INTRODUCTION: It has been demonstrated that vascular endothelial growth factor (VEGF) and its receptors (VEGFR-1 and 2) are expressed in human osteoarthritis (OA) chondrocytes, and that treatment of cultured OA chondrocytes with VEGF enhances production of matrix metalloproteinase (MMP)-1 and MMP-3 but not tissue inhibitor of metalloproteinase (TIMP)-1 or -2 [1], suggesting that VEGF and its receptors play an important role in destruction of cartilage in OA. It has also been reported that peroxisome proliferator-activated receptor (PPAR)-γ is expressed in rat and human chondrocytes [2], that ox-LDL is internalized by scavenger receptors of macrophages resulting in PPAR-γ activation [3], and that ox-LDL upregulates VEGF expression in macrophages and endothelial cells through PPAR-γ activation [4,5].

Recently, Kakinuma et al. [6] and Akagi et al. [7] reported association of ox-LDL and LOX-1 expression in chondrocytes from RA and OA articular cartilage, respectively. They also showed that ox-LDL could penetrate the cartilage matrix and associate with LOX-1, enhancing MMP-3 production from explant cultures of articular cartilage. These data suggest that the binding ox-LDL to LOX-1 may modulate cartilage degradation in RA and OA patients. The purpose of this study is to investigate whether the oxidized low-density lipoprotein (ox-LDL) binding to lectin-like ox-LDL receptor-1 (LOX-1) upregulates vascular endothelial growth factor (VEGF) expression in cultured bovine articular chondrocytes (BACs). Furthermore, to examine whether the upregulation of VEGF expression is mediated by activation of peroxisome proliferator-activated receptor (PPAR)-γ.

METHODS: After the addition of ox-LDL, the time- and dose-dependency of VEGF mRNA expression and protein release by cultured BACs were investigated by reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA), respectively. BACs were also preincubated with anti-LOX-1 mAb. Nuclear extracts were prepared from BACs treated with 50 µg/ml ox-LDL, 50 µg/ml ox-LDL preincubated with 40 µg/ml anti-LOX-1 mAb, or 20 µg/ml 9- and 13-hydroxy-(S)-10,12-oxidodecanedioic acid (9- and 13-HODE, respectively, known as components of ox-LDL), and 20 µg/ml 15-deoxy-A12,14-prostaglandin J2 (PGJ2) or a PPAR-γ antagonist (20 µg/ml GW9662) to cultured BACs.

RESULTS: Ox-LDL markedly increased VEGF mRNA expression and protein release in a time- and dose-dependent manner, which was significantly suppressed by preincubation with anti-LOX-1 mAb (JTX20) (Fig.). Activation of PPAR-γ was evident in BACs after 6 h incubation with ox-LDL and was attenuated by anti-LOX-1 mAb, 9- and 13-HODE, respectively, known as components of ox-LDL, and 20 µg/ml 15-deoxy-A12,14-prostaglandin J2 (PGJ2) or a PPAR-γ antagonist (20 µg/ml GW9662) to cultured BACs.

DISCUSSION: Recently, many lines of evidence have shown that PPAR-γ regulates the expression of several genes important in not only lipid and glucose homeostasis, but also in inflammatory responses, cellular proliferation and differentiation, and apoptosis [8]. PPAR-γ activation results in the inhibition of various inflammatory events such as the production of IL-1β, TNF-α, and IL-6 in monocytes/macrophages [9]. In addition, Fahmi et al. reported an antiinflammatory role for PPAR-γ agonists, which can suppress the expression of iNOS and MMP-13 in human chondrocytes as well as the expression of MMP-1 in human synovial fibroblasts [10]. More recently, Kobayashi et al. reported protective effects of the PPAR-γ agonist pioglitazone on the progression of experimental OA in guinea pigs [11]. However, the data in this study indicate that the natural ligand for PPAR-γ, PGJ2, and the endogenous ligands 9- and 13-HODE (lipid components of ox-LDL) activated PPAR-γ and upregulated VEGF expression in both the mRNA and protein level. Furthermore, GW9662, a synthetic PPAR-γ specific antagonist, significantly suppressed ox-LDL-induced VEGF upregulation. From these findings, it is likely that PPAR-γ activation can cause the induction of VEGF expression in BACs as well as in macrophages and endothelial cells. Interestingly, Shan et al. reported that PGJ2 induces chondrocyte apoptosis through PPAR-γ activation, which is dependent on the states of NF-κB and the MAPK pathway member p38 kinase [12]. Thus, the effects of PPAR-γ ligands on articular chondrocyte gene expression are dependent on the activation state of the cell. These context-dependent effects of PPAR-γ activation may help to explain why ox-LDL-exposed chondrocytes in OA cartilage show chronic rather than fulminant catabolic reactions when the chondrocytes express LOX-1.

Our data suggested that the ox-LDL/LOX-1 system upregulated VEGF expression in BACs through, at least in part, activation of PPAR-γ. These observations may support the hypothesis that the ox-LDL/LOX-1 system is involved in cartilage matrix degradation [6,7].