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Feedback Control of the Arachidonate Cascade in Osteoblastic Cells by 15-deoxy-$\Delta^{12,14}$-Prostaglandin J_2

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Summary **15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) and an anti-diabetic thiazolidinedione, troglitazone (TRO) are peroxisome proliferator-activated receptor (PPAR)-γ ligands, which regulate immuno-inflammatory reactions as well as adipocyte differentiation. We previously reported that 15d-PGJ_2 can suppress interleukin (IL)-1β-induced prostaglandin E_2 (PGE_2) synthesis in synoviocytes of rheumatoid arthritis (RA). IL-1 also stimulates PGE_2 synthesis in osteoblasts by regulation of cyclooxygenase (COX)-2 and regulates osteoclastic bone resorption in various diseases such as RA and osteoporosis. In this study, we investigated the feedback mechanism of the arachidonate cascade in mouse osteoblastic cells, MC3T3-E1 cells, which differentiate into mature osteoblasts. Treatment with 15d-PGJ_2 led to a significant increase in IL-1α-induced COX-2 expression and PGE_2 production in a dose dependent manner. The effect of 15d-PGJ_2 was stronger than that of TRO. However, it did not affect the expression of COX-1. In addition, cell viability of MC3T3-E1 cells was not changed in the condition we established. This means that 15d-PGJ_2 exerts a positive feedback regulation of the arachidonate cascade of PGE_2 in osteoblastic cells. These results may provide important information about the pathogenesis and treatment of bone resorption in a variety of diseases such as RA and osteoporosis.**

Key Words**: 15d-PGJ_2, PPAR-γ, osteoblast, PGE_2, COX-2**

Introduction

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily of ligand activated transcriptional factors that include receptors for steroids, thyroid hormone, vitamin D and retinoic acid [*1*]. Recently three PPAR subtypes, PPAR-α, PPAR-Δ, and PPAR-γ, were identified and their functions have been elucidated [*2*]. PPAR-α is highly expressed in many tissues exhibiting high carbolic rates of fatty acids [*3*, *4*]. PPAR-γ is expressed ubiquitously. PPAR-γ is expressed at high levels in adipose tissue and in parts of the immune system such as the spleen, monocytes, bonemarrow precursors and helper T-cell clones [*5*, *6*]. PPAR-γ relates to many diseases such as inflammation, metabolic diseases (diabetes mellitus and obesity), arteriosclerosis and malignant tumors. PPAR-γ plays a central role in the process of adipocyte differentia-

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tion. This receptor and its heterodimeric partner retinoid X receptor (RXR)-α form a DNA-binding complex that regulates transcription of adipocyte-specific genes [*7*, *8*]. Heterozygous PPAR-γ deficient mice show overexpression and hypersecretion of leptin despite a smaller size of adipocytes and decreased fat mass [*2*, *9*].

PPAR-γ is activated by a range of synthetic and naturally occurring substances, including anti-diabeticthiazolidinediones such as troglitazone (TRO), polyunsaturated fatty acids, prostaglandin D_2 (PGD_2) metabolites, components of oxidized low-density lipoprotein (LDL) and 12/15-lipoxygenase products [*10*, *11*]. We previously demonstrated that immunoreactive PPAR-γ is expressed in macrophages and synoviocytes of rheumatoid arthritis (RA) and its ligands inhibit the growth of synoviocytes *in vitro* through apoptosis [*12*, *13*]. Furthermore, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), which is a PGD_2 metabolite, had 100-fold higher potency in suppressing the chronic inflammation and bone destruction of adjuvant-induced arthritis (AIA) in rats, compared with TRO [*12*]. The suppression of bone destruction in AIA is due to the inhibition of pannus formation, which is thought to be critical for the development of erosive disease and results in irreversible destruction of the cartilage and bone in affected joints. We also previously indicated that 15d-PGJ_2 suppresses interleukin (IL)-1β-induced prostaglandin E_2 (PGE_2) synthesis in RA synoviocytes through the inhibition of cytosolic phospholipase A_2 as well as cyclooxygenase (COX)-2 expression, which are synthetic enzymes for PG. TRO and other prostanoids have no inhibitory effects on PGE_2 synthesis [*13*]. 15d-PGJ_2 may act by a different mechanism from that of TRO in the treatment of RA and negative feedback of the arachidonate cascade regulation by PG in RA synoviocytes may be specific for 15d-PGJ_2.

IL-1 stimulates PGE_2 synthesis in osteoblasts by regulation of COX-2 and regulates osteoclastic bone resorption in various diseases such as RA and osteoporosis [*14*, *15*]. IL-1, especially IL-1α, can promote the differentiation of osteoblast and stimulate the production of various cytokines in osteoblasts [*16*, *17*]. In addition, the function of PPAR-γ in bone metabolism has been clarified from experiments using mesenchymal stem cells, which can differentiate to both osteoblasts and adipocytes. Some studies suggested that the expression of PPAR-γ in mesenchymal stem cells promotes osteoblast differentiation [*18*]. 15d-PGJ_2 is also a direct inhibitor of IκB kinase independent of PPAR-γ, which suggests additional anti-inflammatory effects independent of PPAR-γ [*19*, *20*]. The mechanism of feedback control on the arachidonate cascade is unclear and dependent on the type of cells. In this study, we investigated the feedback mechanism of the arachidonate cascade by 15d-PGJ_2 in mouse osteoblastic cells, MC3T3-E1 cells, which differentiate into mature osteoblasts. Our findings may suggest a novel therapy for inflammatory diseases including RA, and clarify the basic mechanism of the bone metabolism of the mesenchymal osteoblast in inflammatory diseases and aging.

Materials and Methods

Materials

TRO was obtained from Sankyo Co. Ltd. (Tokyo, Japan) and 15d-PGJ_2 was purchased from Cayman Chemical (Ann Arbor, MI). IL-1α was from Genzyme Techne (Minneapolis, MN). Anti-COX-2 antibody and anti-COX-1 antibody were from Santa Cruz Biotechnology Inc (Santa Cruz, CA). Fetal bovine serum (FBS) and penicillin-streptomycin mixture were from BioWhittaker (Walkersville, MD). Phosphate buffered saline (PBS) was obtained from GIBCO BRL (Grand Island, NY).

Cell culture

The MC3T3-E1 mouse preosteoblast cell line [*21*] (Riken Cell Bank, Tsukuba, Japan) was grown in α MEM (Fisher Scientific, Pittsburgh, PA) containing 10% heat-inactivated FBS (Bio Whittaker) supplemented with sodium pyruvate (1 mM), penicillin-streptomycin mixture (100 U/ml) in a humidified 5% CO_2 atmosphere at 37°C. As a pre-incubation for the treatment of reagents, MC3T3-E1 cells were cultured for 24 h with α MEM supplemented with 1% FBS. Then, various concentrations of IL-1α, 15d-PGJ_2 and TRO were added to the cell culture in serum-free α MEM medium for 6 h. Next, Western blot analysis of COX-1 and COX-2, PGE_2 synthesis assay, and cell viability assay were performed.

Western blot analysis of COX-1 and COX-2

IL-1α, 15d-PGJ_2 and TRO were added to MC3T3-E1 cells at concentrations of 5 ng/ml, 0.1, 1 or 10 μM and 0.1, 1 or 10 μM, respectively, in serum-free α MEM medium for 6 h. After incubation, Western blot analysis was performed to determine COX-1 and COX-2 protein expression as described previously [*22*]. Thirty micrograms of each total protein extract from MC3T3-E1 cells was analyzed on blots incubated for 1 h at room temperature with 1:200 dilution of goat anti-COX-2 antibody (Santa Cruz), and washed, followed by incubation for 1 h at room temperature with horseradish peroxidase-linked rabbit anti-goat IgG (1:1500 dilution; EY Laboratories Inc., San Mateo, CA). After being washed again, blots were analyzed using an Amersham enhanced-chemiluminescence (ECL) system (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom), and exposed to Hyperfilm (Amersham Pharmacia Biotech) for 30 s to 5 min, resulting in adequate exposure to visualize the bands.

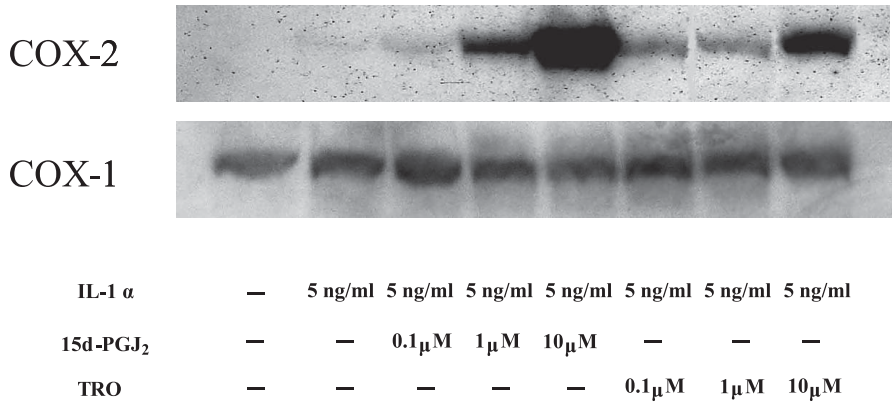

Fig. 1. COX-1 and COX-2 expression in TRO and 15d-PGJ$_2$ treated MC3T3-E1 cells. IL-1α (5 ng/ml)-stimulated MC3T3-E1 cells were incubated with TRO (0.1–10 μM), and 15d-PGJ$_2$ (0.1–10 μM) for 6 h. COX-1 and COX-2 expression in MC3T3-E1 cells were analyzed by Western blotting as described in Materials and Methods. Treatment with 10 μM of 15d-PGJ$_2$ increased IL-1α-induced COX-2 expression more than that with 10 μM of TRO. Three separate experiments were performed.

PGE$_2$ synthesis assay

MC3T3-E1 cells were seeded in 24-well tissue culture plates at a density of 3×10^4 cells/well. 15d-PGJ$_2$ and TRO were added to duplicate wells at concentrations of 0.1, 1 or 10 μM and 10 μM, respectively, with 5 ng/ml of IL-1α in a total volume of 500 μl serum-free α MEM medium for 6 h. The cells were washed three times with PBS, and then incubated in α MEM medium for a further 1 h period. After incubation, the conditioned media from the wells were collected to measure the PGE$_2$ concentration immediately. The concentration of PGE$_2$ was measured with the PGE$_2$ monoclonal enzyme immunoassay (EIA) kit (assay designs, Inc. Ann Arbor MI) according to the manufacturer's instructions.

Cell viability assay

To evaluate the effect of 15d-PGJ$_2$ on cell growth, MTT assay was performed. MC3T3-E1 cells were seeded in 96-well tissue culture plates at a density of 5.0×10^3 cells/well. MC3T3-E1 cells in duplicated wells were treated with 1 or 5 μM of IL-1α, 10 μM of TRO with 5 ng/ml of IL-1α, and 1, 5, or 10 μM of 15d-PGJ$_2$ with 5 μM of IL-1α, in a total volume of 200 μl serum-free α MEM medium for 24 h. Cell viability was measured colorimetrically using the Cell Counting Kit (Dojindo Chemical, Kumamoto, Japan) and a microplate reader (Model 550, Bio-Rad Labs., Hercules, CA) at the test wavelength of 450 nm and the reference wavelength of 650 nm. This assay is based on cleavage of the 2-(2-methoxy-4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt by mitochondrial dehydrogenase in viable cells.

Statistical analysis

The number of experiments analyzed is indicated in the corresponding figure legend. Statistical differences between the mean values (presented with SD) were determined by one-way analysis of variance followed by the Student's *t* test.

Results

15d-PGJ$_2$ increased expression of COX-2 in MC3T3-E1 cells

15d-PGJ$_2$ suppresses IL-1β-induced PGE$_2$ synthesis in RA synoviocytes. IL-1 stimulates PGE$_2$ synthesis in osteoblasts by regulation of COX-2 and regulates osteoclastic bone resorption in various diseases such as RA and osteoporosis. To investigate the effect of 15d-PGJ$_2$ on IL-1α-induced COX expression in MC3T3-E1 cells, we used Western blot analysis. COX-1 protein expression was identical under any treatment. COX-2 protein was expressed in IL-1α (5 ng/m)-stimulated cells. Treatment with 0.1–10 μM of 15d-PGJ$_2$ led to a significant increase of IL-1α-induced COX-2 protein in a dose dependent manner. 10 μM of TRO also increased IL-1α-induced COX-2 protein, to less than that of 10 μM of 15d-PGJ$_2$ (Fig. 1). These data suggest that 15d-PGJ$_2$ can increase the expression of COX-2 in MC3T3-E1 cells and that this effect of 15d-PGJ$_2$ was stronger than that of the PPAR-γ ligand, TRO.

15d-PGJ$_2$ increased PGE$_2$ synthesis in MC3T3-E1 cells

Next we analyzed PGE$_2$ synthesis mediated by 15d-PGJ$_2$ or TRO, in IL-1α (5 ng/ml) - stimulated MC3T3-E1 cells. Treatment with 15d-PGJ$_2$ led to an increase in IL-1α-induced PGE$_2$ synthesis in a dose-dependent manner, while 10 μM of TRO weakly affected IL-1α-induced PGE$_2$ synthesis (Fig. 2).

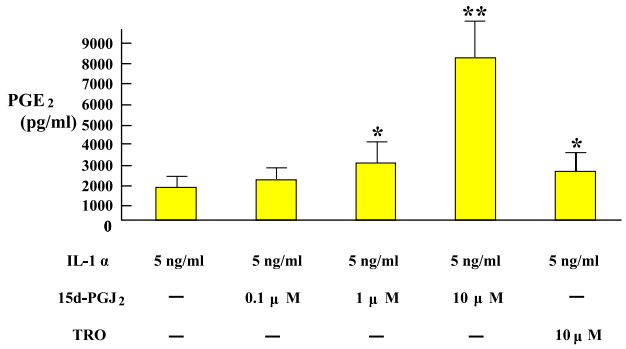

Fig. 2. PGE$_2$ synthesis in TRO and 15d-PGJ$_2$ treated MC3T3-E1cells. MC3T3-E1 cells were incubated with the indicated concentrations of 15d-PGJ$_2$ (0.1–10 μM), TRO (10 μM), with IL-1α (5 ng/ml) for 6 h, and further incubated in α MEM medium for 1 h. Next, PGE$_2$ concentrations of the supernatants were quantified by enzyme immunoassay (EIA). Values are expressed as means ± SD of three experiments with duplicate samples. *Significant difference from control IL-1α (p<0.05). **Significant difference from control IL-1α (p<0.01).

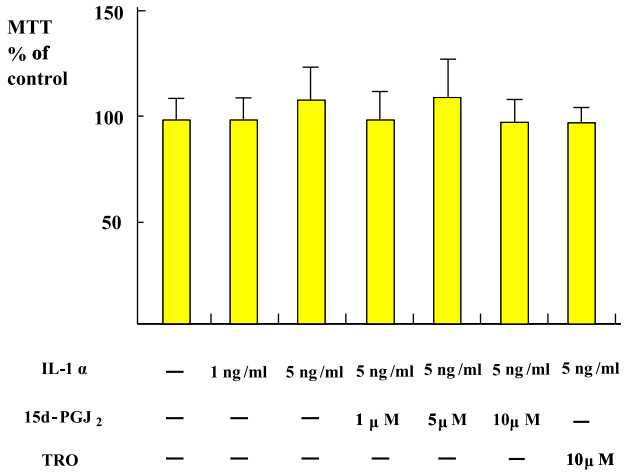

Fig. 3. Cell viability of MC3T3-E1 cells by TRO, 15d-PGJ$_2$ and IL-1α. The viability of MC3T3-E1 cells treated with IL-1α (1, 5 ng/ml), TRO (10 μM), and 15d-PGJ$_2$ (1, 5, 10 μM) for 6 h was measured with an MTT assay and expressed as a percentage of the control. PPAR-γ ligands did not change the viability of MC3T3-E1 cells. Data represent mean ± SD of three experiments with duplicate samples.

Cell viability of MC3T3E-1 cells by PPAR-γ ligands and IL-1α

To investigate the effect of PPAR-γ ligands and IL-1α on MC3T3E-1 cells, we analyzed cell viability *in vitro* by a modified MTT assay. MC3T3E-1 cells were treated with IL-1α, TRO plus IL-1α and 15d-PGJ$_2$ plus IL-1α. Cell counting clearly indicated that neither PPAR-γ ligands nor IL-1α changed the viability of MC3T3E-1 cells (Fig. 3).

Discussion

We examined the production of PGE$_2$ and the expression of COX-1 and COX-2 in IL-1α-stimulated MC3T3-E1 cells *in vitro*. Cell viability of MC3T3-E1 cells was not changed in the condition we established. Treatment with 15d-PGJ$_2$ led to a significant increase in IL-1α-induced COX-2 expression and PGE$_2$ production in a dose dependent manner. In addition, this effect was stronger than that of TRO. However, it did not affect the expression of COX-1. These results show that 15d-PGJ$_2$ exerts a positive feedback regulation on the arachidonate cascade of PGE$_2$ in osteoblastic cells.

PGE$_2$ is produced in most internal organs including bone and exerts various actions [*23*]. PGE$_2$ in bone tissue is produced mainly by osteoblasts. COX is classified as constitutively expressed COX-1 and COX-2, which is induced by stress such as inflammation. In mouse osteoblasts, COX-2 is induced by stimulation of IL-1 or IL-6, and these cytokines can induce bone resorption [*24*]. Therefore it is thought that production of PGE$_2$ is related to inflammatory bone resorption dependent on COX-2. PGE$_2$ acts on osteoblasts and induces a receptor activator of NFκB ligand (RANKL). Furthermore, PGE$_2$ is also an important factor for this synovial proliferation, a tumor-like structure called pannus, invades and erodes the cartilage and the subchondral bone [*25*]. On the other hand, bone formation by PGE$_2$ *in vivo* has been reported in rat, dog, and man, but is thought to be weaker compared with its action of bone resorption. On pre-osteoblastic MC3T3-E1 cells, low-dose PGE$_2$ inhibited cell proliferation and increased alkaline phosphatase activity, a marker of osteoblast differentiation. In contrast, high-dose PGE$_2$ promoted cell proliferation, and decreased alkaline phosphatase activity [*26*]. PGE$_2$ tends to be related with bone resorption, but it's dependent on various conditions.

We previously reported that IL-1β-increased COX-2 expression on RA synoviocytes, and 15d-PGJ$_2$ inhibited COX-2 expression in a dose dependent manner by negative feedback regulation [*13*]. In addition, this effect of 15d-PGJ$_2$ was stronger than that of TRO, which is also a ligand of PPAR-γ. In the current study, we administered 15d-PGJ$_2$ and TRO to IL-1α-stimulated MC3T3-E1 cells. Contrary to our expectation, 15d-PGJ$_2$ and TRO induced more COX-2 expression and PGE$_2$ production on MC3T3-E1 cells. This effect of 15d-PGJ$_2$ in PGE$_2$ synthesis was stronger than that of TRO. Moreover, the number of MC3T3-E1 cells was not changed in this condition, suggesting that COX-2 received positive feedback from 15d-PGJ$_2$ on MC3T3-E1 cells

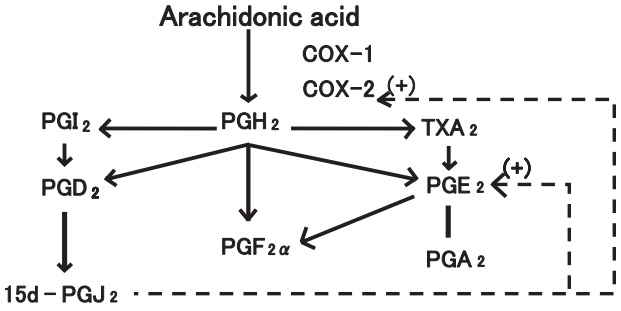

Fig. 4. Positive feedback control of the arachidonate cascade by 15d-PGJ_2 in MC3T3-E1 cells. COX catalyzes the oxidative conversion of arachidonic acid to PGH_2, the first step in the conversion of arachidonic acid to other PGs including 15d-PGJ_2. In MC3T3-E1 cells, 15d-PGJ_2 promotes PGE_2 synthesis via the activation of COX-2 expression.

(Fig. 4). The finding that the effect on COX-2 production by 15d-PGJ_2 was stronger than that by TRO may be due to other mechanisms independent of PPAR-γ. For example, 15d-PGJ_2 binds with SRC-1,-2, and P300 which are co-activators of PGJ_2, whereas TRO is not [*27*]. In addition, 15d-PGJ_2 goes through cell surface receptors unrelated to PPAR-γ and has an anti-inflammatory effect. On MCF-7 breast cancer cells, 15d-PGJ_2 has an anti-inflammatory effect mediated by inhibition of COX-2 through a cell membrane receptor restraining IκB kinase, which is not related to PPAR-γ. Moreover osteoblasts may have different mechanisms through receptors except PPAR-γ in synoviocytes, but they have not yet been fully elucidated.

In this study 15d-PGJ_2 and TRO enhanced COX-2 expression of IL-1α-stimulated MC3T3-E1 cells. IL-1 is a potent inducer of bone resorption. Induction of PGE_2 production through COX-2 overexpression of osteoblasts might stimulate bone resorption, and it was thought to be one of the various effects of PPAR-γ ligands. In addition, 15d-PGJ_2 also suppressed the differentiation of osteoclast *in vitro* [*28*]. Bone resorption is greater than bone formation in osteoporosis, so bone mineral density is decreased. Thus, 15d-PGJ_2 is an effective treatment reagent for bone resorption in inflammatory diseases such as RA because of its anti-inflammatory effects. However the response between COX-2 and 15d-PGJ_2 may be different in other bone resorption diseases. Our results including the feedback regulation of the arachidonate cascade in osteoblasts provide important information about the pathogenesis and treatment of bone resorption in a variety of diseases.

Acknowledgment

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Abbreviations

15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; IL, interleukin; PGE_2, prostaglandin E_2; TRO, troglitazone; PPAR, peroxisome proliferator-activated receptor; RA, rheumatoid arthritis; RXR, retinoid X receptor; PGD_2, prostaglandin D_2; LDL, Low-density lipoprotein; AIA, adjuvant-induced arthritis; COX, cyclooxygenase; PBS, Phosphate Buffered Saline; FBS, Fetal bovine serum; MEM, Minimum Essential Medium; ECL, enhanced-chemiluminescence; EIA, enzyme immunoassay; RANKL, receptor activator of NFκB ligand; TXA, thromboxane A.

References

[1] Issemann, I. and Green, S.: Activation of a member of the steroid hormone receptor superfamily by Peroxisome proliferator. *Nature*, **347**, 645–650, 1990.

[2] Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R.M.: The nuclear receptor superfamily: the second decade. *Cell*, **83**, 835–839, 1995.

[3] Kliewer, S.A., Forman, B.M., Blumberg, B., Ong, E.S., Borgmeyer, U., Mangelsdorf, D.J., Umesono, K., and Evans, R.M.: Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci.*, **91**, 7355–7359, 1994.

[4] Braissant, O., Foufelle, F., Scotto, C., Dauca, M., and Wahli, W.: Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology*, **137**, 354–366, 1996.

[5] Kliewer, S.A., Umesono, K., Noonan, D.J., Heyman, R.A., and Evans, R.M.: Convergence of 9-*cis* retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. *Nature*, **358**, 771–774, 1992.

[6] Daynes, R.A. and Jones, D.C.: Emerging roles of PPARs in inflammation and immunity. *Nat. Rev. Immunol.*, **2**, 748–759, 2002.

[7] Schmidt, A., Endo, N., Rutledge, S.J., Vogel, R., Shinar, D., and Rodan, G.A.: Identification of a new member of the steroid hormone receptor superfamily that is activated by peroxisome proliferator and fatty acid. *Mol. Endocrinol.*, **6**, 1634–1641, 1992.

[8] Tontonoz, P., Singer, S., Forman, B.M., Sarraf, P., Fletcher, J.A., Fletcher, C.D., Brun, R.P., Mueller, E., Altiok, S., Oppenheim, H., Evans, R.M., and Spiegelman, B.M.: Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor γ and the retinoid X receptor. *Proc. Natl. Acad. Sci. USA*, **94**, 237–241, 1997.

[9] Kubota, N., Terauchi, Y., Miki, H., Tamemoto, H., Yamauchi, T., Komeda, K., Satoh, S., Nakano, R., Ishii, C., Sugiyama, T., Eto, K., Tsubamoto, Y., Okuno, A., Murakami, K., Sekihara, H., Hasegawa, G., Naito, M., Toyoshima, Y.,

Tanaka, S., Shiota, K., Kitamura, T., Fujita, T., Ezaki, O., Aizawa, S., and Kadowaki, T.: PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol. Cell*, **4**, 597–609, 1999.

[10] Vamecq, J. and Latruffe, N.: Medical significance of peroxisome proliferator-activated receptors. *Lancet*, **354**, 141–148, 1999.

[11] Huang, J.T., Welch, J.S., Ricote, M., Binder, C.J., Willson, T.M., Kelly, C., Witztum, J.L., Funk, C.D., Conrad, D., and Glass, C.K.: Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase. *Nature*, **400**, 378–382, 1999.

[12] Kawahito, Y., Kondo, M., Tsubouchi, Y., Hashiramoto, A., Bishop-Bailey, D., Inoue, K., Kohno, M., Yamada, R., Hla, T., and Sano, H.: 15-deoxy-delta (12, 14)-PGJ (2) induces synoviocyte apoptosis and suppresses adjuvant-induced arthritis in rats. *J. Clin. Invest.*, **106**, 189–197, 2000.

[13] Tsubouchi, Y., Kawahito, Y., Kohno, M., Inoue, K., Hla, T., and Sano, H.: Feedback Control of the Arachidonate Cascade in Rheumatoid Synoviocytes by 15-deoxy-delta (12, 14)-PGJ (2). *Biochem. Biophys. Res. Commun.*, **284**, 750–755, 2001.

[14] Dayer, J.M., de Rochemonteix, B., Burrus, B., Demczuk, S., and Dinarello, C.A.: Human recombinant interleukin 1 stimulates collagenase and prostaglandin E_2 production by human synovial cells. *J. Clin. Invest.*, **77**, 645–648, 1986.

[15] Yoshida, T., Horiuchi, T., Sakamoto, H., Inoue, H., Takayanagi, H., Nishikawa, T., Yamamoto, S., and Koshihara, Y.: Production of parathyroid hormone-related peptide by synovial fibroblasts in human osteoarthritis. *FEBS Lett.*, **433**, 331–334, 1998.

[16] Miwa, M., Kozawa, O., Tokuda, H., and Uematsu, T.: Mitogen-activated protein (MAP) kinases are involved in interleukin-1 (IL-1)-induced IL-6 synthesis in osteoblasts: modulation not of p38 MAP kinase, but of p42/p44 MAP kinase by IL-1-activated protein kinase C. *Endocrinology*, **140**, 5120–5125, 1999.

[17] Yang, S., Takahashi, N., Yamashita, T., Sato, N., Takahashi, M., Mogi, M., Uematsu, T., Kobayashi, Y., Nakamichi, Y., Takeda, K., Akira, S., Takada, H., Udagawa, N., and Furusawa, K.: Muramyl dipeptide enhances osteoclast formation induced by lipopolysaccharide, IL-1 alpha, and TNF-alpha through nucleotide-binding oligomerization domain 2-mediated signaling in osteoblasts. *J. Immunol.*, **175**, 1956–1964, 2005.

[18] Jackson, S.M. and Demer, L.L.: Peroxisome proliferator-activated receptor activators modulate the osteoblastic maturation of MC3T3-E1 preosteoblasts. *FEBS Lett.*, **471**, 119–124, 2000.

[19] Rossi, A., Kapahi, P., Natoli, G., Takahashi, T., Chen, Y., Karin, M., and Santoro, M.G.: Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase. *Nature*, **403**, 103–118, 2000.

[20] Straus, D.S., Pascual, G., Li, M., Welch, J.S., Ricote, M., Hsiang, C.H., Sengchanthalangsy, L.L., Ghosh, G., and Glass, C.K.: 15-deoxy-delta 12,14-prostaglandin J_2 inhibits multiple steps in the NF-kappa B signaling pathway. *Proc. Natl. Acad. Sci. USA*, **97**, 4844–4849, 2000.

[21] Kodama, H., Amgoai, Y., Sudo, H., and Hamamoto, S.: Establishment of a clonal osteogenic cell line from new born mouse calvaria. *Japan J. Oral. Biol.*, **23**, 899–901, 1999.

[22] Yamada, R., Sano, H., Hla, T., Hashiramoto, A., Ukui, W., Miyazaki, S., Kohno, M., Tsubouchi, Y., Kusaka, Y., and Kondo, M.: Auranofin inhibits interleukin-1beta-induced transcript of cyclooxygenase-2 on cultured human synoviocytes. *Eur. J. Pharmacol.*, **385**, 71–79, 1999.

[23] Narumiya, S., Sugimoto, Y., and Ushikubi, F.: Prostanoid receptors: structures, properties, and functions. *Physiol. Rev.*, **79**, 1193–1226, 1999.

[24] Tai, H., Miyaura, C., Pilbeam, C.C., Tamura, T., Ohsugi, Y., Koishihara, Y., Kubodera, N., Kawaguchi, H., Raisz, L.G., and Suda, T.: Transcriptional induction of cyclooxygenase-2 in osteoblasts is involved in interleukin-6-induced osteoclast formation. *Endocrinology*, **138**, 2372–2379, 1997.

[25] Firestein, G.S.: Evolving concepts of rheumatoid arthritis. *Nature*, **423**, 356–361, 2003.

[26] Hakeda, Y., Ikeda, E., Kurihara, N., Nakatani, Y., Maeda, N., and Kumegawa, M.: Induction of osteoblastic cell differentiation by forskolin. Stimulation of cyclic AMP production and alkaline phosphatase activity. *Biochim. Biophys. Acta*, **838**, 49–53, 1985.

[27] Wang, C., Fu, M., D'Amico, M., Albanese, C., Zhou, J.N., Brownlee, M., Lisanti, M.P., Chatterjee, V.K., Lazar, M.A., and Pestell, R.G.: Inhibition of cellular proliferation through IkappaB kinase-independent and peroxisome proliferator-activated receptor gamma-dependent repression of cyclin D1. *Mol. Cell Biol.*, **21**, 3057–3070, 2001.

[28] Lin, T.H., Yang, R.S., Tang, C.H., Lin, C.P., and Fu, W.M.: PPARgamma inhibits osteogenesis via the down-regulation of the expression of COX-2 and iNOS in rats. *Bone*, available on line Jul 4, 2007.