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ORIGINAL ARTICLE

Peroxisome Proliferator-Activated Receptor γ Negatively Regulates Allergic Rhinitis in Mice

Naoko Fukui[1], Kohei Honda[1], Eiko Ito[1] and Kazuo Ishikawa[1]

ABSTRACT

Background: Peroxisome proliferator-activated receptor γ (PPAR-γ) has been shown to play an important role in the control of inflammatory responses acting on macrophages, mast cells, T cells, and eosinophils. The present study was aimed at investigating the effects of PPAR-γ agonist on nasal symptoms and eosinophil accumulations in nasal mucosa by using a murine allergic rhinitis model. Furthermore, we examined the expression of PPAR-γ in the nasal mucosa in mice.

Methods: BALB/c mice were sensitized and challenged intranasally with ovalbumin. Ciglitazone, a PPAR-γ agonist, was administered orally 6 hours before each nasal challenge.

Results: Administration of PPAR-γ agonist significantly decreased the number of nasal rubs, nasal histamine responsiveness, serum IgE, IL-5 production from the spleen, and eosinophilic infiltration in the nasal mucosa. Furthermore, PPAR-γ was expressed in eosinophils and epithelial cells in the nasal mucosa by immunohistochemistry.

Conclusions: PPAR-γ was expressed in eosinophils and epithelial cells in the nasal mucosa. Also, the oral administration of ciglitazone is effective in upper airway allergic inflammation in mice.

KEY WORDS

allergic rhinitis, eosinophils, murine model, ovalbumin

INTRODUCTION

The prevalence of allergic rhinitis has increased in recent years and appears to be at least 300% more likely to occur in a patient with asthma.[1] The vast majority of patients with asthma have rhinitis, and rhinitis is a major independent risk factor for asthma.[2] Allergic rhinitis has been defined as an inflammatory disease of the nasal mucosa characterized by the following symptoms: nasal obstruction, sneezing, and rhinorrhea. Eosinophils are prominent in nasal washings, smears, and biopsies of the nasal mucosa in patients with allergic rhinitis.[3] Eosinophils are considered to be the major inflammatory cells in allergic rhinitis, since eosinophils release proinflammatory mediators, such as leukotriene C_4 and platelet-activating factor which increase permeability and are a potent chemotactic factor for eosinophils.[4] Eosinophils also produce a number of potent, highly positively charged proteins which are toxic to the human respiratory epithelium.[5] Peroxisome proliferators-activated receptor-γ (PPAR-γ), a member of the nuclear hormone receptor superfamily, has a critical role in adipogenesis, glucose metabolism.[6] One group of synthetic PPAR-γ agonistic ligands are glitazones. Glitazones are drugs used in the treatment of diabetic patients.[7] One of the natural ligands for PPAR-γ is 15-deoxy-Δ-prostaglandin J_2 (15d-PGJ_2), an arachidonic acid metabolite.[8] Furthermore, PPAR-γ also plays an important role in the control of the inflammatory response acting on T cells,[9] macrophages,[10] dendritic cells,[11] mast cells,[12] and eosinophils.[13,14] In monocytes and macrophages PPAR-γ agonists inhibit the expression of proinflamatory cytokines, such as TNF-α, IL-1β, and IL-6.[10] In human cultured mast cells (HCMC) PPAR-γ agonists attenuated the production of

[1]Department of Sensory Medicine, Division of Otorhinolaryngology Head and Neck Surgery, Akita University, School of Medicine, Akita, Japan.
Correspondence: Kohei Honda, Department of Sensory Medicine, Division of Otorhinolaryngology Head and Neck Surgery, Akita University, School of Medicine, 1–1–1 Hondo, Akita 010–8543, Japan.
Email: kohei@med.akita–u.ac.jp
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granulocyte-macrophage colony-stimulating factor by anti-IgE-stimulated HCMC.[12] It was reported that administration of PPAR-γ agonist inhibited the development of allergic inflammation, including pulmonary eosinophilia, airway hyperresponsiveness (AHR), cytokine production, GATA-3 expression, and the serum level of antigen-specific IgE in a murine model of asthma.[13,15] In addition, PPAR-γ is expressed in airway epithelium and smooth muscle, and PPAR-γ agonist nebulization reduces airway remodeling.[16]

However, the effects of PPAR-γ on allergic rhinitis, upper airway IgE-mediated inflammation have not been clarified. The present study was aimed at investigating the effect of PPAR-γ agonist on nasal symptoms and eosinophils accumulations in the nasal mucosa by using a murine allergic rhinitis model. Furthermore, we examined the expression of PPAR-γ in the nasal mucosa in mice.

METHODS

MURINE ALLERGIC RHINITIS MODEL

Because the complete inactivation of the PPAR-γ gene leads to embryonic lethality, the PPAR-γ agonist, ciglitazone, was used to investigate the potential role of PPAR-γ as a regulator of airway inflammation. Female 6–8 week-old BALB/c mice, housed under pathogen-free conditions, were sensitized by means of intraperitoneal injection of 50 μg ovalbumin (OVA) (Sigma, Steinheim, Germany) in 100 μl of alum (Pierce, Rockford, USA) or received alum only and were challenged intranasally (i.n.), on day 23 to 27 with OVA, 20 μl of PBS containing 500 μg of OVA. Ciglitazone (20 mg/kg in 1% carboxymethylcellulose, Sigma) was administered orally 6 hours before the OVA challenge on day 22 to 26. Serum was collected on day 28. Mice were killed 12 hours after i.n. provocation with OVA. The Institutional Review Board of the Akita University School of Medicine approved all animal experiments.

CLINICAL SYMPTOMS

Nasal symptoms were evaluated by counting the number of sneezes and nasal rubs that occurred in the 10 minutes after OVA i.n. provocation, by the same dose of OVA as given during the daily challenge on day 27. 12 hours after intranasal provocation on day 27, nasal histamine responsiveness (NHR) was measured. It was measured by determining the limiting concentration of histamine that caused sneezes and rubs. Nasal challenges with 20 μl of serially diluted histamine solution were administered.

SERUM OVA-SPECIFIC IgE LEVEL

Serum anti-OVA IgE concentration was measured by ELISA, using a specific ELISA kit. (Shibayagi, Gunma, Japan)

HISTOLOGY

Nasal mucosa samples were fixed with formalin for 3 days at 4℃. After dehydration with ethanol, they were embedded in paraffin. The embedded tissues were cut into 3 μm thin sections using a microtome, and stained with Eosinostain-Hansel (Torii, Tokyo, Japan). To evaluate eosinophilic infiltration in the nasal mucosa, we counted the number of eosinophils, which was the same part of the coronal slice of the nasal mucosa.

IMMUNOHISTOCHEMISTRY

Nasal mucosa samples were fixed with Immunohistofix (Intertiles, Brussels, Belgium) for 3 days at 4℃. After dehydration in graded alcohol baths embedded in Immunohistowax (Intertiles), the embedded tissues were cut into 3 μm thin sections using a microtome. Dewaxed and TBS-washed sections were incubated with 3% H_2O_2 in methanol for 20 minutes to block endogenous peroxidases. Sections were washed with TBS, and incubated with 5% skimmed milk in TBS for 20 minutes to block endogenous peroxidases. And then, sections were incubated with rabbit anti-human PPAR-γ (SC-7196: Santa Cruz Biotechnology, CA, USA) for 2 hours at room temperature. After washing, sections were incubated with ENVISION+ Rabbit/HRP (K4002: Dako, Denmark) for 30 minutes at room temperature. Sections were counterstained with hematoxilin.

CYTOKINES IN SPLEEN TISSUE MEASUREMENT

Spleen samples were homogenized in 300 μl of buffer containing 1% NP-40, 150 mM NaCl, 50 mM Hepes, PMSF, and Complete protease inhibitor cocktail. IL-5, IL-13, IFN-γ were measured using specific ELISA kits. (ELM-IFNgamma-001, ELM-IL5-001, ELM-IL13-001, RayBiotech, GA, USA)

STATISTICAL ANALYSIS

Values were expressed as means ± SEMs. Data was compared by using the unpaired t test. A P value of less than 0.05 was considered significant.

RESULTS

CLINICAL SYMPTOMS

The number of nasal rubs after the OVA challenge in the OVA-sensitized group was significantly higher than that in the unsensitized group. Administration of ciglitazone decreased the number of nasal rubs significantly (Fig.1A). The number of sneezes after the OVA challenge in the OVA-sensitized group was significantly higher than the number in the unsensitized group. Administration of ciglitazone decreased the number of sneezes (not statistically significant; Fig.1B). NHR in the OVA-sensitized group was significantly higher than the NHR in the unsensitized group. Administration of ciglitazone decreased the

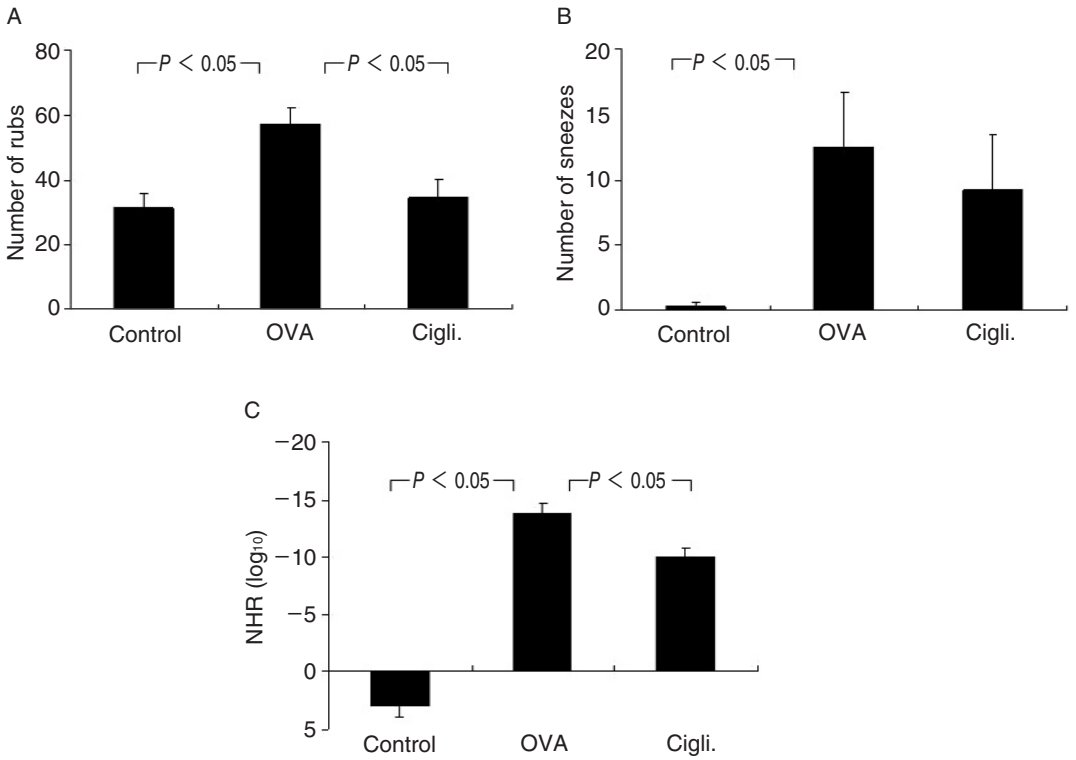

Fig. 1 Clinical symptoms in the unsensitized (control), the OVA-sensitized (OVA), and the ciglitazone administrated mice (Cigli.). **A**: The number of nasal rubs that occurred in the 10 minutes after the OVA i.n. provocation. **B**: The number of sneezes that occurred in the 10 minutes after the OVA i.n. provocation. **C**: NHR is represented by the limiting concentration of histamine that caused sneezes and rubs.

NHR significantly (Fig.1C).

OVA ANTIGEN SPECIFIC SERUM IgE ANTIBODY LEVEL

Serum levels of OVA-specific IgE in the OVA-sensitized group were significantly higher than that in the unsensitized group. Administration of ciglitazone significantly decreased the serum levels of OVA-specific IgE (Fig.2A).

CYTOKINES IN SPLEEN TISSUE MEASUREMENT

IL-5 levels in the spleen tissue in the OVA-sensitized group were significantly higher than the IL-5 levels in the OVA-unsensitized group. Administration of ciglitazone significantly decreased the IL-5 levels in the spleen (Fig.2B). IL-13 levels in the spleen tissue in the OVA-sensitized group were also significantly higher than that in the OVA-unsensitized group. Administration of ciglitazone decreased IL-13 levels in the spleen (not statistically significant; Fig.2C). IFN-γ levels in the spleen tissue brought about no significant differences in the all 3 groups (Fig.2D).

EOSINOPHILS INFILTRATION IN NASAL MUCOSA

In the OVA-unsensitized mice, few eosinophils infiltrated into the submucosa (Fig.3A). On the other hand, many eosinophils infiltrated into the submucosa in the OVA-sensitized mice (Fig.3B). In the ciglitazone-administered mice, eosonophilic infiltration was decreased (Fig.3C). The number of eosinophils in the nasal mucosa was counted to evaluate eosinophilic infiltration. The number of eosinophils of nasal mucosa in the OVA-sensitized group was significantly higher than that in OVA-unsensitized group. Administration of ciglitazone significantly decreased eosinophil infiltration (Fig.4).

PPAR-γ EXPRESSION IN NASAL MUCOSA

Immunohistochemistry of nasal mucosa sections in the OVA-sensitized mice revealed that PPAR-γ was expressed in infiltrated cells in the submucosa and epithelial cells (Fig.5B). Serial slices of each section were stained by the eosinostain, and it was shown that the cells infiltrated into the submucosa were eosinophils (Fig.5C). On the other hand, PPAR-γ was not expressed in the OVA-unsensitized mice.

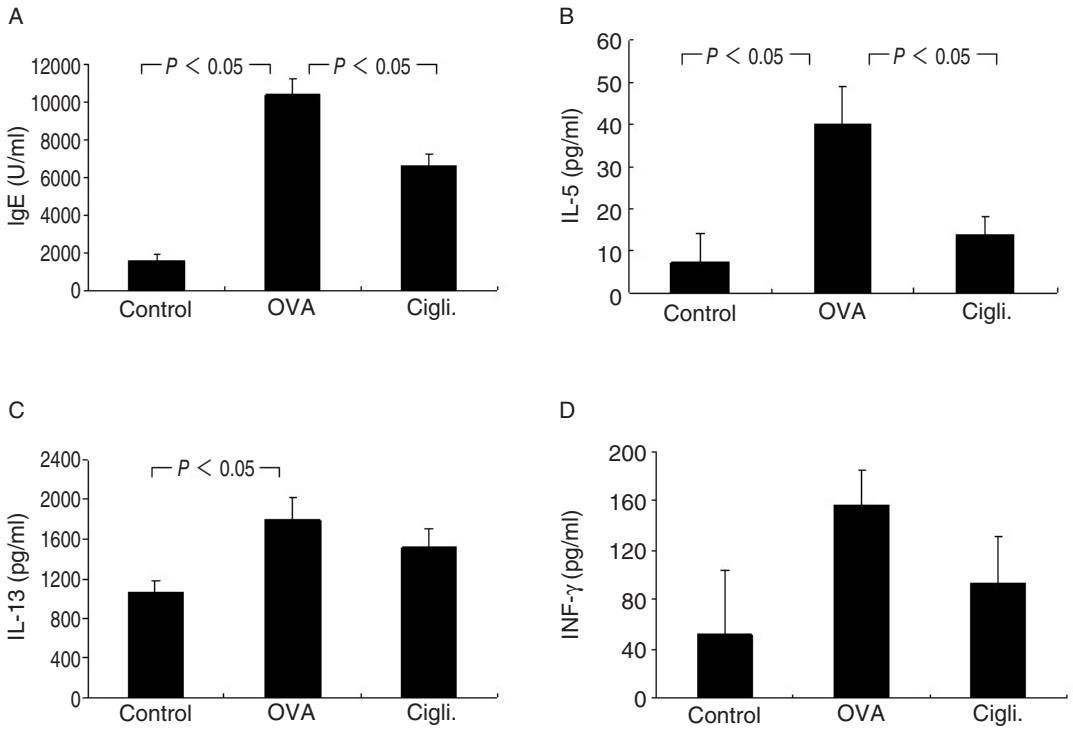

Fig. 2 OVA-specific serum IgE levels and cytokine levels in spleen tissues in the unsensitized (control), the OVA-sensitized (OVA), and the ciglitazone administrated mice (Cigli.). **A**: OVA-specific serum IgE levels. **B**: IL-5 levels of spleen tissues. **C**: IL-13 levels of spleen tissues. **D**: IFN-γ levels of spleen tissues.

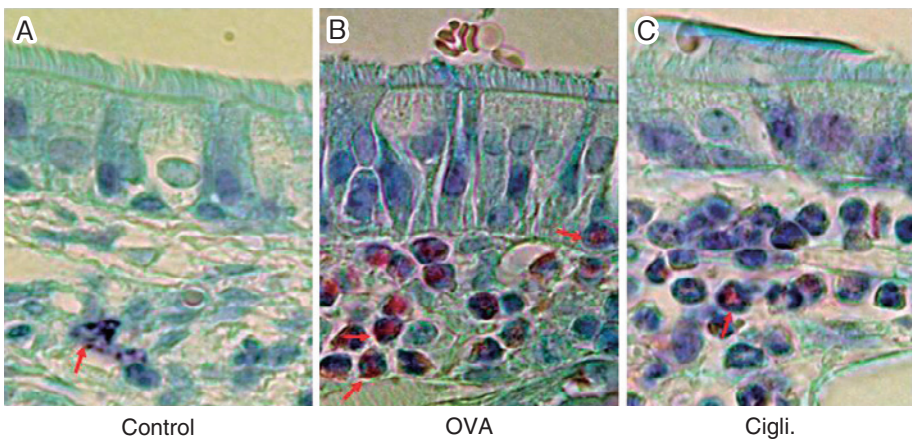

Fig. 3 Nasal mucosa stained with Eosinostain-Hansel. **A**: control; OVA-unsensitized mice. **B**: OVA; OVA-sensitized mice. Many eosinophils infiltrated into the submucosa. Arrows indicate eosinophils. **C**: Cigli.; Ciglitazone administration decreased eosinophilic infiltrations.

DISCUSSION

Allergic rhinitis is characterized by a two-phase allergic reaction. In the early-phase inflammatory response allergen-IgE dependent activation of mast cells and basophils results in the production of pharmacologically active mediators such as histamine, prostaglandins, leukotrienes, and cytokines which produce sneezing, rhinorrhea, and itching. The late-phase response begins 2 to 4 hours following allergen exposure at which point inflammatory cells, including eosinophils, basophils, and T cells, infiltrate the local tissue. Recruitment of inflammatory cells results in further release of histamine and leukotrienes, as well

as other compounds including proinflamatory cytokines and chemokines, sustaining the allergic response and promoting the late phase response.[17] We have demonstrated, in a murine allergic rhinitis model, that oral administration of PPAR-γ agonist reduced IgE production, nasal symptoms, NHR, eosinophils accumulation in nasal mucosa, and that PPAR-γ expressed itself in eosinophils and airway epithelium in nasal mucosa. According to the parameters (IgE production and NHR) measured, this inhibitory effect was totally abrogated by co-administration of GW9662 a PPAR-γ antagonist (data not shown), thus indicating that most ciglitazone-mediated effects may occur through PPAR-γ stimulation in this model. Our data shows that PPAR-γ regulates not only the early-phase inflammatory response but also the late-phase response. It has been shown that HCMC and human basophilic cells express PPAR-γ and PPAR-γ ligands, 15-d-PGJ$_2$ and troglitazone suppressed cytokine production and histamine release.[12,18] PPAR-γ was also expressed in eosinophils.[13,14] Their activation inhibits chemotaxis and antibody-dependent cellular cytotoxicity. In contrast to these findings, a recent study revealed that 15d-PGJ$_2$ appears to possess not only anti-inflammatory activities but also a proinflamatory potential depending on its concentration. At low concentrations, 15d-PGJ$_2$ enhances eotaxin-induced chemotaxis in eosinophils through its ligation with PPAR-γ.[19] 15d-PGJ$_2$ binds two receptors, PPAR-γ and CRTH2, which are both expressed on eosinophils in

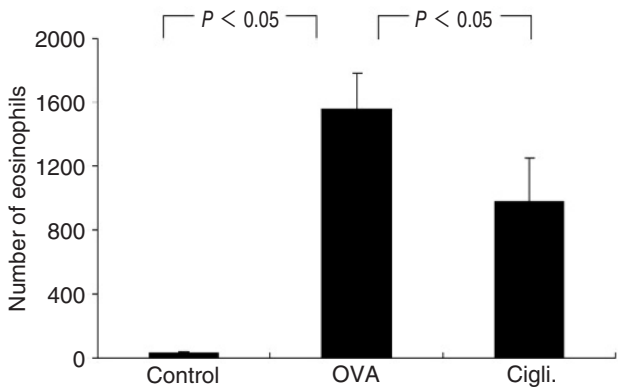

Fig. 4 The number of eosinophils of nasal mucosa in the unsensitized (control), the OVA-sensitized (OVA), and the ciglitazone administrated mice (Cigli.) (original magnification, 400×). Administration of ciglitazone significantly decreased eosinophils infiltration.

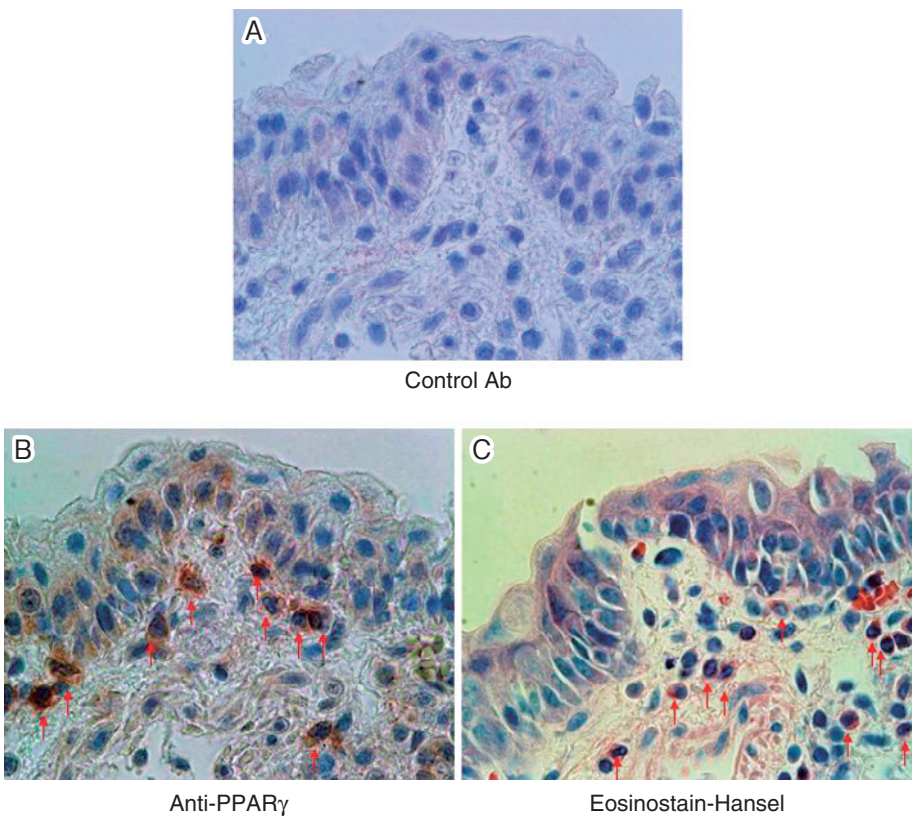

Fig. 5 PPAR-γ expression in the nasal mucosa. **A**: OVA-sensitized mouse with control antibody. **B**: OVA-sensitized mouse with anti- PPAR-γ. **C**: OVA-sensitized mouse with Eosinostain-Hansel (original magnification, 400×). Arrows indicate eosinophils, PPAR-γ was expressed in eosinophils and epithelial cells in the nasal mucosa.

allergic inflammation.[20] Further investigation will be required to reveal the role of PPAR-γ in eosinophils. Airway epithelial cells constitutively express PPAR-γ, mRNA, and protein and PPAR-γ agonists inhibit the cytokine-mediated induction of inflammatory mediators. Furthermore IL-4 treatment significantly upregulated PPAR-γ expression in airway epithelial cells.[21] This enhanced PPAR-γ expression may support our results that PPAR-γ expressed airway epithelium in nasal mucosa in OVA-sensitized mice due to the development of a Th2 response but did not express itself in control mice. There are numerous studies about PPAR-γ and inflammation, but the studies about PPAR-γ and airway inflammation were limited. In a previous study about PPAR-γ and lower airway inflammation, it was demonstrated in a murine model of asthma, that PPAR-γ agonists decrease antigen-induced AHR, pulmonary eosinophilia, cytokine production, and GATA-3 expression as well as serum levels of antigen-specific IgE. Furthermore, PPAR-γ is expressed in airway epithelium and smooth muscle on antigen challenge and PPAR-γ agonist nebulization reduced airway remodeling including collagen deposition and production of TGF-β. Similar findings have been reported by others.[15] In a human sample, PPAR-γ is augmented in the bronchial submucosa, the airway epithelium, and the smooth muscle of asthmatic patients, as compared with control subjects.[22] These studies indicated that PPAR-γ may decrease AHR regulating eosinophils, T cells, and airway epithelial cells in lower airway inflammation.

We showed that oral administration of PPAR-γ agonist reduced IgE production, nasal symptoms, NHR, eosinophils accumulation in nasal mucosa, and that PPAR-γ expressed itself in eosinophils and airway epithelium in nasal mucosa in a murine model of allergic rhinitis. Considered together, activation of PPAR-γ in this model appears to regulate upper airway allergic inflammation by down regulating IL-5 production acting on epithelial cells and eosinophils.

In a human sample study of allergic rhinitis, the expression levels of PPAR-γ, m-RNA, and protein in the nasal mucosa was significantly higher in patients with perennial allergic rhinitis compared with the control.[23] In another group, no differences in the expression of PPARs were obtained in nasal biopsies from both patients with seasonal allergic rhinitis and healthy volunteers.[24] These studies suggested that PPAR-γ which expresses nasal mucosa, eosinophils, and other inflammatory cells might regulate upper airway allergic inflammation in humans.

Finally, we have demonstrated that PPAR-γ was expressed in eosinophils and epithelial cells in nasal mucosa and that the oral administration of ciglitazone is effective at inhibiting eosinophil accumulations in nasal mucosa and nasal symptoms. Our results provide for the therapeutic potential of the PPAR-γ agonist for allergic rhinitis.

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