Peroxisome proliferator-activated receptors are expressed in mouse bone marrow-derived mast cells

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Abstract **We examined the expression of peroxisome proliferator-activated receptors (PPARs) and the role of PPARs in cytokine production in mouse bone marrow-derived mast cells (mBMMCs). mBMMCs expressed PPARβ strongly and γ slightly, but not α. Activation of mBMMCs with antigen or calcium ionophore resulted in the increased expression of PPARγ mRNA specifically. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) and troglitazone, all PPARγ ligands, attenuated the antigen-induced cytokine production by mBMMCs. Carbaprostacyclin, a PPARβ ligand, also inhibited cytokine production, whereas PPARα ligands did not. These results suggest that PPARβ and γ might be included in the negative regulation of mast cell activation.**

Key words: Peroxyzome proliferator-activated receptor; Mast cell; Cytokine

1. Introduction

Mast cell plays a pivotal role in allergic inflammation by releasing chemical mediators, such as histamine and lipid mediators including peptidyl leukotrienes, prostaglandins, and platelet-activating factor, in response to antigen or to neuropeptides released from peripheral neurons [1]. In addition, mouse mast cells produce several cytokines including tumor necrosis factor α (TNFα), interleukin-4 (IL-4), IL-5 and granulocyte macrophage colony-stimulating factor (GM-CSF), which act in the late phase of allergic reactions by recruiting inflammatory cells into the reaction site [2–5]. Furthermore, fibrogenic cytokines such as transforming growth factor β (TGFβ), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) derived from mast cells have been thought to contribute to the pathogenesis of tissue fibrosis [6–8].

Peroxisome proliferator-activated receptor (PPAR) is a member of the nuclear receptor superfamily. Three different subtypes of PPAR (α, β/δ, and γ) have been described [9,10]. PPARα is expressed dominantly in liver and has been focused in its role in lipid metabolism [11]. On the other hand, PPARγ is found in adipose tissue, spleen, and other tissues [12] and has been implicated in adipocyte differentiation [13] and glucose metabolism [14,15].

We hypothesized that PPAR is a candidate of a negative regulator of mast cell activation, because PPARγ has been demonstrated to modulate the activation of macrophages [16,17]. To confirm our hypothesis, we examined the expression of PPARs and the effect of PPAR ligands on the function of mouse bone marrow-derived mast cells (mBMMCs).

2. Materials and methods

2.1. Chemicals

15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), and carbaprostacyclin were purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). 5,8,11,14-Eicosatetraynoic acid (ETYA) and benzafibrate were from Sigma Chemical Co. (St. Louis, MO, USA). Troglitazone was obtained from Sankyo Co. These drugs were dissolved in ethanol, with the final concentration of ethanol being 0.5%. This concentration had no effect on any experiment described in this paper.

2.2. Cell culture

mBMMCs were obtained as previously described [18] with some modifications. Briefly, bone marrow cells obtained from 2-month-old female BALB/c mice (Charles River Japan Inc., Yokohama, Japan) were cultured at a density of 1×10^5 cells in 1 ml in RPMI 1640 medium (GIBCO RBL, Life Technologies Inc, Gaithersburg, MD, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS, GIBCO RBL) and 4 ng/ml recombinant mouse IL-3 (Pepro Tech EC Inc., London, UK) at 37°C in a humidified atmosphere containing 5% CO_2. The culture medium was changed weekly. mBMMCs were obtained after 5 weeks of culture. The purity of the mast cell population was determined by toluidine blue and May-Gruenwald and Giemsa staining.

Rat peritoneal mast cells (RPMCs) were obtained from Sprague Dawley male rats (Charles River Japan, Inc.) weighing 400–500 g. The RPMCs were purified from rat peritoneal cells by sedimentation through isotonic Percoll solution [19].

2.3. Stimulation of cells

For antigen stimulation, mast cells were sensitized with 10 μg/ml of anti-TNP mouse IgE (Pharmingen, San Diego, CA, USA) at 37°C for 12 h. The sensitized cells were then washed, and stimulated with 20 ng/ml DNP-BSA (LSL Ltd., Tokyo, Japan) as antigen at 37°C. Several concentrations of drugs were added to the cells simultaneously with IgE and incubated at 37°C for 12 h. No cytotoxicity was observed following these treatments, as estimated by trypan blue exclusion.

For A23187 stimulation, A23187 was added to cells at a concentration of 0.1 μg/ml, and incubation was carried out at 37°C.

2.4. Analysis of the mRNA expression by reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from mBMMCs with an RNA extraction

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Abbreviations: PPAR, peroxisome proliferator-activated receptor; mBMMC, mouse bone marrow-derived mast cell; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2

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kit (QIAGEN Inc., Valencia, CA, USA). cDNA was synthesized from 100–500 ng of total RNA by use of random hexamers (first-strand cDNA synthesis kit, Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). cDNA (20 ng of total RNA) was amplified with the following specific primers: mouse TNFα, ATG-AGC-ACA-GAA-AGC-ATG-ATC (upstream) and TAC-AGG-CTT-GTC-ACT-CGA-AYY (downstream) [20]; mouse glyceraldehyde-3-phosphate dehydrogenase (G3PDH), ATG-CCC-CAT-GTT-TGT-GAT-G (upstream) and ATG-GCA-TGG-ACT-GTG-GTC-AT (downstream) [21]; mouse PPARα, CCT-GCA-GAG-CAA-CAA-TCC-AG (upstream) and CCC-GTT-ATT-TAA-TGG-CGA-A (downstream) [22]; mouse PPARβ, GTC-ATG-GAT-CCG-CCA-CAG-GAG-GAG-ACC-CCT (upstream) and (downstream) [10]; mouse PPARγ, GAG-ATG-CCA-TTC-TGG-CCC-ACC-AAC-TTC-GGA (upstream) and TAT-CAT-AAA-TAA-GCA-TCA-ATC-GGA-TGG-TTC (downstream) [10]; mouse GM-CSF, GAA-AGG-CTA-AGG-TCC-TGA-GGA-G (upstream) and TAA-GGC-TGT-CTA-TGA-AAT-CCG (downstream) [23]. We detected PPARα, β, and γ with these primers for PPARs in mouse liver, brain, and spleen, respectively. The primers for mouse IL-4 and IL-5 were obtained from Clontech Laboratories, Inc. (Palo Alto, CA, USA). Taq polymerase was purchased from Takara Shuzo Co. (Tokyo, Japan). Each PCR cycle was composed of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 2 min. The PCR products were visualized by agarose gel electrophoresis followed by ethidium bromide staining.

2.5. Measurement of TNFα and GM-CSF by enzyme-linked immunosorbent assay (ELISA)

mBMMCs (1×10^5) in culture medium were stimulated with antigen as described above. The supernatants were collected and analyzed by ELISA kits: Duo Sets for mouse TNFα (Genzyme Diagnostics, Cambridge, MA, USA) and Quantikine mouse GM-CSF Immunoassay for GM-CSF (R and D Systems, Inc., Minneapolis, MN, USA).

2.6. Histamine release

The sensitized cells (1×10^5) were suspended in physiological saline solution (154 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$, 5 mM HEPES and 0.1% glucose), and stimulated with 20 ng/ml DNP-BSA at 37°C for 30 min. After centrifugation, histamine contents of both the supernatants and the cell pellets were determined by histamine autoanalyzer (Bran Luebbe Analyzing Technologies, Tokyo, Japan). Then the percent release was calculated.

2.7. Immunoblot analysis

The protein contents of the cell homogenates were determined with a using BCA protein assay kit (Pierce, Rockford, IL, USA). Twenty micrograms of protein was separated by 10–20% polyacrylamide gel (ATTO, Tokyo, Japan) and transferred to a polyvinylidene difluoride membrane (Millipore Co., Bedford, MA, USA). To detect the PPARγ protein, we used a rabbit polyclonal antibody against PPARγ (Santa Cruz, CA, USA). This antibody is not cross-reactive with PPARα or PPARβ. Immunoreactive protein was visualized by using a detection reagent for alkaline phosphatase (Pierce).

3. Results

3.1. Expression of PPARs in mBMMCs

Expression of PPARα, β and γ mRNA in mBMMCs was examined by RT-PCR. mBMMCs expressed PPARβ mRNA constitutively, whereas PPARγ mRNA was detected only slightly and PPARα not at all (Fig. 1A). Stimulation of mBMMCs with antigen resulted in a time-dependent increase in the mRNA level of PPARγ, with the peak at 4 h after the stimulation. A23187 also induced PPARγ mRNA, and the highest level was observed 8 h after the stimulation. These stimulants had no effect on the expression of PPARα or β mRNA. The induction of PPARγ was also observed in RPMCs (Fig. 1C), which expressed PPARβ but not PPARα, as in the case of mBMMCs (data not shown).

PPARγ protein was also detected by immunoblot analysis, by which the induced expression was observed after antigen or A23187 stimulation (Fig. 1B).

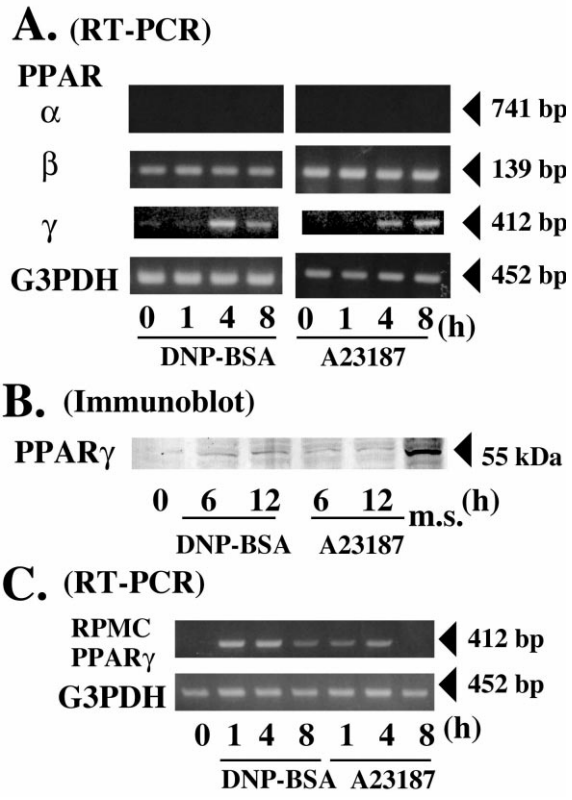

Fig. 1. Expression of PPARs in mBMMCs. A: mBMMCs were stimulated with 10 ng/ml DNP-BSA or 0.1 μg/ml A23187 for 0–8 h. Total RNA was extracted and analyzed by RT-PCR. B: Immunoblot analysis of PPARγ protein. mBMMCs were stimulated for 6 or 12 h as described. Cell homogenates (20 μg protein) were separated on SDS–PAGE and probed with anti-PPARγ antibody. A homogenate of mouse spleen (m.s., 20 μg protein) was used as a positive control. C: PPARγ mRNA expression in RPMCs analyzed by RT-PCR.

3.2. PPAR ligands inhibit cytokine production and histamine release

To investigate a possible role of PPARs in mast cell, we examined the effects of PPAR ligands on the induction of TNFα in antigen-stimulated mBMMCs. 15d-PGJ_2, which was reported to activate PPARγ [24], inhibited TNFα production in a dose-dependent manner over a range of 1–10 μM (Fig. 2A). The IC_{50} value of 15d-PGJ_2 was 6.5 μM. With troglitazone, a relatively higher concentration was required to suppress TNFα production (IC_{50} = 68.2 μM). Carbaprostacyclin, a PPARβ ligand, also inhibited the production of TNFα at 1 μM, whereas ETYA and bezafibrate, PPARα agonists, did not do so even at 100 μM (Fig. 2B). GM-CSF production was also attenuated by these PPARγ ligands, of which effects were observed at relatively lower concentrations (Fig. 2C). The IC_{50} values of 15d-PGJ_2 and troglitazone were less than 1 μM and 12.8 μM, respectively.

These suppressive effects of PPARγ and β ligands were observed at the mRNA level. Induced expression of TNFα mRNA after the antigen stimulation was lowered by 15d-PGJ_2, troglitazone and carbaprostacyclin (Fig. 3), suggesting that PPAR ligands suppressed the transcription of the TNFα

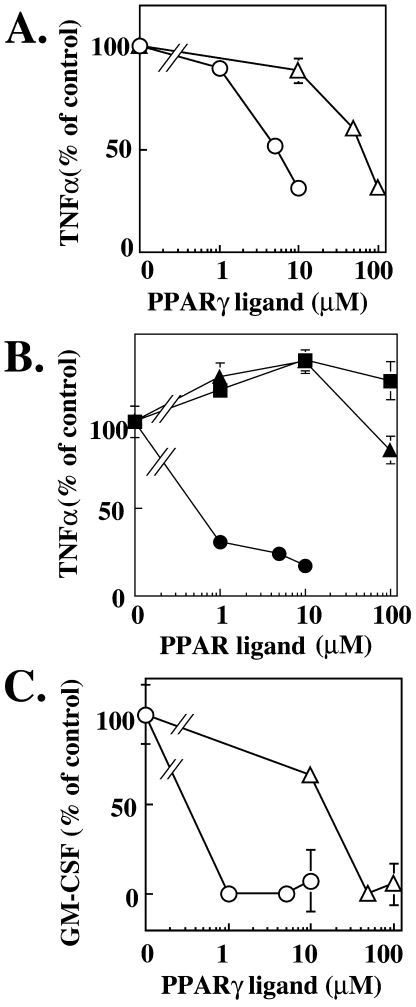

Fig. 2. Effects of PPAR ligands on cytokine production by mBMMCs. mBMMCs were stimulated with 10 ng/ml DNP-BSA at 37°C for 6 h (TNFα, A and B) or 24 h (GM-CSF, C). Spontaneous and induced production of TNFα were 0.05 and 7.50 ng/10^6 cells, respectively. Those of GM-CSF were less than 0.03 and 0.145 ng/10^6 cells, respectively. A, C: PPARγ ligands, 1–10 μM 15d-PGJ_2 (open circle) and 10–100 μM troglitazone (open triangle). B: PPARα ligands, 1–100 μM bezafibrate (closed triangle) and ETYA (closed square), and β ligand, 1–10 μM carbaprostacyclin (closed circle). Each point represents the mean ± S.D. of triplicate experiments.

gene. The induction of mRNAs of GM-CSF, IL-4 and IL-5 was observed 4 h after the antigen stimulation, and was also reduced by 15d-PGJ_2, troglitazone and carbaprostacyclin, but not by bezafibrate.

In addition, PPARβ and γ ligands also inhibited antigen-induced histamine release, whereas bezafibrate had little effect on it (Fig. 4).

4. Discussion

In this study, we found that PPARβ and γ, but not α, are expressed in mouse mast cell. In addition, PPARγ was induced in antigen- or A23187-stimulated mast cells. The expressions of PPARβ and γ were also observed in rat mast cells. However, the role of PPARs in mast cell still remains unknown. To investigate a possible role of PPARs in mast cell, we examined the effects of PPAR ligands on the function of mBMMCs. Mast cell is known as a major cytokine-producing cell at the site of allergic inflammation [2–5].

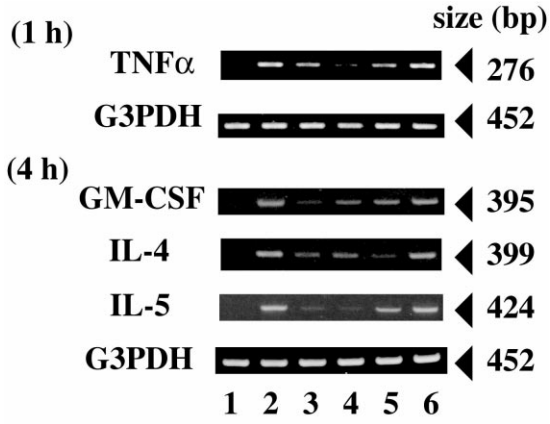

Fig. 3. Effects of PPAR ligands on the expression of cytokine mRNAs. mBMMCs were treated at 37°C for 12 h with 10 μM 15d-PGJ_2 (lane 3), 50 μM troglitazone (lane 4), 10 μM carbaprostacyclin (lane 5), 100 μM bezafibrate (lane 6) or vehicle (lanes 1, 2). These cells were stimulated with antigen (lanes 2–6) for 1 h (TNFα) or 4 h (GM-CSF, IL-4 and IL-5) or not (lane 1), and then analyzed by RT-PCR.

Anti-inflammatory corticosteroid and an immunosuppressive drug cyclosporin A are reported to suppress the production of cytokines not only by lymphocytes, but also by mast cells [25], resulting in a reduced level of allergic inflammation. Recent studies have shown that PPARγ negatively regulates the production of inflammatory cytokines and nitric oxide synthetase by the mechanism of interrupting other transcription factors in human macrophages [17,18]. As shown in this paper, PPARβ and γ ligands suppressed antigen-induced cytokine production by mBMMCs at both protein and mRNA levels, whereas PPARα ligands did not. These results sug-

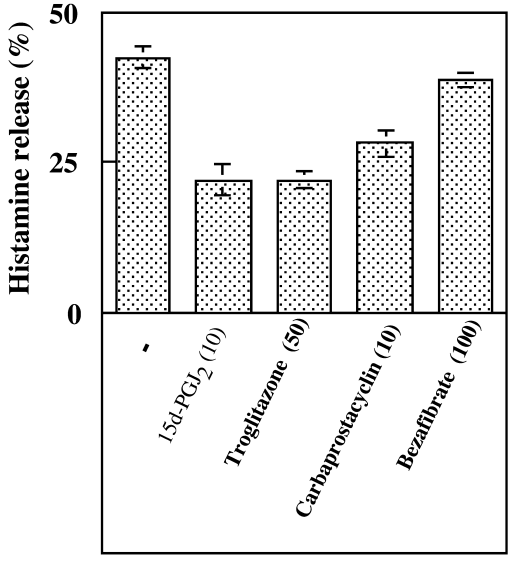

Fig. 4. Effects of PPAR ligands on histamine release from mBMMCs. mBMMCs were sensitized with IgE and treated with drugs as described in the text. Cells were stimulated with 10 ng/ml DNP-BSA at 37°C for 30 min. The concentrations of PPAR ligands are indicated in parentheses (μM). Data are expressed as means ± S.D. of triplicate experiments.

gested that PPARβ and γ might be involved in the negative regulation of cytokine production in mast cell and that a ligand of PPAR might be a candidate of a new therapeutic drug for allergic disorders.

However, it is uncertain whether the action of 15d-PGJ_2 and troglitazone is mediated by PPARγ, because the level of PPARγ expression was very low in the mBMMC used in this study. Nevertheless, a small amount of PPARγ in the mast cell may be enough to mediate the inhibitory action of these ligands. Otherwise another mechanism aside from PPARγ might operate in mast cells. However, no receptor for 15d-PGJ_2 on the cell membrane has not yet been reported [26]. PPARγ is the only receptor that has been reported to mediate the action of 15d-PGJ_2. Furthermore, troglitazone, a specific PPARγ ligand, also attenuated the cytokine production by the mast cell. These evidences suggest that PPARγ contributes to the action of PPAR ligands used here at least partially. Induced expression of PPARγ after the activation of the mast cell likely supports the inhibitory effect of PPARγ ligands. The observation that PPARγ ligands suppressed GM-CSF more effectively than TNFα (Fig. 2) may be due to the induced expression of PPARγ after antigen stimulation, because the induction of GM-CSF occurs later than that of TNFα.

Interestingly, mast cells produce PGD_2 in response to specific antigen [1]. 15d-PGJ_2 is a metabolite of PGD_2, which is rapidly converted to Δ^{12}-PGJ_2 in the culture medium of mBMMC [27]. In addition, these three prostanoids are all PPARγ ligands [24]. These facts raise the possibility that a negative feedback mechanism via PGD_2-PPARγ pathway exists in mast cell. Namely, PGD_2 (or its metabolites of PGJ_2), once produced after the activation of the mast cell, acts on mast cell itself via PPARγ and attenuates the production of inflammatory cytokines. Consequently, it prevents proceeded activation of mast cells and leads to the cessation of inflammatory responses.

It is reported that inhibition of inducible cyclooxygenase-2 (COX-2) resulted in exacerbated inflammation in carrageenin-induced pleurisy in rats [28]. This result indicates the anti-inflammatory property of COX-2, which contribute to the increased production of PGD_2 and 15d-PGJ_2. This report seems to support our hypothesis that PGD_2 and PGJ_2, downstream metabolites of COX-2, have an anti-inflammatory property against mast cells via the PPARγ pathway.

In conclusion, we demonstrated that PPARβ and γ are expressed in mouse mast cells. In addition, we suggested a possible role of PPARs in negative regulation of mast cell activation. Further studies are required to establish the role of PPAR in mast cell, but this finding may bring a new sight into the mechanism of the regulation of mast cell activation.

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