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Ciglitazone inhibits the antigen-induced leukotrienes production independently of PPARγ in RBL-2H3 mast cells

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Abstract

Peroxisome prolifelator-activated receptor γ (PPARγ) is a ligand-activated transcription factor, through which PPARγ agonists have been demonstrated to down-regulate inflammatory cell functions. Recently, the agonists are reported to exert, in some conditions, their inhibitory actions independently of PPARγ. Previously, we showed that a PPARγ agonist, troglitazone, inhibited cycteinyl (Cys)-leukotrienes production in RBL-2H3 cells after IgE receptor triggering. Here we examined whether the inhibition of cycteinyl-leukotrienes production in the cells was dependent on the activation of PPARγ. A PPARγ agonist, ciglitazone, significantly inhibited Cys-leukotrienes, but not prostaglandin D_2, production. The inhibition was not attenuated by the pretreatment with a PPARγ antagonist. Ciglitazone did not alter the mRNA expression of acyl-coenzyme A binding protein, the gene expression of which is up-regulated by PPARγ, nor induce the nucleus translocation of PPARγ. These results suggest that the inhibition by PPARγ agonists of Cys-leukotrienes production in RBL-2H3 cells after IgE receptor triggering is not through the activation of PPARγ.

Keywords: Ciglitazone; Peroxisome prolifelator-activated receptor γ; Cycteinyl-leukotrienes; Mast cell; Independence

1. Introduction

In allergic inflammation in asthmatic airways, mast cells play an essential role by releasing chemical mediators such as histamine, lipid mediators such as cysteinyl (Cys)-leukotrienes, and cytokines in response to specific antigens (Bousquet et al., 2000; Marone et al., 2005). Cys-leukotrienes including leukotriene (LT) C_4 and its metabolites, LTD_4 and LTE_4, which are synthesized from arachidonic acid upon antigen stimulation, have potent biological activities implicated in acute airflow limitation and chronic airway inflammation in allergic asthma. Cys-leukotrienes cause bronchoconstriction, mucus hypersecretion, increased microvascular permeability and bronchial hyper-responsiveness. They are also involved in the pathogenesis of aspirin-induced asthma (Lams and Lee, 1996; Coffey and Peters-Golden, 2003). Therefore, interference with the formation and action of Cys-leukotrienes has a therapeutic efficacy in allergic inflammation, as recommended in a guideline (NHLBI/WHO Workshop report. Global initiative for asthma, global strategy for asthma management and prevention. NIH 02-3659, 2002), for the treatment and management of chronic asthma worldwide.

Peroxisome prolifelator-activated receptors (PPARs), ligand-activated transcription factors, are members of the nuclear receptor superfamily. Three subtypes of PPARs (α, β/δ and γ) have been identified, and are known to play different biological roles in association with the variation in their expression according to the tissue. PPARγ is highly expressed in white and brown adipose tissue, and regulates adipocyte gene expression, cell differentiation and glucose metabolism (Chinetti et al., 2000). Recent studies reported that PPARγ is expressed also in inflammatory cells such as T cells (Harris and Phipps, 2001), monocytes/macrophages (Ricote et al., 1998), eosinophils (Ueki et al., 2003) and

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mast cells (Sugiyama et al., 2000a,b). PPARγ has been shown to mediate anti-inflammatory activities in those inflammatory cells. In monocytes/macrophages, PPARγ activation by endogeneous PPARγ agonists such as 15-deoxy-$\Delta^{12,14}$-prostaglangin J_2 (15d-PGJ_2) and exogeneous PPARγ agonists such as BRL49653, pioglitazone, ciglitazone and troglitazone, developed as insulin-sensitizing thiazolidinediones, inhibited the phorbol ester- and lipopolysaccharide (LPS)-induced production of inflammatory mediators including tumor necrosis factor α (TNF-α), interleukin (IL)-1β, IL-6, IL-12, regulated upon activation normal T cells expressed and secreted (RANTES), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 and gelatinaseB (Colville-Nash et al., 1998; Ricote et al., 1998; Jiang et al., 1998; Subbaramaiah et al., 2001; Alleva et al., 2002). In mast cells, PPARγ agonists attenuated antigen-induced TNF-α and granulocyte-macrophage colony-stimulating factor (GM-CSF) production (Sugiyama et al., 2000a,b). These actions of PPARγ agonists were proven through the PPARγ-dependent repression of activities of the transcription factors, nuclear factor-κB (NF-κB), activator protein-1 (AP-1), signal transducer and activator of transcription 1 (STAT1) and transcription factor Sp1 in monocytes/macrophages (Ricote et al., 1998). On the other hand, recent studies have demonstrated that PPARγ agonists can also function in a receptor-independent manner. For example, 15d-PGJ_2 has been shown to directly inhibit NF-κB-mediated gene expression by covalent modification of the DNA-binding domains of NF-κB in monocytes/macrophages (Castrillo et al., 2000; Chawla et al., 2001). However, the mechanisms by which PPARγ agonists inhibit the inflammatory functions of mast cells are poorly characterized in terms of the dependency on PPARγ. We previously demonstrated that troglitazone suppressed the antigen-induced production of LTB_4 and Cys-leukotrienes in RBL-2H3 mast cells sensitized with antigen specific-IgE. In that study, the inhibition of LTB_4 production was achieved even in the absence of nuclear fraction, suggesting that the agent might exhibit the inhibitory action in a PPARγ-independent manner (Yamashita et al., 2000). However, it has not yet been examined in detail in detail whether the effects of PPARγ agonists on mast cell function are independent of or dependent on PPARγ.

Therefore, we attempted to clarify the involvement of PPARγ in the anti-inflammatory activities of the agonists by evaluating the cellular events following PPARγ activation in antigen-stimulated RBL-2H3 cells.

2. Materials and methods

2.1. Cell culture

Rat basophilic leukemia (subline 2H3) (RBL-2H3) cells and J774A.1 (a mouse monocyte/macrophage cell line) cells were obtained from the Human Science Research Bank (Osaka, Japan). For IgE receptor triggering, RBL-2H3 cells were passively sensitized and stimulated with specific antigen as previously described (Yamashita et al., 2000). Briefly, the cells (5×10^5 cells/ml) were suspended in Eagle's minimum essential medium containing anti dinitrophenol (DNP)-specific mouse monoclonal IgE (Nippon Biotest Laboratories Inc., Tokyo, Japan) (100 ng/ml), and incubated for 20 h. Then the cells were washed and stimulated with DNP-conjugated human serum albumin (DNP-HSA, Sigma Chemical Co., Louis, MI, USA) (50 ng/ml) or medium alone for 30 min. In some experiments, several concentrations of ciglitazone (Sigma), a PPARγ agonist, and GW9662 (2-chloro-5-nitrobenzanilide, Cayman Chemical, Ann Arbor, USA), a PPARγ antagonist, were added to the cell culture 2 and 3 h, respectively, before the antigen stimulation. The agents were also present through the whole period of the antigen stimulation. The agents were dissolved in ethanol so that the final concentration of ethanol was less than 0.2 v/v% in the culture medium. The agent vehicle was used as the control of the agents. J774A.1 cells (5×10^5 cells/ml) were cultured in Dulbecco's minimal essential medium (DMEM; 9.5 g/l) (Nissui Seiyaku, Tokyo, Japan) supplemented with 10 v/v% heat-inactivated fetal bovine serum (ICN, Costa Mesa, USA), 4 mM L(+)-glutamine (Wako, Osaka, Japan), 1.6 mg/ml $NaHCO_3$ (Wako, Osaka, Japan), 100 μg/ml strepomycin sulfate (Meiji Seika, Tokyo, Japan) and 100 U/ml penicillin G potassium (Meiji Seika), in the presence or absence of ciglitazone (30 μM) for the indicated period.

2.2. Determination of the degranulation of RBL-2H3 cells

The degranulation of RBL-2H3 cells was evaluated by β-hexosaminidase release into the culture medium (Yamashita et al., 2000). The total amount of β-hexosaminidase in the sensitized cells before the antigen stimulation (T) and its content of that in the culture supernatants of the sensitized cells 30 min after the stimulation with medium alone (B) or the antigen (A) were measured in triplicate as previously described (Yamashita et al., 2000). The degranulation of RBL-2H3 cells after IgE receptor triggering was expressed as follows: degranulation (%)= $(A-B)\times100/(T-B)$.

2.3. Measurement of LTB_4, LTC_4, LTE_4, and prostaglandin (PG) D_2 released from RBL-2H3 cells

LTB_4, LTC_4, LTE_4 and PGD_2 contents in the culture supernatants of sensitized RBL-2H3 cells 30 min after the antigen stimulation were determined by each enzyme immunoassay (EIA) kit (Cayman Chemical) according to the manufacturer's protocol. The sensitivity of the kit for LTB_4 is 3.9 pg/ml, LTC_4 7.8 pg/ml, LTE_4 7.8 pg/ml and PGD_2 3.9 pg/ml.

Table 1
Primer sequences used for PCR amplification

Gene		Sequences	Product size (bp)
ACBP	sense	5′-AGCCAACTGATGAAGAGATG-3′	260
	antisense	5′-AGGCATTATGTCCTCACAGG-3′	
PPAP	sense	5′-CCGAGAAGGAGAAGCTGTTG-3′	445
	antisense	5′-TTATTCATCAGGGAGGCCAG-3′	
GAPDH	sense	5′-TGATGACATCAAGAAGGTGGTGAAG-3′	249
	antisense	5′-TCCTTGGAGGCCATGTAGGCCAT-3′	

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

Total RNA was extracted from sensitized RBL-2H3 cells after the antigen stimulation in the presence or absence of ciglitazone (30 μM) as described above using a total RNA extraction kit (RNeasy-mini, QIAGEN Inc., Valencia, CA) according to the manufacturer's protocol. cDNA was synthesized from 1 μg of total RNA using random hexamers with GenAmp PCR kit (Perkin-Elmer, Branchburg, Germany). Synthesized cDNA was amplified with the specific primers (Table 1) using Ready-To-Go PCR Beads (Amersham Pharmacia Biotech Inc., Piscataway, NJ). The PCR cycling conditions were as follows: PPARγ, 36 cycles of denaturation at 94 °C for 1 min, annealing at 61 °C for 1.5 min and extension at 72 °C for 1 min, acyl-coenzyme (Co) A-binding protein (ACBP), 24 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 1 min and extension at 72 °C for 1 min, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 24 cycles of denaturation at 94 °C for 1 min, annealing at 67 °C for 1 min and extension at 72 °C for 1 min. The PCR products were visualized by 1.5% agarose gel electrophoresis followed by ethidium bromide staining, and the images were digitalized using software NIH image 1.56 software. Quantification of the mRNA expression levels was determined as previously described (Kitabatake et al., 2004).

2.5. Immunocytochemical analysis

Immunostaining was performed on sensitized RBL-2H3 cells after the antigen stimulation in the presence or absence of ciglitazone (30 μM) as described above, and J774A.1 cells cultured in the presence or absence of ciglitazone (30 μM) for 3 h. The cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (pH 7.4) for 15 min. Immunolocalization of PPARγ protein was detected with anti-human PPARγ polyclonal rabbit IgG (5 μg/ml) or normal rabbit IgG (5 μg/ml) as a control antibody, using biotinylated anti-rabbit IgG goat IgG and alkaline phosphatase-conjugated avidin-biotin complex as previously described (Maeda et al., 1998). PPARγ protein was visualized with the Fast Red Substrate System (DAKO corporation, Carpinteria, USA) according to the manufacturer's protocol.

2.6. Statistical analysis

The data are expressed as mean±S.D. Significant differences between groups were determined using the Mann–Whitney *U*-test. A *P*-value of less than 0.05 was taken as significant.

3. Results

3.1. The effects of ciglitazone on eicosanoid production in RBL-2H3 cells with IgE receptor triggering

RBL-2H3 cells sensitized with antigen-specific IgE secreted LTC_4, LTB_4, LTE_4 and PGD_2 upon stimulation with antigen for 30 min (Table 2). The exposure to ciglitazone significantly reduced antigen-induced LTC_4 production by sensitized RBL-2H3 cells in a dose-dependent manner over the range of 1–30 μM (1 μM; 87.00±10.43% (18.70±2.47 ng/ml), 10 μM; 50.04±8.58% (9.63±0.21 ng/ml), 30 μM; 20.32±5.82% (4.33±0.40 ng/ml)) (Fig. 1A). The suppressive effect of 30 μM of ciglitazone was also observed on LTB_4 (34.93±5.82%) and LTE_4 (21.85±18.83%) production, while the PGD_2 production (105.98±20.24%) was not affected by ciglitazone (Fig. 1B). On the other hand, the treatment with ciglitazone did not affect the degranulation induced by antigen stimulation, as determined by the release of hexosaminidase (44.25±4.41% without and 45.92±3.73% with the treatment) (Fig. 2). GW9662 (0.1 μM), a specific PPARγ antagonist, which has been shown to antagonize PPARγ activation by PPARγ agonists in other types of cells (Huang et al., 1999), did not reverse at all the attenuation of LTC_4 production by ciglitazone (30 μM) (31.97±15.87% with and 25.89±15.18% without GW9662). The antagonist also didn't affect the LTC_4

Table 2
The production of eicosanoids by sensitized RBL-2H3 cells after antigen stimulation

Stimulation	LTB_4	LTC_4	LTE_4	PGD_4
DNP-HAS	3.45±0.16 *	20.50±2.46	15.00±0.76	199.00±10.08
Control	0.02±0.00	0.05±0.01	0.01±0.00	ND

* ng/ml (n=4) ND, not detected.

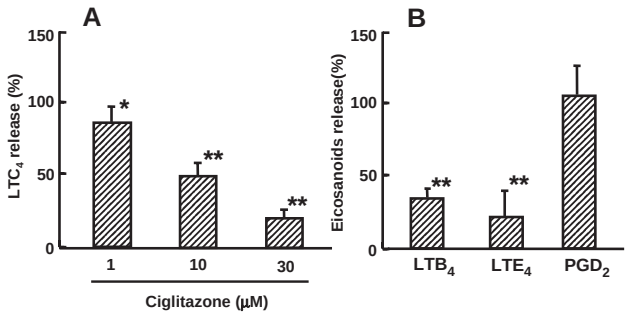

Fig. 1. Effects of ciglitazone on the production of eicosanoids by sensitized RBL-2H3 cells after antigen stimulation. Sensitized RBL-2H3 cells were treated with the indicated concentrations of ciglitazone and stimulated with DNP-HSA (50 ng/ml) as described in Materials and Methods. LTC_4 release in the cells with ciglitazone is shown as percentages of that in the cells without ciglitazone (A). The cells were treated with 30 μM of ciglitazone and stimulated with DNP-HSA (50 ng/ml) as described in Materials and Methods. LTB_4, LTE_4, and PGD_2 release in the cells with ciglitazone is shown as percentages of that in the cells without ciglitazone (B). Data are expressed as mean±S.D. (n=4). *P<0.05, ** P<0.01; compared with the cells without ciglitazone.

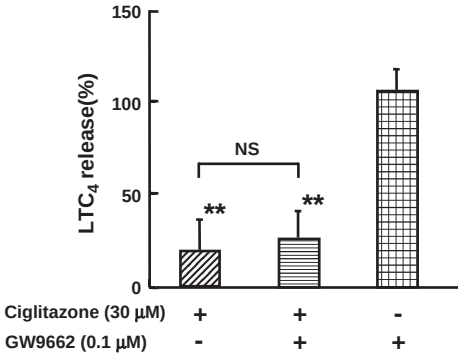

Fig. 3. Influence of GW9662 on the inhibitory effect of ciglitazone on leukotriene C_4 production by sensitized RBL-2H3 cells after antigen stimulation. Sensitized RBL-2H3 cells were preincubated with or without GW9662 (0.1 μM) before the addition of 30 μM of ciglitazone, and stimulated with DNP-HSA (50 ng/ml) as described in Materials and Methods. LTC_4 release in each condition is shown as a percentage of that in the cells without ciglitazone and GW9662. Data are expressed as mean±S.D. (n=4). **P<0.01; compared with the cells without ciglitazone and GW9662. NS; not significant.

production by sensitized RBL-2H3 cells not exposed to ciglitazone (Fig. 3).

3.2. The effect of ciglitazone on ACBP mRNA expression in RBL-2H3 cells

It was reported that ACBP gene is up-regulated by the binding of PPARγ upon activation to PPAR-response element in the promoter region. Furthermore, a PPARγ agonist has been shown to increase the expression of ACBP mRNA both in vivo and in vitro in rat adipocytes (Helledie et al., 2002). In sensitized RBL-2H3 cells, ACBP mRNA was constitutively expressed (data not shown). The stimulation of the sensitized cells with neither antigen nor medium alone led to the change of the mRNA expression. The incubation with ciglitazone, unexpectedly, did not evoke a significant increase in the expression of the ACBP transcript (Fig. 4A, B).

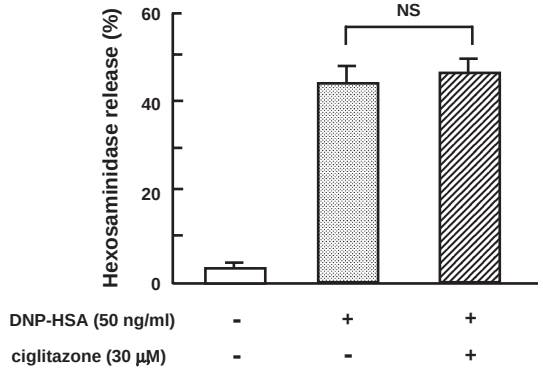

Fig. 2. Effects of ciglitazone on hexosaminidase release from sensitized RBL-2H3 cells after antigen stimulation. Sensitized RBL-2H3 cells were treated with or without 30 μM of ciglitazone, and stimulated with or without DNP-HSA (50 ng/ml) as described in Materials and Methods. Hexosaminidase release was determined as described in Materials and Methods. Data are expressed as mean±S.D. (n=4). NS; not significant.

3.3. The effect of ciglitazone on PPARγ mRNA expression in RBL-2H3 cells

The constitutive expression of PPARγ mRNA was detected in both non-sensitized and sensitized RBL-2H3 cells (data not shown). Neither the control nor antigen stimulation affected the expression in the sensitized cells. The treatment with ciglitazone induced no significant change in the PPARγ mRNA expression (Fig. 4A, C).

3.4. The effect of ciglitazone on the localization of PPARγ protein in RBL-2H3 cells

The immunoreactivity of PPARγ was detected in the cytosol of RBL-2H3 cells and J774A.1 cells without the exposure to ciglitazone by immunocytochemistry. PPARγ once activated by its agonist, translocates into the nucleus to exert its regulatory function in gene expression (Shibuya et al., 2002). After the treatment with ciglitazone, there was an increase of PPARγ immunoreactivity in the nucleus of J774A.1 cells, whereas the immunoreactive protein of PPARγ was not translocated from the cytosol into the nucleus in RBL-2H3 cells (Fig. 5).

4. Discussion

We here demonstrated for the first time that the inhibition by ciglitazone, a PPARγ agonist, of LTB_4 and Cys-leukotrienes release from RBL-2H3 mast cells after IgE receptor triggering was independent of PPARγ, with the following observations. First, the suppressive effect of the

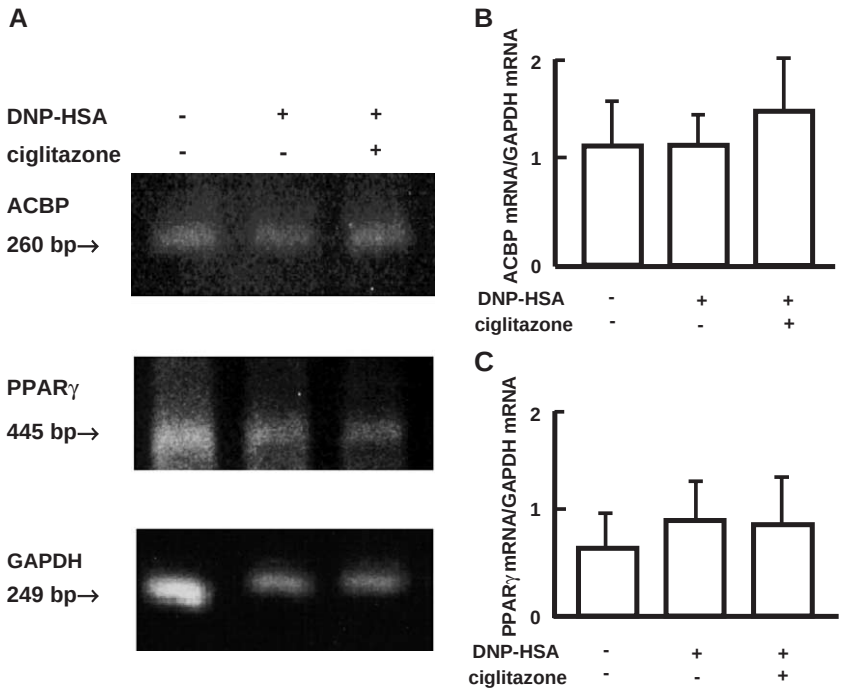

Fig. 4. The effect of ciglitazone on the expression of ACBP and PPARγ mRNA in sensitized RBL-2H3 cells. Sensitized RBL-2H3 cells were treated with or without 30 μM of ciglitazone and stimulated with or without DNP-HSA (50 ng/ml) as described in Materials and Methods. Total RNA was extracted and subjected to RT-PCR with primers for ACBP, PPARγ and GAPDH mRNAs. Representative photos of gel electrophoresis of RT-PCR products are shown in (A). Relative levels of ACBP (B) and PPARγ (C) mRNA were determined as described in Materials and Methods. Data are expressed as mean±S.D. (n=4).

agonist was not reversed pharmacologically by GW9662, a PPARγ antagonist. GW9662 is a potent and selective antagonist of PPARγ with nanomolar IC_{50} versus PPARγ in binding experiments (Leesnitzer et al., 2002). The concentration (0.1 μM) of GW9662 used here was demonstrated, based on the PPARγ-dependent promoter activity, to block the activation of PPARγ by a PPARγ agonist, BRL49653, in murine macrophages (Huang et al., 1999). Second, ciglitazone did not affect the expression of ACBP mRNA. ACBP gene is known as a housekeeping gene that plays a role in the intracellular transport of medium

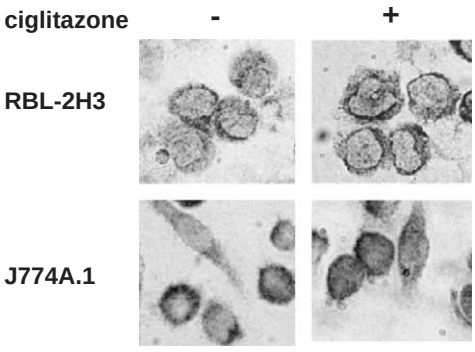

Fig. 5. Immunolocalization of PPARγ in sensitized RBL-2H3 cells and J774A.1 cells. Sensitized RBL-2H3 cells and J774A.1 cells were treated with (+) or without (−) ciglitazone (30 μM). Immunolocalization of PPARγ was detected with anti-mouse PPARγ antibody as described in Materials and Methods. Positive staining was not detected in any cells with the control antibody. (×1000).

to long chain acyl-CoA esters (Kragelund et al., 1999). Helledie et al. reported that ACBP gene is a PPARγ target gene and that activated PPARγ up-regulates the transcription of ACBP through an intronic PPAR-response element. The authors also showed that the exposure to BRL49653 for 6 h increased the expression of ACBP mRNA in rat adipocytes (Helledie et al., 2002). In the current study, the up-regulated expression of ACBP gene was not observed after the exposure to ciglitazone even for 6 h in RBL-2H3 cells (data not shown). Although the type of cells used in the present study is different from that in the study (Helledie et al., 2002), RBL-2H3 cells were originated from rat and were confirmed to express both ACBP mRNA and PPARγ protein in the present study. Therefore, the PPARγ agonist is very likely not to activate PPARγ in RBL-2H3 cells. Third, ciglitazone did not induce the nucleus translocation of PPARγ in RBL-2H3 cells. Activation of the PPARγ pathway starts in the cytosol by the binding of specific ligands to PPARγ, which subsequently translocates into the nucleus. PPARγ has been observed to translocate into the nucleus upon activation by PPARγ agonists in a murine macrophage cell line and rat synoviocytes (Shibuya et al., 2002; Kawahito et al., 2000). We found the translocation of the protein in a murine macrophage cell line, J774A.1 cells, but not in RBL-2H3 cells after stimulation with ciglitazone. Accordingly, our findings concerning the intracellular localization of PPARγ protein, as for ACBP gene transcription, indicate that PPARγ in RBL-2H3 cells was not activated by the agonist. Finally, the inhibition of LTB_4

and Cys-leukotrienes release was not due to the cytotoxicity of the agonist, because the agonist did not affect hexosaminidase degranulation.

PPARγ is mainly expressed in adipose tissue and is important in adipocyte differentiation (Chinetti et al., 2000). Recently, PPARγ has been shown to be expressed in inflammatory cells such as T cells (Harris and Phipps, 2001), monocytes/macrophages (Ricote et al., 1998) and eosinophils (Ueki et al., 2003). With regard to the mast cell/basophil lineage, the expression of PPARγ mRNA and protein has been reported in human cord blood-derived mast cells (Sugiyama et al., 2000a), mouse bone marrow-derived mast cells and rat peritoneal mast cells (RPMC) (Sugiyama et al., 2000b). PPARγ mRNA was detected in human basophilic KU812 cells (Fujimura et al., 2002). We found here that RBL-2H3 cells expressed both PPARγ mRNA and protein. In contrast to our findings in RBL-2H3 cells, the activation of sensitized mouse bone marrow-derived mast cells and human cord blood-derived mast cells with antigen resulted in an increase of the mRNA and protein expression of PPARγ and PPARγ2, respectively (Sugiyama et al., 2000a,b). The regulatory mechanisms of PPARγ expression in RBL-2H3 cells might be different from those in mast cells because of differences in the species or type of cells.

PPARγ has been suggested to have a regulatory function in inflammatory responses based on findings that endogenous and exogenous PPARγ agonists exhibit anti-inflammatory effects on inflammatory cells. 15d-PGJ_2 and troglitazone attenuated the production of GM-CSF, TNF-α, IL-5 and macrophage inflammatory protein-1α in antigen stimulated human cord blood-derived mast cells (Sugiyama et al., 2000a). In mouse bone marrow-derived mast cells, 15d-PGJ_2 and troglitazone inhibited the production of GM-CSF, TNF-α, IL-4 and IL-5 after antigen stimulation (Sugiyama et al., 2000b). 15d-PGJ_2 decreased the high-affinity IgE receptor FcεRI expression in KU812 cells (Fujimura et al., 2002). 15d-PGJ_2 and troglitazone inhibited the antigen-induced degranulation in mouse bone marrow-derived mast cells (Sugiyama et al., 2000b), but not in human cord blood-derived mast cells (Sugiyama et al., 2000a) and RBL-2H3 cells in this study. The different effect of PPARγ agonists on the degranulation suggests the existence of differences in the involvement of PPARγ according to the type of cell. The attenuation of antigen-induced leukotrienes synthesis by troglitazone and ciglitazone was observed also in human cord blood-derived mast cells (Sugiyama et al., 2000a). However, it has not been assessed whether PPARγ is involved in the inhibitory effects of PPARγ agonists on these cellular responses to IgE receptor triggering in mast cells and basophils. Interestingly, Emi and Maeyama reported that 15d-PGJ_2 induced bifunctional effects on RBL-2H3 cells, in which proliferation occurred at lower concentrations and apoptosis was observed at higher concentrations. However, thiazolidinediones didn't evoke any effects on the viability and apoptosis (Emi and Maeyama, 2004). Furthermore, in mouse bone marrow-derived mast cells, the up-regulation by 15d-PGJ_2 of cytokine-dependent PG endoperoxide synthase-2 (COX-2) expression and IL-6 generation is independent of PPARγ, although this is a case of enhancement (Diaz et al., 2002). These reports might support our findings.

The aggregation of FcεRI induces both degranulation and arachidonate metabolism such as PGs and leukotrienes secretion in mast cells and basophils. In the present study, ciglitazone inhibited leukotrienes release, but did not affect PGD_2 release and degranulation in antigen-stimulated RBL-2H3 cells, as in human cord blood-derived mast cells. Therefore, the molecules targeted by PPARγ agonists in RBL-2H3 cells would be in the leukotriene synthesis pathway following the liberation of arachidonic acid by cytoplasmic phopholipase A_2 ($cPLA_2$) (Reischl et al., 1999). Arachidonic acid is converted to leukotrienes via the activation of 5-lipoxygenase (5-LO) and its activating protein, 5-LO activation protein (FLAP). 5-LO is localized to the cytosolic compartment, whereas FLAP is localized to the inner and outer nuclear membrane. When mast cells and basophils are activated, 5-LO translocates to the nuclear envelope where FLAP then mediates the interaction between 5-LO and arachidonic acid yielding both 5(*S*)-hydroperoxyeicosatetraenoic acid and LTA_4 (Werz, 2002). The metabolism of LTA_4 by LTA_4 hydrolase results in the production of the potent chemoattractant LTB_4 (Yokomizo et al., 1995). Alternatively, LTA_4 can be conjugated with glutathione by LTC_4 synthase to produce LTC_4 and its metabolites LTD_4 and LTE_4, collectively referred to as Cys-leukotrienes (Lam, 2003). In our previous report, troglitazone inhibited LTB_4 production by the supernatant fraction of the RBL-2H3 cells lysate, avoiding the influence of FLAP (Yamashita et al., 2000). Accordingly, it is assumed that ciglitazone reduced the release of LTB_4 and Cys-leukotrienes through the suppression of the action of key enzymes for leukotrienes synthesis including 5-LO, LTA_4 hydrolase, and LTC_4 synthase. The suppression of the enzyme action is not likely to be due to the inhibition of the enzyme expression, because the inhibitory effect was achieved by the incubation of the cells with ciglitaozne for only 2 h and, in the previous study with troglitazone, even for only 15 min (Yamashita et al., 2000). PPARγ-independent mechanisms by which endogenous and exogenous PPARγ agonists exert either their inhibitory or enhancing activity have been investigated in other types of cells. 15d-PGJ_2 inhibits NF-κB nuclear translocation via the inhibition of IκB degradation in monocytes/macrophages without PPARγ–PPAR-response element binding (Castrillo et al., 2000; Chawla et al., 2001; Straus et al., 2000). Troglitazone inhibited cyclin D1 expression and cell cycling in cultured mouse skin keratinocytes in which PPARγ was not expressed at functional levels (He et al., 2004). Chen and Harrison reported that the cellular proliferation enhanced by ciglitazone in colon cancer cells was associated with the activation with NF-κB transcriptional activity without PPARγ-PPAR-response element binding (Chen and Harrison, 2005).

The administration of thiazolidinediones has been shown to inhibit the development of asthmatic phenotypes upon antigen inhalation in a murine model of allergic asthma (Trifilieff et al., 2003, Woerly et al., 2003, Honda et al., 2004). However, the target cells for PPARγ agonists within the airways and the mechanisms by which they suppress allergic inflammation are poorly characterized. In addition, the anti-asthmatic effects of ciglitazone were not totally abolished by GW9662 (Woerly et al., 2003). These findings suggest the possibility that the in vivo effects of thiazolidinediones on the asthmatic pathophysiology are mediated, at least in part, by the PPARγ-independent pathway in mast cells, as observed in the current study. Therefore, it would be of value to develop thiazolidinediones as new anti-asthma drugs by investigating further the anti-inflammatory mechanisms of the agents in inflammatory cells such as mast cells and basophils.

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