Feedback Control of the Arachidonate Cascade in Osteoblastic Cells by 15-deoxy-\(\Delta^{12,14}\)-Prostaglandin J\(\text{\textsubscript{2}}\)

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Summary 15-deoxy-\(\Delta^{12,14}\)-prostaglandin J\(\text{\textsubscript{2}}\) (15d-PGJ\(\text{\textsubscript{2}}\)) and an anti-diabetic thiazolidinedione, troglitazone (TRO) are peroxisome proliferator-activated receptor (PPAR)-\(\gamma\) ligands, which regulate immuno-inflammatory reactions as well as adipocyte differentiation. We previously reported that 15d-PGJ\(\text{\textsubscript{2}}\) can suppress interleukin (IL)-1\(\beta\)-induced prostaglandin E\(\text{\textsubscript{2}}\) (PGE\(\text{\textsubscript{2}}\)) synthesis in synoviocytes of rheumatoid arthritis (RA). IL-1 also stimulates PGE\(\text{\textsubscript{2}}\) 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\(\text{\textsubscript{2}}\) led to a significant increase in IL-1\(\alpha\)-induced COX-2 expression and PGE\(\text{\textsubscript{2}}\) production in a dose dependent manner. The effect of 15d-PGJ\(\text{\textsubscript{2}}\) 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\(\text{\textsubscript{2}}\) exerts a positive feedback regulation of the arachidonate cascade of PGE\(\text{\textsubscript{2}}\) 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\(\text{\textsubscript{2}}\), PPAR-\(\gamma\), osteoblast, PGE\(\text{\textsubscript{2}}\), 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-\(\alpha\), PPAR-\(\Delta\), and PPAR-\(\gamma\), were identified and their functions have been elucidated [2]. PPAR-\(\alpha\) is highly expressed in many tissues exhibiting high carboxyl rates of fatty acids [3, 4]. PPAR-\(\gamma\) is expressed ubiquitously. PPAR-\(\gamma\) 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-\(\gamma\) relates to many diseases such as inflammation, metabolic diseases (diabetes mellitus and obesity), arteriosclerosis and malignant tumors. PPAR-\(\gamma\) plays a central role in the process of adipocyte differentia-
fatty acids, prostaglandin D 

PPAR-γ is activated by a range of synthetic and naturally occurring substances, including anti-diabetic thiazolidinediones 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-Δ$^{12,14}$-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 IkB 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.
H. Ishino et al.

**PGE2 synthesis assay**

MC3T3-E1 cells were seeded in 24-well tissue culture plates at a density of $3 \times 10^4$ cells/well. 15d-PGJ2 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 αMEM medium for 6 h. The cells were washed three times with PBS, and then incubated in αMEM medium for a further 1 h period. After incubation, the conditioned media from the wells were collected to measure the PGE2 concentration immediately. The concentration of PGE2 was measured with the PGE2 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-PGJ2 on cell growth, MTT assay was performed. MC3T3-E1 cells were seeded in 96-well tissue culture plates at a density of $5.0 \times 10^3$ 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-PGJ2 with 5 µM of IL-1α, in a total volume of 200 µl serum-free αMEM 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-PGJ2 increased expression of COX-2 in MC3T3-E1 cells**

15d-PGJ2 suppresses IL-1β-induced PGE2 synthesis in RA synoviocytes. IL-1 stimulates PGE2 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-PGJ2 on IL-1α-induced COX expression in MC3T3-E1 cells, we used Western blot analysis. COX-2 protein expression was identical under any treatment. COX-2 protein was expressed in IL-1α (5 ng/ml)-stimulated cells. Treatment with 0.1–10 µM of 15d-PGJ2 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-PGJ2 (Fig. 1). These data suggest that 15d-PGJ2 can increase the expression of COX-2 in MC3T3-E1 cells and that this effect of 15d-PGJ2 was stronger than that of the PPAR-γ ligand, TRO.

**15d-PGJ2 increased PGE2 synthesis in MC3T3-E1 cells**

Next we analyzed PGE2 synthesis mediated by 15d-PGJ2 or TRO, in IL-1α (5 ng/ml) - stimulated MC3T3-E1 cells. Treatment with 15d-PGJ2 led to an increase in IL-1α-induced PGE2 synthesis in a dose-dependent manner, while 10 µM of TRO weakly affected IL-1α-induced PGE2 synthesis (Fig. 2).
Controls the Arachidonate Cascade in MC3T3-E1 Cells

To investigate the effect of PPAR-γ ligands and IL-1α on MC3T3-E1 cells, we analyzed cell viability in vitro by a modified MTT assay. MC3T3-E1 cells were treated with IL-1α, TRO plus IL-1α and 15d-PGJ2 plus IL-1α. Cell counting clearly indicated that neither PPAR-γ ligands nor IL-1α changed the viability of MC3T3-E1 cells (Fig. 3).

Discussion

We examined the production of PGE2 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-PGJ2 led to a significant increase in IL-1α-induced COX-2 expression and PGE2 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-PGJ2 exerts a positive feedback regulation on the arachidonate cascade of PGE2 in osteoblastic cells.

PGE2 is produced in most internal organs including bone and exerts various actions [23]. PGE2 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 PGE2 is related to inflammatory bone resorption dependent on COX-2. PGE2 acts on osteoblasts and induces a receptor activator of NFκB ligand (RANKL). Furthermore, PGE2 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 PGE2 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 PGE2 inhibited cell proliferation and increased alkaline phosphatase activity, a marker of osteoblast differentiation. In contrast, high-dose PGE2 promoted cell proliferation, and decreased alkaline phosphatase activity [26]. PGE2 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-PGJ2 inhibited COX-2 expression in a dose dependent manner by negative feedback regulation [13]. In addition, this effect of 15d-PGJ2 was stronger than that of TRO, which is also a ligand of PPAR-γ. In the current study, we administered 15d-PGJ2 and TRO to IL-1α-stimulated MC3T3-E1 cells. Contrary to our expectation, 15d-PGJ2 and TRO induced more COX-2 expression and PGE2 production on MC3T3-E1 cells. This effect of 15d-PGJ2 in PGE2 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-PGJ2 on MC3T3-E1 cells.
cytes, but they have not yet been fully elucidated.

Mechanisms through receptors except PPAR-α are related to PPAR-γ in breast cancer cells, 15d-PGJ₂ and prostaglandin E₂ (PGE₂) are inducers of bone resorption. Induction of PGE₂ is mediated by inhibition of COX-2 through a cell surface receptor restraining IkB 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-PGJ₂ and TRO enhanced COX-2 expression of IL-1α-stimulated MC3T3-E1 cells. IL-1α is a potent inducer of bone resorption. Induction of PGE₂ 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-PGJ₂ 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-PGJ₂ 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-PGJ₂ 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.

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Abbreviations

15d-PGJ₂, 15-deoxy-Δ12,14-prostaglandin J₂; IL, interleukin; PGE₂, prostaglandin E₂; TRO, troglitazone; PPAR, peroxisome proliferator-activated receptor; RA, rheumatoid arthritis; RXR, retinoid X receptor; PGD₂, prostaglandin D₂; 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-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. Endocrinology, 137, 354–366, 1996.

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

[3] Kliwer, S.A., Forman, B.M., Blumberg, B., Nong, E.S., Borgmeyer, U., Mangelsdorf, D.J., Umesono, K., 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.

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

[5] Schmidt, A., Endo, N., Rutledge, S.I., 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.

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

[7] 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., et al. J. Clin. Biochem. Nutr.
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., Witzum, 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-prostaglandin J2 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 the Arachidonate Cascade in Rheumatoid Synoviocytes by 15-deoxy-delta(12,14)-PGJ2. *Biochem. Biophys. Res. Commun.*, **284**, 750–755, 2001.

[14] Dayer, J.M., de Rochemonteix, B., Burres, B., Demczuk, S., and Dinarello, C.A.: Human recombinant interleukin 1 stimulates collagenase and prostaglandin E2 production by human synovial cells. *J. Clin. Invest.*, **77**, 645–648, 1986.

[15] Yoshida, T., Horiiuchi, 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] Strauss, 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 J2 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.: Aurano in inhibits interleukin-1beta-induced transcript of cyclooxygenase-2 on cultured human synovioocytes. *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., Oh sugi, 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 Kume gawa, 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., Alb anese, 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 online Jul 4, 2007.