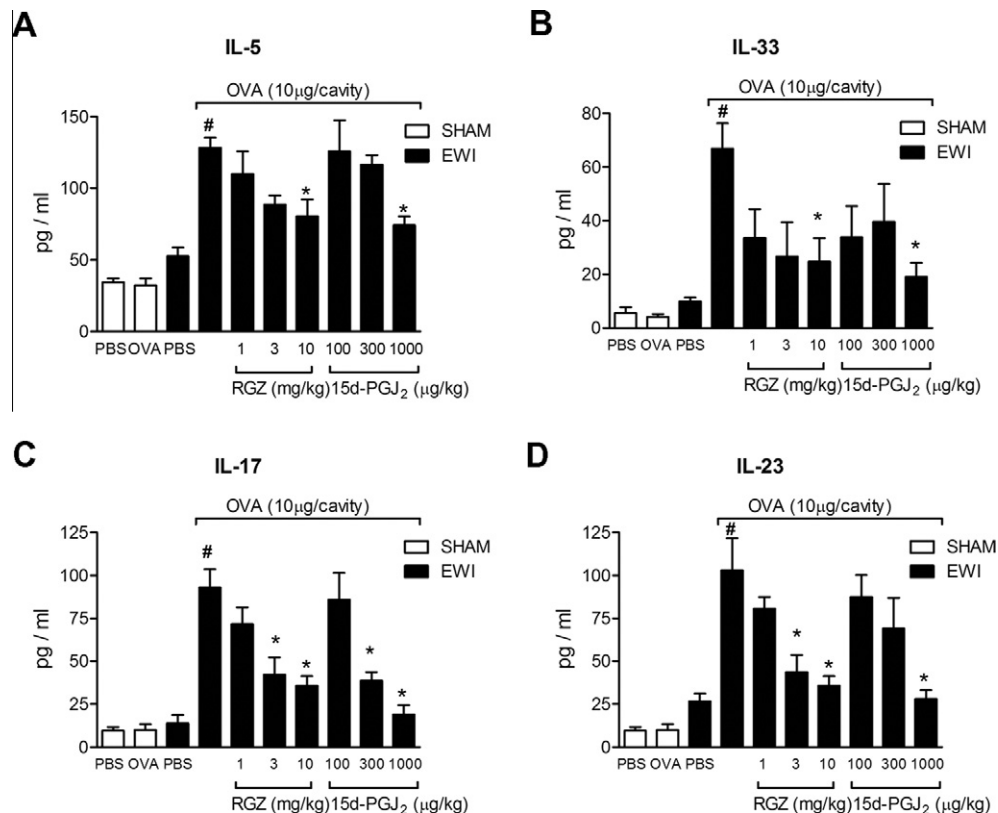


Fig. 4. Effect of PPAR- γ agonists on cytokines release. The OVA-challenge EWI mice were submitted to the treatments with RGZ or 15d-PGJ₂. After 48 h of the challenge, the peritoneal exudates were harvested and the levels of IL-5(A), IL-33(B), IL-17(C) and IL-23(D) were detected through ELISA assay. Data are the mean $\pm$ SD and are representative of 6 mice/group. # P < 0.05 compared with control groups. * P < 0.05 compared with OVA-challenge EWI without treatment.

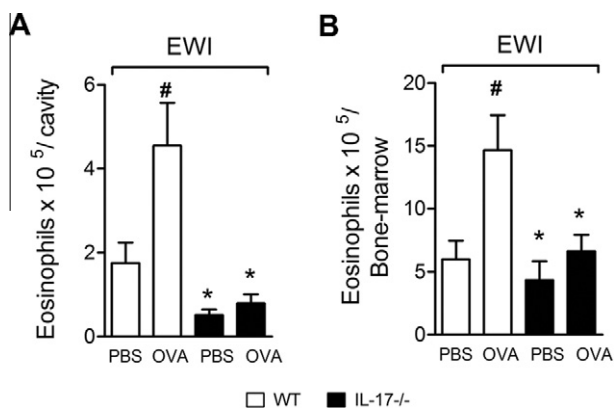


Fig. 5. IL-17 is essential to eosinophil migration and eosinopoiesis in EWI model. C57/B6 WT and C57/B6 IL-17^{-/-} mice submitted to a subcutaneous implant of heat-coagulated egg white (EWI) were challenged with OVA (10 μ g/cavity) 15 days thereafter. The eosinophils were harvested from the peritoneal cavity (A) or bone-marrow (B) from OVA-challenged mice treated with different doses of RGZ. The eosinophils migration (A) to peritoneal cavity or eosinopoiesis on bone-marrow (B) were determined in the peritoneal exudates 48 h after challenge of C57/B6 WT (white bars) and C57/B6 IL-17^{-/-} (black bars) mice after challenge with OVA or PBS. # P < 0.05 compared with EWI-PBS challenge WT mice (first white bar). Data are the mean $\pm$ SD and are representative of 6 mice/group, and each count was carried out at least twice, with differences of less than 10%. * P < 0.05 compared with EWI-OVA-challenge WT mice.

PGJ₂ and RGZ) which may in part explain their effectiveness. IL-5 is indispensable to long-term eosinophilia, as it is the major factor inducing eosinophil differentiation from lineage-committed precursors. Furthermore, IL-5 extends the lifespan of infiltrating eosinophils and further activates or enhances their effector capabilities [31].

Moreover, the discovery of IL-33 as the ligand for the orphan Th2 associated receptor ST2 has opened an assortment of different directions for this pathway, once the IL-33/ST2 axis is thought to be intimately involved in the promotion and maintenance of allergic inflammation [32]. It has been proposed that IL-33 is released by damaged cells undergoing necrotic cell death in the presence of pathogens or allergens, suggesting it may function as an endogenous danger signal or 'alarmin' which may propagate the exacerbations of asthma disease [33]. Also, IL-33 promotes secretion of IL-5 and IL-13 from Th2 cells which will stimulate the eosinophil commitment [34]. Thus, the ability to reduce this cytokine is a very useful drug to control a Th2-disease.

On the other hand, in our system, we have detected the pro-inflammatory cytokine IL-23 which is linked with Th17 cell responses, through expansion and/or maintenance of the Th17 cells [35]. IL-17 a cytokine mainly released by Th17 cells, is very important for an array of inflammatory disease. Previous report demonstrated that eosinophils are a potential source of IL-17 within asthmatic airways, suggest that IL-17 might have the potential to amplify inflammatory responses [36]. Both IL-17 and IL-23 were reduced in animals treated with PPAR- γ agonists and consistent with it, the IL-17 knockout mice did not recruit an important number of eosinophils after OVA-challenge. In addition, it was demonstrated the importance of IL-23/IL-23R signaling in the development of allergic airway inflammation by acting on Th2 cells differentiation [21]. The neutralization of IL-23 decreased antigen-induced eosinophil recruitment and Th2 cytokine production in the airways of sensitized mice upon antigen inhalation [20].

Finally we have observed that only 15d-PGJ₂ at 1000 μ g/kg was able to decrease the IgE in the serum. Previously it was suggested that IL-17 may contribute to allergic airway inflammation through

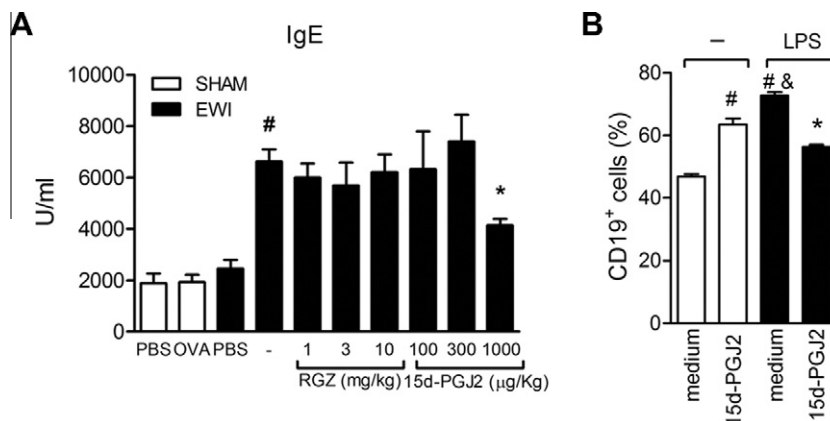


Fig. 6. 15d-PGJ₂ decrease IgE level and inhibits LPS-induced B cell proliferation. In (A), the OVA-challenge EWI mice were submitted to the treatments with RGZ or 15d-PGJ₂, in different doses. After 48 h of the challenge, blood was drawn by puncturing the orbital plexus and centrifuged. Serum was used for IgE measurements by ELISA. [#]*P* < 0.05 compared with control groups. ^{*}*P* < 0.05 compared with OVA-challenge EWI without treatment. Data are the mean ± SD and are representative of 6 mice/group. In (B), spleen cells (1 × 10⁶ cells/ml) were pre-incubated with 5 μM of 15-PGJ₂ for 1 h and then stimulated with LPS (500 ng/ml) for 24 h. B cells were determined by CD19 positivity by flow cytometry using specific antibody. Data are the mean ± SD and are representative of one single experiment made in triplicate. [#]*P* < 0.05 compared with medium alone. ^{*}*P* < 0.05 compared with LPS stimuli.

the recruitment of airway inflammatory cells, Th2-mediated immune responses, and altering the levels of allergen-specific antibody such as IgE [11]. Furthermore, it was demonstrated that 15d-PGJ₂ is capable of decrease the expression of FcεRI thus avoiding the IgE binding to the cells which in turn decreases the release of some important mediators to activate the allergic inflammation [37]. Moreover, previous report showed that 15d-PGJ₂ inhibit the IgE-switch in the B cell by suppressing the STAT-6 phosphorylation [38].

5. Conclusion

In conclusion, the results presented here indicate that PPAR-γ agonists, mainly 15d-PGJ₂, reduce eosinophil infiltration after OVA-challenge by decreasing the levels of main Th2 cytokines (IL-5 and IL-33) as well as IL-23 and IL-17 cytokines, which in turn decrease the amount of IgE. Our data support the idea that PPAR-γ activation could be a therapeutic strategy for eosinophil-mediated disorders.

Acknowledgments

This work was supported by grants from Fundação de Amparo a Pesquisa de Minas Gerais (#PPM 097/09). T.S.F. received a master scholarship from Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil. The authors are responsible for the content and writing of the paper and declare that do not possess any financial interest.

References

- [1] T. Varga, L. Nagy, Nuclear receptors, transcription factors linking lipid metabolism and immunity: the case of peroxisome proliferator-activated receptor gamma, *Eur. J. Clin. Invest.* 38 (2008) 695–707.
- [2] B. Díez-Dacal, D. Pérez-Sala, Anti-inflammatory prostanooids: focus on the interactions between electrophile signaling and resolution of inflammation, *Sci. World J.* 10 (2010) 655–675.
- [3] D.W. Gilroy, T. Lawrence, M. Perretti, A.G. Rossi, Inflammatory resolution: new opportunities for drug discovery, *Nat. Rev. Drug Discov.* 3 (2004) 401–416.
- [4] S. Cuzzocrea, N.S. Wayman, E. Mazzon, L. Dugo, R. Di Paola, I. Serrano, D. Britti, P.K. Chatterjee, A.P. Caputi, C. Thiemermann, The cyclopentenone prostaglandin 15-deoxy-Δ^{12,14}-prostaglandin J₂ attenuates the development of acute and chronic inflammation, *Mol. Pharmacol.* 61 (2002) 997–1007.
- [5] M.H. Napimoga, S.M. Vieira, D. Dal-Secco, A. Freitas, F.O. Souto, F.L. Mestriner, J.C. Alves-Filho, R. Grespan, T. Kawai, S.H. Ferreira, F.Q. Cunha, Peroxisome proliferator-activated receptor-γ ligand, 15-deoxy-Δ^{12,14}-prostaglandin J₂, reduces neutrophil migration via NO pathway, *J. Immunol.* 180 (2008) 609–617.

- [6] M.H. Napimoga, G.R. Souza, T.M. Cunha, L.F. Ferrari, J.T. Clemente-Napimoga, C.A. Parada, W.A. Verri Jr., F.Q. Cunha, S.H. Ferreira, 15d-Prostaglandin J₂ inhibits inflammatory hypernociception: involvement of peripheral opioid receptor, *J. Pharmacol. Exp. Ther.* 324 (2008) 313–321.
- [7] D.R. Pena-Dos-Santos, F.P. Severino, S.A.L. Pereira, D.B.R. Rodrigues, F.Q. Cunha, S.M. Vieira, M.H. Napimoga, J.T. Clemente-Napimoga, Activation of peripheral κ/δ opioid receptors mediates 15-deoxy-Δ^{12,14}-prostaglandin J₂ induced-antinociception in rat temporomandibular joint, *Neuroscience* 163 (2009) 1211–1219.
- [8] M.Y. Hassumi, V.J. Silva-Filho, J.C. Campos-Júnior, S.M. Vieira, F.Q. Cunha, P.M. Alves, J.B. Alves, T. Kawai, R.B. Gonçalves, M.H. Napimoga, PPAR-γ agonist rosiglitazone prevents inflammatory periodontal bone loss by inhibiting osteoclastogenesis, *Int. Immunopharmacol.* 9 (2009) 1150–1158.
- [9] C. Alves, N. de Melo, L. Fraceto, D. de Araújo, M. Napimoga, Effects of 15d-PGJ₂-loaded poly(D,L-lactide-co-glycolide) nanocapsules on inflammation, *Br. J. Pharmacol.* 162 (2011) 623–632.
- [10] M.G. Belvisi, D.J. Hele, M.A. Birrell, Peroxisome proliferator-activated receptor γ agonists as therapy for chronic airway inflammation, *Eur. J. Pharmacol.* 533 (2006) 101–109.
- [11] S.J. Park, K.S. Lee, S.R. Kim, K.H. Min, Y.H. Choe, H. Moon, H.J. Chae, W.H. Yoo, Y.C. Lee, Peroxisome proliferator-activated receptor gamma agonist down-regulates IL-17 expression in a murine model of allergic airway inflammation, *J. Immunol.* 183 (2009) 3259–3267.
- [12] S. Ueki, T. Adachi, J. Bourdeaux, H. Oyama, Y. Yamada, K. Hamada, A. Kanda, H. Kayaba, J. Chihara, Expression of PPAR-γ in eosinophils and its functional role in survival and chemotaxis, *Immunol. Lett.* 86 (2003) 183–189.
- [13] Y. Matsuwaki, S. Ueki, T. Adachi, H. Oyama, Y. Kamada, K. Yamaguchi, A. Kanda, K. Hamada, H. Kayaba, J. Chihara, The synthetic PPAR-γ agonist troglitazone inhibits IL-5-induced CD69 upregulation and eosinophil-derived neurotoxin release from eosinophils, *Pharmacology* 74 (2005) 169–173.
- [14] G. Woerly, K. Honda, M. Loyens, J. Papin, J. Auwerx, B. Staels, M. Capron, D. Dombrowicz, Peroxisome proliferator-activated receptor α and γ down-regulate allergic inflammation and eosinophil activation, *J. Exp. Med.* 198 (2003) 411–421.
- [15] N. Fukui, K. Honda, E. Ito, K. Ishikawa, Peroxisome proliferator-activated receptor γ negatively regulates allergic rhinitis in mice, *Allergol. Int.* 58 (2009) 247–253.
- [16] H. Hammad, B.N. Lambrecht, Recent progress in the biology of airway dendritic cells and implications for understanding the regulation of asthmatic inflammation, *J. Allergy Clin. Immunol.* 118 (2006) 331–336.
- [17] M. Kurowska-Stolarska, P. Kewin, G. Murphy, R.C. Russo, B. Stolarski, C.C. Garcia, M. Komai-Koma, N. Pitman, Y. Li, W. Niedbala, A.N. McKenzie, M.M. Teixeira, F.Y. Liew, D. Xu, IL-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4, *J. Immunol.* 181 (2008) 4780–4790.
- [18] M. Komai-Koma, D. Xu, Y. Li, A.N. McKenzie, I.B. McInnes, F.Y. Liew, IL-33 is a chemoattractant for human Th2 cells, *Eur. J. Immunol.* 37 (2007) 2779–2786.
- [19] H. Li, J.A. Bradbury, R.T. Dackor, M.L. Edin, J.P. Graves, L.M. DeGraff, P.M. Wang, C.D. Bortner, S. Maruoka, F.B. Lih, D.N. Cook, K.B. Tomer, A.M. Jetten, D.C. Zeldin, Cyclooxygenase-2 regulates Th17 cell differentiation during allergic lung inflammation, *Am. J. Respir. Crit. Care Med.* 184 (2011) 37–49.
- [20] H. Wakashin, K. Hirose, Y. Maezawa, S. Kagami, A. Suto, N. Watanabe, Y. Saito, M. Hatano, T. Tokuhisa, Y. Iwakura, P. Puccetti, I. Iwamoto, H. Nakajima, IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice, *Am. J. Respir. Crit. Care Med.* 178 (2008) 1023–1032.
- [21] J. Peng, X.O. Yang, S.H. Chang, J. Yang, C. Dong, IL-23 signaling enhances Th2 polarization and regulates allergic airway inflammation, *Cell Res.* 20 (2010) 62–71.

- [22] B. Zingarelli, J.A. Cook, Peroxisome proliferator-activated receptor-gamma is a new therapeutic target in sepsis and inflammation, *Shock* 23 (2005) 393–399.
- [23] A.B. Cheraim, P. Xavier-Elsas, S.H. de Oliveira, T. Batistella, M. Russo, M.I. Gaspar-Elsas, F.Q. Cunha, Leukotriene B4 is essential for selective eosinophil recruitment following allergen challenge of CD4+ cells in a model of chronic eosinophilic inflammation, *Life Sci.* 83 (2008) 214–222.
- [24] S. Ueki, Y. Matsuwaki, H. Kayaba, H. Oyamada, A. Kanda, A. Usami, N. Saito, J. Chihara, Peroxisome proliferator-activated receptor- γ regulates eosinophil functions: a new therapeutic target for allergic airway inflammation, *Int. Arch. Allergy Immunol.* 134 (2004) 30–36.
- [25] R. Rajakariar, M. Hilliard, T. Lawrence, S. Trivedi, P. Colville-Nash, G. Bellington, D. Fitzgerald, M.M. Yaqoob, D.W. Gilroy, Hematopoietic prostaglandin D2 synthase controls the onset and resolution of acute inflammation through PGD2 and 15-deoxyDelta12 14 PGJ2, *Proc. Natl. Acad. Sci. USA* 104 (2007) 20979–20984.
- [26] G.M. Denning, L.L. Stoll, Peroxisome proliferator-activated receptors: potential therapeutic targets in lung disease? *Pediatr. Pulmonol.* 41 (2006) 23–34.
- [27] L. Benayoun, S. Letuve, A. Druilhe, J. Boczkowski, M.C. Dombret, P. Mechighel, J. Megret, G. Leseche, M. Aubier, M. Pretolani, Regulation of peroxisome proliferator-activated receptor γ expression in human asthmatic airways: relationship with proliferation, apoptosis, and airway remodeling, *Am. J. Respir. Crit. Care Med.* 164 (2001) 1487–1494.
- [28] K.S. Lee, S.J. Park, S.R. Kim, K.H. Min, S.M. Jin, H.K. Lee, Y.C. Lee, Modulation of airway remodeling and airway inflammation by peroxisome proliferator-activated receptor in a murine model of toluene diisocyanate-induced asthma, *J. Immunol.* 177 (2006) 5248–5257.
- [29] S.J. Park, Y.C. Lee, Peroxisome proliferator-activated receptor as a novel therapeutic target in asthma, *J. Asthma* 45 (2008) 1–8.
- [30] L. Cohn, J.A. Elias, G.L. Chupp, Asthma: mechanisms of disease persistence and progression, *Ann. Rev. Immunol.* 22 (2004) 789–815.
- [31] K. Takatsu, H. Nakajima, IL-5 and eosinophilia, *Curr. Opin. Immunol.* 20 (2008) 288–294.
- [32] C.M. Lloyd, IL-33 family members and asthma – bridging innate and adaptive immune responses, *Curr. Opin. Immunol.* 22 (2010) 800–806.
- [33] C. Moussion, N. Ortega, J.P. Girard, The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel “alarmin”? *PLoS ONE* 3 (2008) e3331.
- [34] M.D. Smithgall, M.R. Comeau, B.R. Park-Yoon, D. Kaufman, R. Armitage, D.E. Smith, IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK Cells, *Int. Immunol.* 20 (2008) 1019–1030.
- [35] C.L. Langrish, Y. Chen, W.M. Blumenschein, J. Mattson, B. Basham, J.D. Sedgwick, T. McClanahan, R.A. Kastelein, D.J. Cua, IL-23 drives a pathogenic T cell population that induces autoimmune inflammation, *J. Exp. Med.* 201 (2005) 233–240.
- [36] S. Molet, Q. Hamid, F. Davoine, E. Nutku, R. Taha, N. Pagé, R. Olivenstein, J. Elias, J. Chakir, IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines, *J. Allergy Clin. Immunol.* 108 (2001) 430–438.
- [37] Y. Fujimura, H. Tachibana, K. Yamada, Peroxisome proliferator-activated receptor ligands negatively regulate the expression of the high-affinity IgE receptor Fc epsilon RI in human basophilic KU812 cells, *Biochem. Biophys. Res. Commun.* 297 (2002) 193–201.
- [38] Y. Miyazaki, H. Tachibana, K. Yamada, Inhibitory effect of peroxisome proliferator-activated receptor-gamma ligands on the expression of IgE heavy chain germline transcripts in the human B cell line DND39, *Biochem. Biophys. Res. Commun.* 295 (2002) 547–552.