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Forum Minireview

Nuclear Receptors as Targets for Drug Development: Peroxisome Proliferator-Activated Receptor γ in Mast Cells: Its Roles in Proliferation and Differentiation

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Abstract. Mast cells are derived from stem cells in bone marrow and their proliferation and differentiation are regulated by stimulation of stem cell factor derived from fibroblasts and/or IL-3 from T lymphocytes. The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily and ligand-activated transcription factors. It has been reported that PPARγ is expressed in mast cells, but its roles remain uncertain. Since mast cells produce and release prostaglandin D_2, which is metabolized to 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, a candidate for the endogenous PPARγ agonist, mast cells play roles in inflammation and immunological response via the PPARγ pathway. We will mainly discuss the contribution of PPARγ to the proliferation and functions in murine cultured bone marrow derived mast cells.

Keywords: peroxisome proliferator-activated receptor (PPAR) γ, mast cell, histamine, proliferation, differentiation

Introduction

The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily and ligand-activated transcription factors. PPAR promotes the transcription of target genes by forming heterodimers with the retinoid X receptor (RXR) and binding to specific motifs termed PPAR-responsive elements (1 – 3). There are three different subtypes of PPAR (α, β/δ, and γ). PPARγ is found in adipose tissues, spleen, and vascular smooth muscle cells. The activation of PPARγ regulates lipid and glucose homeostasis and its agonists have been developed as novel antidiabetic drugs. Moreover, PPARγ was found in inflammatory cells such as lymphocytes or macrophages, and it has been reported that PPARγ plays roles in inflammatory and immunological responses. Especially in the monocyte/macrophage system, the expression of PPARγ and the negative regulation of cytokine production has been reported (4, 5). There are not so many reports discussing PPARγ expression and its function in mast cells. In this minireview the roles of PPARγ in mast cells will be discussed.

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Proliferation and differentiation of mast cells

Mast cells, which are derived from bone marrow stem cells and differentiate at the peripheral tissues, contain various chemical mediators such as histamine and proteases in granules that are released immediately after antigen stimulation. They also produce and release arachidonic acid derivatives such as prostaglandins and leukotrienes within a half hour and finally synthesize several cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-4, IL-5, and granulocyte macrophage colony-stimulating factor (GM-CSF) in several hours. These cytokines are important for recruiting other inflammatory cells into the inflammation site and cause the late phase of allergic responses. Since mast cells also store TNF-α, which is released immediately after antigen stimulation, mast cells are thought to have important roles in regulating not only the allergic reaction but also innate immunity (6, 7).

The differentiation of mast cells derived from bone marrow stem cells are regulated by kit signal or IL-3 (shown in Fig. 1). In contrast to basophils, which differentiate completely in the bone marrow and are circulated

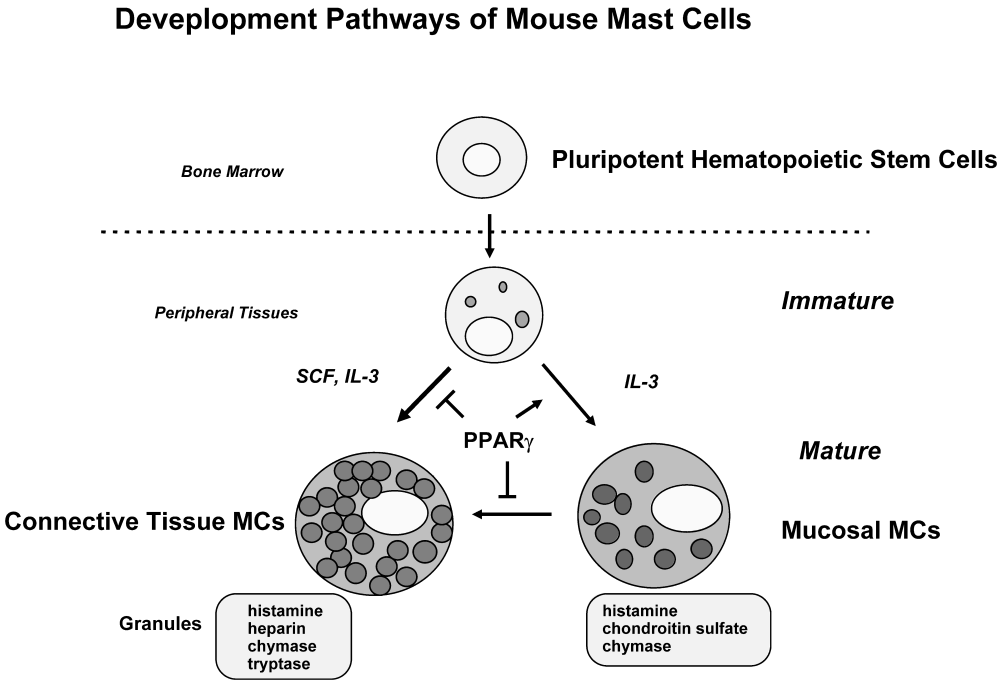

Fig. 1. Scheme of the differentiation pathway of mast cells. Mast cells derive from bone marrow stem cells and flow into the blood stream in the immature stage. These immature mast cells differentiate to two types of mast cells, connective tissue mast cells and mucosal mast cells, after stimulation with stem cell factor (SCF) expressed on the fibroblast cell membrane and IL-3 released from T lymphocytes. The proliferation and differentiation or conversion from mucosal mast cells to connective tissue mast cells may be regulated via the PPARγ pathway.

in the blood, mast cells flow out from the bone marrow in the immature stage and differentiate at the peripheral tissues. In rodents, mast cells are classified mainly into two types, connective tissue mast cells (CTMCs) and mucosal mast cells (MMCs), that are clearly separated by their morphological, biochemical, and pharmacological characteristics. That is, CTMCs contain more histamine and serotonin in the granules than MMCs. The proteoglycan in the granules is heparin in CTMCs, whereas it is chondroitin sulfate in MMCs. The changes in the negative charge of these proteoglycans effect the histological staining. Using toluidine blue staining at the low pH (less than 2), only CTMCs are stained, but both types of mast cells are stained at pH higher than 5. Using safranin, CTMCs are clearly stained red. The different pattern of responses to secretagouges, such as cationic peptides or compound 48/80, are known in each type of mast cells. CTMCs highly respond to these compounds, resulting in remarkable histamine release, but MMCs do not. CTMCs are located in the skin dermis, submucosal area, and peritoneal cavity. MMCs are observed at the epithelial layer of the respiratory and intestinal tract.

After the differentiation mechanisms were clarified and the growh factors were obtained, the culture method for mast cells in vitro was established. In the presence of IL-3, granurocytes of bone marrow cells differentiate to MMCs and in the presence of stem cell factor, to CTMCs. Even though we can mimic the process of mast cells maturation in vitro using these growth factors, it is too simplified to estimate the real maturation steps and functions of mast cells in vivo. Takada et al. report the cross-talk between PPARγ and cytokine signaling in this series of minireviews (8). Suppression of PPARγ function by TNF-α or IL-1 through NF-κB activated by the TAK1/TAB1-NIK cascade inhibits adipogenesis and induces osteoblastogenesis in mesenchymal stem cells. In human mast cells, IL-1β induces expression of IL-13 and monocyte chemoattractant protein-1 by IL-1 receptor-binding and subsequent signaling via nuclear translocation of NF-κB (9). The differentiation of mast cells may be partly regulated by the cross-talk between PPARγ and cytokines signaling. The contribution of PPARγ to the mast cell proliferation was studied using cultured bone marrow-derived mast cells (BMMCs) obtained from PPARγ heterozygotic knockout mice.

Analysis of BMMCs derived from the PPARγ gene-targeted mice

PPARγ gene-targeted mice were useful for demonstrating the PPARγ regulating function. Complete elimination of a functional PPARγ gene results in embryonic lethality, whereas PPARγ heterozygote knockout mice (PPAR$\gamma^{+/-}$) exhibit resistance to high-fat diet-induced obesity and insulin resistance. This heterozygote shows a 50% reduction in PPARγ (10). To get BMMCs, bone marrow cells obtained from mutant PPAR$\gamma^{+/-}$ and wild type (+/+) mice were cultured in the presence of IL-3 for 4 weeks and combination of IL-3 and stem cell factor for a further 4 weeks. As shown

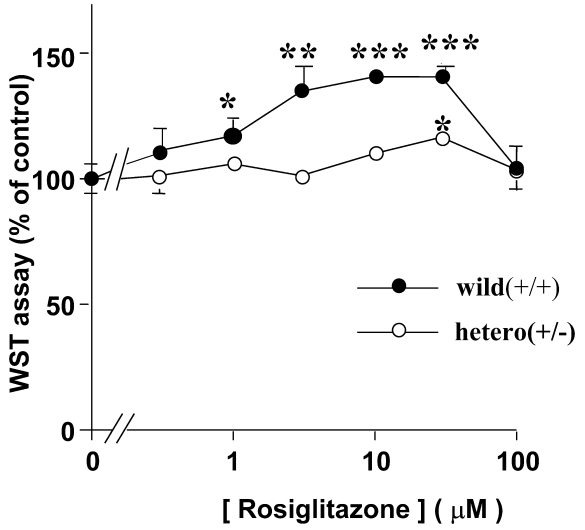

Fig. 2. Effect of rosiglitazone (BRL), a PPARγ agonist, on proliferation of bone marrow-derived mast cells (BMMCs) obtained from heterozygous PPARγ-deficient (+/–) and wild (+/+) mice. BMMCs were obtained after 4 weeks of culture with IL-3 and a further 4 weeks of culture with stem cell factor and IL-3. BMMCs were disseminated in a 96-well plate at a density of 1.25×10^4 cells/0.2 ml per well. Cell proliferation was measured using the cell proliferation reagent kit WST-1 (Roche, Mannheim, Germany) in the presence of various concentrations of rosiglitazone. Results are the mean ± S.E.M. of three experiments. *P<0.05, **P<0.01, ***P<0.001, significantly different from the non-treated group.

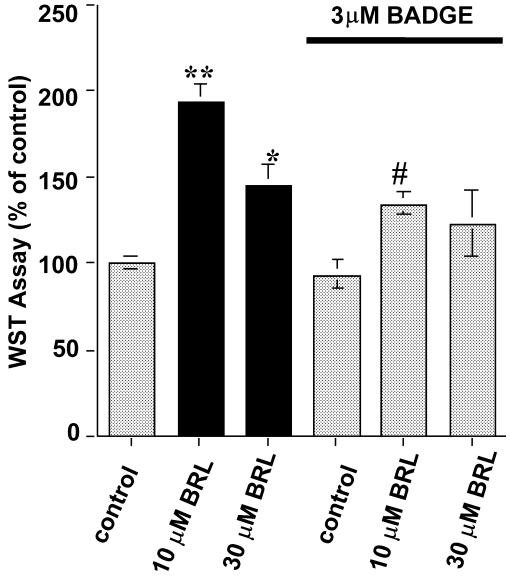

Fig. 3. Inhibitory effect of bisphenol A diglycidyl ether (BADGE), a PPARγ antagonist, on enhanced proliferation induced by rosiglitazone (BRL). BMMCs obtained from wild mice were cultured according to the method described in Fig. 2. In the presence of 3 μM of BADGE, BMMCs were stimulated with the indicated concentrations of BRL, and the cell proliferation was measured using the cell proliferation reagent kit WST-1. Results are the mean ± S.E.M. of three experiments. *P<0.05, **P<0.001, significantly different from the non-treated group; $^{\#}P$<0.01, significantly different from the group treated with 10 μM BRL in the absence of BADGE.

in Fig. 2, the proliferation of BMMCs are regulated by PPARγ. Rosiglitazone (BRL), a PPARγ agonist, increased viability of BMMCs from +/+ mice by 30% at 3 – 30 μM, whereas no increase were observed in the BMMCs from PPAR$\gamma^{+/-}$ mice at these drug concentration except at 30 μM. This increase of viability was suppressed after treatment of 3 μM bisphenol A diglycidyl ether (BADGE), a PPARγ antagonist, (11) (Fig. 3).

PPARγ and inflammation

PPARγ is expressed in many kinds of inflammatory cells, including monocytes/macrophages, lymphocytes, and neutrophils, and known to regulate inflammatory responses. In monocytes and monocyte-derived macrophages, the activation of PPARγ inhibits the expression of inflammatory cytokines such as TNF-α, IL-1α, and IL-6 (4, 5).

PPARγ activation also modifies lymphocyte function. Setoguchi et al. (8) reported that haploinsufficiency of PPARγ affects B cells but not T cells. This result indicates that PPARγ plays a critical role in regulating homeostasis of B cell function. These findings led to the proposal that manipulation of PPARγ has potential as a therapeutic strategy for controlling inflammatory and immunological diseases. In mast cells, the expression and functions of PPARγ are not so clear. Sugiyama et al. showed first that PPARγ is expressed in mouse BMMCs (12). Antigen stimulation increased the mRNA level of PPARγ, time-dependently, with the peak at 4 h. The protein levels of PPARγ also increased after antigen or Ca^{2+} ionophore stimulation. Functionally PPARγ plays roles in negative control of cytokine production. TNF-α and GM-CSF production induced by antigen stimulation was inhibited dose-dependently by PPARγ agonists, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) and troglitazone. In human cultured mast cells, PPARγ is expressed and PGD_2, 15d-PGJ_2, and troglitazone attenuated the production of GM-CSF stimulated by IgE receptors (13). In human basophilic KU812 cells, mRNA for PPARγ is expressed and PPAR agonists negatively regulate the expression of high-affinity IgE receptor (14). Taken these data together, allergic and inflammatory roles of mast cells are regulated or modified via the PPARγ pathway.

The effects of chronic treatment of PPARγ agonists on mast cell differentiation and function

BMMCs were cultured in the presence of IL-3 for 4

weeks and both IL-3 and stem cell factor (SCF) for a further 4 weeks. The chronic effect of BRL on proliferation was determined morphologically and functionally. As shown in Fig. 4, control BMMCs show representative characteristics of CTMCs, that is, stained red with safranin alcian blue and stained purple with toluidine blue. BRL treatment resulted in clear changes such as faint color and swelling of granules. The expressions of c-*kit* receptor and FcεRI were not affected by treatment of BRL.

The functional effects of BRL on histamine synthesis and release were determined. A 6-week treatment of BRL suppressed both histamine synthesis and secretion as shown in Fig. 5. Histamine content in BMMCs was decreased by BRL treatment to 30% of that with vehicle treatment. This decrease of histamine content was confirmed by the evidence of attenuation of the expression of histidine decarboxylase, the histamine synthesizing enzyme, after treatment of BRL. Histamine release induced by antigen stimulation, dinitrophenylated bovine serum albumin (DNP-BSA), is also suppressed to 55% of the control level. Chronic treatment of PPARγ suppresses the histamine levels and release from BMMCs, suggesting the negative control of PPARγ in allergic status. From the morphological and functional changes, we speculate that PPARγ affects the differenti-

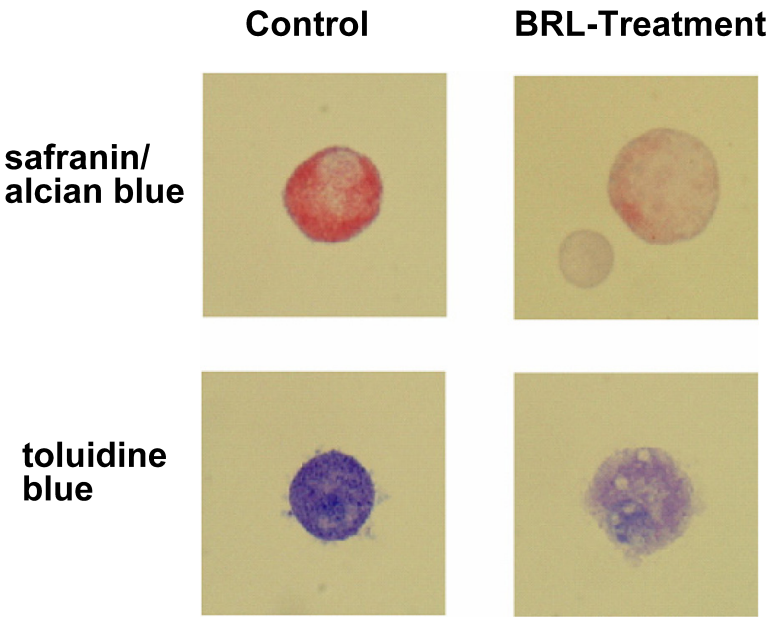

Fig. 4. Effect of chronic treatment of a PPARγ agonist on maturation of bone marrow-derived mast cells (BMMCs). Bone marrow cells were collected from the femur of BALB/c mice and differentiated in the enriched RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and IL-3 (2 ng/ml). After 4 weeks, stem cell factor (50 ng/ml) was added to the culture medium. Rosiglitazone (BRL, 10 μM), a PPARγ agonist, was administered to the culture medium at 2 weeks after the start of culture. At 8 weeks, BMMCs were fixed in Carnoy's solution and stained with safranin/alcian blue or toluidine blue.

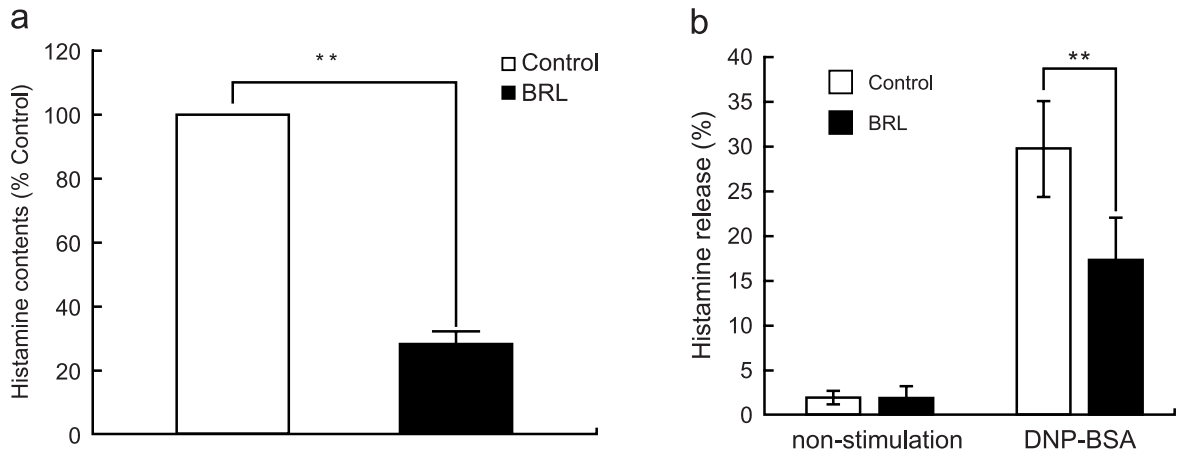

Fig. 5. Effect of a PPARγ agonist on antigen stimulated histamine release from bone marrow-derived mast cells (BMMCs). BMMCs were cultured and treated with rosiglitazone (BRL) in the same way as described in Fig. 4. BMMCs were sensitized with monoclonal IgE against dinitrophenylated bovine serum albumin (DNP-BSA) and stimulated with 20 ng/ml DNP-BSA as an antigen for 30 min. Histamine content in BMMCs (a) and released histamine in the supernatant (b) were measured by HPLC-flurometry. Results are the mean ± S.E.M. of 5 – 7 experiments. $^{**}P<0.01$, significantly different from the vehicle group.

ation steps of mast cells and inhibits conversion to CTMCs (Fig. 1).

Candidates of endogenous agonists for PPARγ

Several endogenous agonists for PPARγ have been considered. One candidate is 15d-PGJ_2, the metabolite of PGD_2. Mast cells have PGD_2 synthase and are thought to be a main source of PGD_2 in the body. It is uncertain that 15d-PGJ_2 acts as an agonist of PPARγ in vivo because the production pathway of 15d-PGJ_2 is not clear. PGD_2 and PGJ_2 in rat basophilic leukemia (RBL-2H3) cells were determined using the LC-MS method. PGD_2 level was 95.1 nmol/10^6 cells at 10 min after antigen stimulation, which was 40.8-fold over the unstimulated level, but PGJ_2, Δ^{12}-PGJ_2, and 15d-PGJ_2 were not detected. At 24 h after stimulation, PGD_2 was decreased to 8.65 nmol/10^6 cells, whereas PGJ_2, Δ^{12}-PGJ_2, and 15d-PGJ_2 levels were increased to 3.57, 0.312, and 0.42 nmol/10^6 cells, respectively, showing an increase of 43-, 19.5-, and 140-fold over the unstimulated levels, respectively. 15d-PGJ_2 produced in mast cells may act directly on themselves or surrounding cells via PPARγ and regulate their inflammatory actions. Lysophosphatidic acid (LPA) has been also thought to be a candidate for a PPARγ agonist. In inflammation, LPA shows activity as a growth factor through LPA receptors. Recently, it is reported that LPA can also induce proliferation in an LPA-receptor-independent manner, that is, serving as a transcellular agonist of PPARγ in human cultured mast cells. LPA-mediated proliferation was attenuated by GW9662, a selective antagonist of PPARγ and rosiglitazone modestly increased proliferation (15). Endogenous agonists should be clarified to understand the pathology of inflammation.

Conclusion

PPARγ is expressed in mast cells and regulates mast cell proliferation and various functions such as synthesis and release of inflammatory mediators. From these results obtained from in vitro experiments, the roles of PPARγ in mast cells are too variable to conclude a simple regulatory mechanism of inflammation, but further studies including in vivo experiments will give us the real regulatory mechanism via PPARγ in mast cells.

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