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# 15-Deoxy-$\Delta^{12,14}$-$PGJ_2$, but not troglitazone, modulates IL-1β effects in human chondrocytes by inhibiting NF-κB and AP-1 activation pathways

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**Abstract The activation of peroxisome proliferator-activated receptor γ (PPARγ) has been shown to inhibit the production and the effects of proinflammatory cytokines. Since interleukin-1β (IL-1β) directly mediates cartilage degradation in osteoarthritis, we investigated the capability of PPARγ ligands to modulate IL-1β effects on human chondrocytes. RT-PCR and Western blot analysis revealed that PPARγ expression was decreased by IL-1β. 15-Deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ (15d-$PGJ_2$), in contrast to troglitazone, was highly potent to counteract IL-1β-induced cyclooxygenase-2 and inductible nitric oxide synthase expression, NO production and the decrease in proteoglycan synthesis. Western blot and gel-shift analyses demonstrated that 15d-$PGJ_2$ inhibited NF-κB activation, while troglitazone was ineffective. Although 15d-$PGJ_2$ attenuated activator protein-1 binding on the DNA, it potentiated c-jun migration in the nucleus. The absence or the low effect of troglitazone suggests that 15d-$PGJ_2$ action in human chondrocytes is mainly PPARγ-independent.** 

*Key words:* Peroxisome proliferator-activated receptor; Human chondrocyte; Interleukin-1β; 15-Deoxy-$\Delta^{12,14}$-$PGJ_2$; Nuclear factor-κB; Activator protein-1

## 1. Introduction

Progressive destruction of articular cartilage is a common feature of rheumatoid arthritis and osteoarthritis. It is largely accepted that interleukin-1 (IL-1) initiates a number of events which lead to the inhibition of the synthesis of the extracellular matrix components and promotes their degradation [1–3]. Thus, cellular responses to IL-1 within the joint may constitute potential targets for disease intervention.

Peroxisome proliferator-activated receptor-γ (PPARγ) belongs to the superfamily of nuclear receptors which are ligand-activated transcription factors. They regulate gene expression by binding with their heterodimeric partner, retinoid X receptor to specific response elements located in the promoter of target genes. These genes are mainly involved in lipid metabolism, glucose homeostasis regulation and in cell differentiation [4–6]. Besides these functions, this nuclear receptor has been shown to play an important role in the control of inflammatory processes. The possibility to modulate cell activation by the use of PPARγ ligands has been demonstrated in many cell types, including aortic smooth muscle cells [7], monocytes and macrophages [8–10], astrocytes and glial cells [11,12], T lymphocytes [13], vascular endothelial cells [14] or in animal models [15]. However, to date, this receptor has been poorly studied in cartilage.

The modulation of the effects of IL-1β is of great interest for the protection of cartilage in arthritic diseases. We recently described the presence of PPARα and PPARγ in rat cartilage, and we found that 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ (15d-$PGJ_2$), a PPARγ ligand, modulated the IL-1β-induced decrease in proteoglycan (PG) synthesis [16].

The aim of the present work was to investigate the possibility to counteract IL-1β effects in human chondrocyte cultures by the use of PPARγ ligands, and to study the inhibitory effects on the activator protein-1 (AP-1) and nuclear factor-κB (NF-κB) transcriptional pathways. Controversy exists about the identification of intracellular targets involved in the mechanism of action of cyclopentenone, and especially of 15d-$PGJ_2$ [17]. Some of its effects have been shown to be through the transcriptional inhibition exerted by activated PPARγ [8–10]. However, other works, which take into account the lack of effect of synthetic PPARγ ligands, suggest a main contribution of PPARγ-independent mechanisms on the anti-inflammatory effect of 15d-$PGJ_2$ [11,17–19]. No data is available concerning the mechanism of action of cyclopentenone prostaglandins in cartilage cells.

In the present study, we demonstrate that 15d-$PGJ_2$ was much more potent than troglitazone to counteract the decrease in PG synthesis and the expression of inductible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) induced by IL-1β. We also found that this prostaglandin exerts its effects at least partly by antagonizing the activation of AP-1, and especially NF-κB pathways. These results, and especially the much lower effect of troglitazone, strongly suggest

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*Abbreviations:* IL-1, interleukin-1; PPAR, peroxisome proliferator-activated receptor; 15d-$PGJ_2$, 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$; iNOS, inductible nitric oxide synthase; COX-2, cyclooxygenase-2; AP-1, activator protein-1; NF-κB, nuclear factor-κB; FCS, fetal calf serum; EMSA, electrophoretic mobility shift assay

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that the main effects of 15d-$PGJ_2$ in human chondrocytes are PPARγ-independent.

## 2. Materials and methods

### 2.1. Chondrocyte isolation and culture

Articular cartilage was obtained after routine joint surgery from informated donors who had undergone hip replacement due to fracture. Cartilage specimens were examined thoroughly, both macroscopically and microscopically. Only those with neither lesions nor alterations were further processed. Slices of cartilage were aseptically dissected and chondrocytes were obtained by sequential digestions with Pronase and collagenase B (Roche Molecular Biochemicals, Meylan, France) as previously described [20]. Cells were cultured to confluence at 37°C in a humidified atmosphere containing 5% $CO_2$, in complete medium [Dulbecco's modified Eagle's medium/Ham's F12 supplemented with L-glutamine (2 mM), gentamicin (50 μg $ml^{-1}$) and 10% fetal calf serum (FCS)]. All experiments were performed with primary or first passage chondrocytes to avoid any problems of de-differentiation. For the PG synthesis studies, chondrocytes were also encapsulated in alginate beads (1.2%, w/v) (from *Macrocystis pyrifera*, Sigma, St. Quentin Fallavier, France) and cultured in three-dimensional matrix according to the method of Haüselmann [21] and modified by us [22].

### 2.2. Recombinant human IL-1β (rhIL-1β) treatments in the presence of specific ligands

Chondrocyte cultures were treated with rhIL-1β in the range of 0–250 U $ml^{-1}$ for 14 h (PPARγ mRNA analysis) or with 50 U $ml^{-1}$ IL-1β for 6 h (iNOS and COX-2 mRNA analysis). The ligands or the vehicle (0.1% $Me_2SO$ in final concentration) were added to cells 4 h before rhIL-1β. The ligands tested were troglitazone (10 μM) and 15d-$PGJ_2$ (10 μM) (France-Biochem, Meudon, France). At 1 μM, both ligands were ineffective, and at 100 μM, they were highly toxic for the cells.

### 2.3. RNA extraction and quantitative RT-PCR analysis

Total RNA was extracted from cell cultures by a single-step guanidinium thiocyanate–phenol chloroform method using Trizol reagent (Gibco BRL, Cergy-Pontoise, France). PPARγ, iNOS and COX-2 mRNA from human chondrocyte cultures were assayed using a quantitative multistandard RT-PCR method that takes advantage of both interest gene and β-actin sequence conservation between human and rat [23]. This method allowed us to normalize the amount of the gene mRNA to be measured with respect to that of β-actin mRNA. For each sample, human RNA was mixed with a constant amount of total RNA prepared from rat chondrocyte cultures, which brought both competitive rat β-actin and interest gene sequences, and thus acted as a multistandard source. PCRs for each gene amplification were undertaken with oligonucleotide primers that are able to hybridize with human and rat sequences with the same efficiency. For PPARγ amplification, the direct primer extended from nucleotides 841 to 866 and the reverse primer from nucleotides 1364 to 1339, in accordance with the human sequence (GenBank accession number U79012). For iNOS amplification, the direct primer extended from nucleotides 1489 to 1512 and the reverse primer from nucleotides 1921 to 1897, in accordance with the human sequence (GenBank accession number L09210). For COX-2 amplification, the direct primer extended from nucleotides 410 to 432 and the reverse primer from nucleotides 850 to 830, in accordance with the human sequence (GenBank accession number M90100).

Each amplification product was then distinguished by restriction site polymorphism between the two species. The bands were quantitated after electrophoresis and analysis of ethidium bromide-stained gels. Results are expressed as the ratio $(gene_{human}/gene_{rat}) \times (\beta\text{-}actin_{rat}/\beta\text{-}actin_{human})$ in arbitrary units.

### 2.4. Protein analysis

Immunoblotting analysis of PPARγ was performed on human chondrocytes treated or not with rhIL-1β at 250 U $ml^{-1}$ for 14 h. Proteins were isolated from cells in lysis buffer (10 mM HEPES, 400 mM KCl, 1.5 mM $MgCl_2$, 0.5% Igepal) containing a protease-inhibitor mixture (Roche). Cell lysates were centrifuged, and the supernatants were collected and subjected to Western blot analysis using an anti-PPARγ antibody [24]. Immunoblotting analysis of p65 and c-jun were performed on nuclear extracts of cells stimulated with IL-1β (50 U $ml^{-1}$) for 1 h after a pre-incubation of 4 h with the ligand or $Me_2SO$ (0.1%). Nuclear extracts were obtained as described for electrophoretic mobility shift assay (EMSA) experiments (see below). For immunocytochemical analysis, chondrocyte layers were fixed in 3% formaldehyde in phosphate-buffered saline (PBS) for 10 min and permeabilized in methanol for 20 min at 4°C. They were then exposed to the primary anti-PPARγ antibody (diluted 1:2000) for 30 min at 37°C. Cells were washed twice and incubated with the secondary fluorescein-conjugated antibody (diluted 1:100 in PBS) for 30 min at 37°C. Cells were then mounted in Vectashield medium and photographed with a Polyvar microscope (Reichert-Jung, Vienna, Austria).

### 2.5. PG synthesis and quantification of nitrites

After 8 days of culture, chondrocytes cultured in alginate beads were treated as described before. The culture medium was then collected to measure the concentration of nitrites according to the method of Griess [25] using $NaNO_2$ as standard. The beads were incubated in medium containing only 1% FCS with 10 μCi $ml^{-1}$ radiolabeled sodium sulfate ($Na_2^{35}SO_4$) for 4 h at 37°C. After six washes with 0.15 M NaCl, the alginate beads were solubilized in Soluene 350 overnight, and the $^{35}SO_4$-labeled PG content was measured by liquid scintillation with a LKB 1214 counter (Wallac, France).

### 2.6. Preparation of nuclear extracts and EMSA

For EMSA experiments, nuclear proteins were isolated as described before [26], after few modifications. Briefly, cells were scraped in a lysis buffer [10 mM HEPES, pH 7.9, 10 mM KCl, 1 mM dithiothreitol (DTT)] containing a protease-inhibitor cocktail and 0.5% Igepal, and were incubated for 15 min on ice. Nuclei were collected by centrifugation at 2000×*g* for 5 min at 4°C and resuspended in 50 μl of the same buffer without Igepal and KCl, but with 420 mM NaCl. After a 30-min incubation on ice, nuclear debris were removed by centrifugation at 13 000×*g* for 10 min at 4°C, and the supernatants were collected and stored at −80°C before use.

The DNA sequences of the double-stranded oligonucleotide specific of NF-κB were 5′-GAT CCA GTT GAG GGG ACT TTC CCA GGC G-3′ and 5′-GAT CCG CCT GGG AAA GTC CCC TCA ACT G-3′. Those specific to AP-1 were 5′-GAT CCG CTT GAT GAC TCA GCC GGA AG-3′ and 5′-GAT CCT TCC GGC TGA GTC ATC AAG CG-3′. Complementary strands were annealed and double-stranded oligonucleotides were labeled with [$^{32}$P]dCTP using the Klenow fragment of DNA polymerase (Gibco BRL, Cergy-Pontoise, France). Five micrograms of nuclear proteins were incubated for 10 min at 4°C in a binding buffer (20 mM Tris–HCl, pH 7.9, 5 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM EDTA and 20% glycerol) in the presence of 2 μg of poly(dI-dC). The extracts were then incubated for 30 min at 4°C with 10 000 cpm of $^{32}$P-labeled NF-κB or AP-1 probes. The samples were loaded on a 5% native polyacrylamide gel, and run in 0.5× TBE buffer. NF-κB- and AP-1- specific bands were confirmed by competition with a 100-fold excess of the respective unlabeled probe, which resulted in no shifted band. For supershift experiments, after the addition of the labelled probe, the extracts were incubated for 30 min at 4°C in the presence of the specific antibody (anti-p65 or anti-p50 for NF-κB; anti-c-fos or anti-c-jun for AP-1).

## 3. Results

### 3.1. PPARγ mRNA and protein expression in human chondrocytes is decreased by IL-1β

RT-PCR followed by digestion with a specific restriction enzyme led to products of the expected size (524 bp for human uncut product, 291+208+24 bp for rat digested products; Fig. 1A). These results, as well as those of the Western blot analysis (Fig. 1B), indicate that PPARγ is constitutively expressed in human chondrocytes at both the mRNA and the protein levels. An immunocytochemical analysis was further performed on normal chondrocyte primary cultures in order to investigate the intracellular localization of the transcription factor. Fluorescence signals specific of PPARγ were mainly observed in nuclei (Fig. 1C), which is in accordance with their

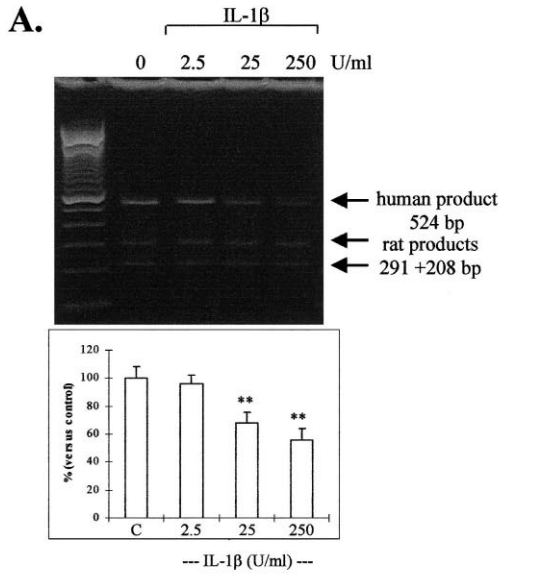


Fig. 1. PPARγ is constitutively expressed in human chondrocyte cultures and is down-regulated by IL-1β treatment. A: Electrophoretic profile of RT-PCR products for PPARγ after specific amplification of mRNA from a mixture of human and rat cells, which brought both competitive rat β-actin and PPARγ sequences. Top: RT-PCR products for PPARγ. mRNA was extracted from chondrocyte cultures treated or not with increased concentrations of rhIL-1β. The amplification products were distinguished by restriction site polymorphism between the two species. Bottom: PPARγ transcripts were quantified as described in Section 2 using the β-actin from the same cDNA pools as an internal control. B: Top: Western blot analysis of PPARγ protein expression in cultured chondrocytes treated or not with IL-1β (250 U $ml^{-1}$) for 14 h. Bottom: The intensity of the bands was determined by densitometric analysis and fixed at 100% for the control. This result is the mean of three independent experiments. C: Intracellular localization of PPARγ in cultured chondrocytes. Immunocytochemical analysis of PPARγ expression in human osteoarthritis chondrocyte cultures was performed using polyclonal anti-PPARγ antibody. PPARγ protein is mainly observed in nuclei, but not in nucleoles, with a low cytoplasmic localization (**$P < 0.05$).

site of action. No signal was detected in nucleoles. A low cytoplasmic localization was also observed.

Because IL-1β has a key role in the events leading to cartilage damage, we have investigated its effects on the expression of PPARγ mRNA and protein. Chondrocyte cultures were treated with IL-1β in the range of 0–250 U $ml^{-1}$ for 14 h. Fig. 1A shows that PPARγ mRNA expression was significantly decreased in a dose-dependent manner. A Western blot analysis furthermore showed that the mRNA level encoding PPARγ upon treatment with IL-1β (250 U $ml^{-1}$) could be related to the variation of the corresponding protein (Fig. 1B).

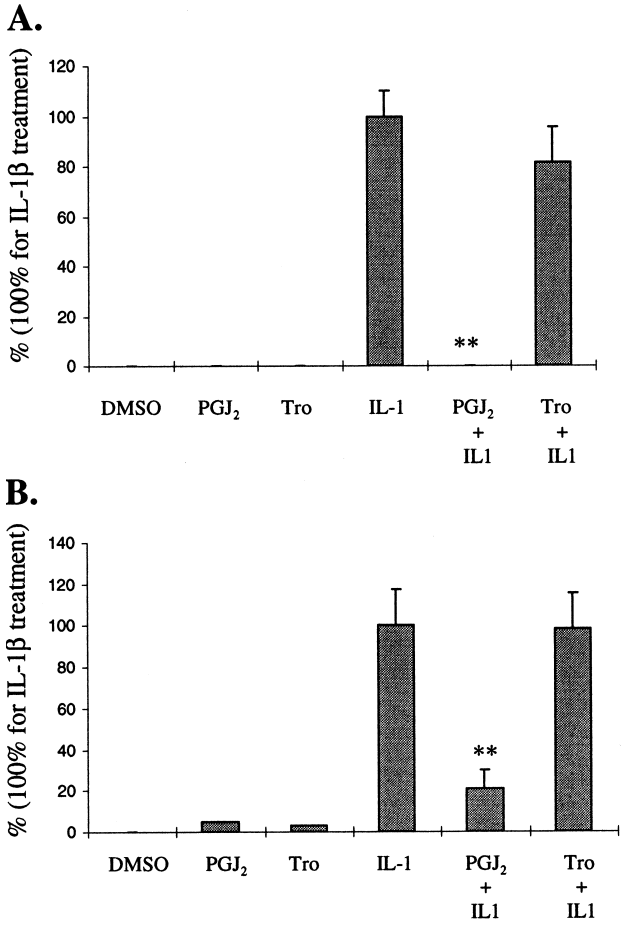


Fig. 2. Effect of troglitazone and 15d-$PGJ_2$ on IL-1β-induced iNOS (A) and COX-2 (B) mRNA expression. Human chondrocytes were cultured in 1% FCS-containing medium for 4 h, then in the presence of the ligands (troglitazone or 15d-$PGJ_2$) before being stimulated with 50 U $ml^{-1}$ IL-1β for 6 h. Total RNA was immediately extracted for RT-PCR experiments. Results are expressed as relative arbitrary units with a value of 100 for the IL-1β treatment. Results represent mean values ± S.D. ($n = 5$; **$P < 0.05$).

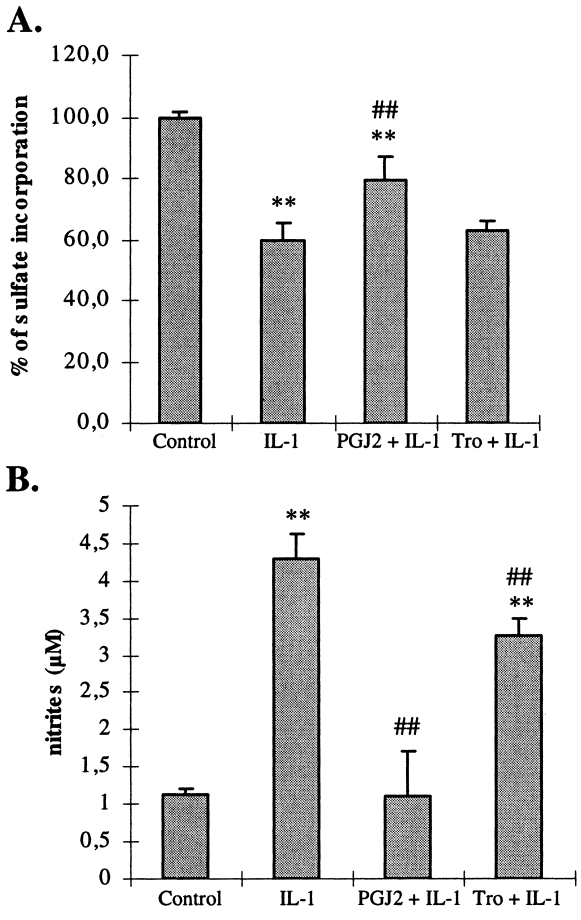


Fig. 3. Effect of PPARγ ligands on IL-1β-induced decrease in PG synthesis (A) and NO release (B) in human chondrocytes. Chondrocytes were cultured in alginate beads at a concentration of $5\times10^6$ cells $ml^{-1}$. Beads were cultured in 1% FCS-containing medium for 12 h before the treatments. Cultures were incubated in the presence of the ligands 4 h before the addition of IL-1β (50 U $ml^{-1}$) in the medium, and the chondrocytes were stimulated for 24 h. PG synthesis was assessed by incorporation of radiolabeled sodium sulfate for 4 h at 37°C. NO production was determined spectrophotometrically according to the Griess method. Results represent mean values ± S.D. ($n=5$; $^{**}P<0.05$ versus control; $P<0.05$ versus IL-1).

### 3.2. *Effects of PPARγ ligands on IL-1β-induced iNOS and COX-2 mRNA expression*

We further investigated the capability of PPARγ ligands to modulate the effects of IL-1β on human chondrocyte cultures. IL-1β is well known to induce the expression of inflammatory enzymes, such as iNOS and COX-2, and the associated production of metabolites, such as NO and prostaglandin-E2. To this end, chondrocyte cultures were treated with IL-1β (50 U $ml^{-1}$) for 6 h in the presence or absence of ligands and mRNA expression for iNOS and COX-2 was assessed. IL-1β induced a strong expression of both iNOS and COX-2 mRNA, and we found that 15d-$PGJ_2$ at 10 μM was highly efficient to inhibit the mRNA expression of both enzymes. After 4 h of pre-incubation, it completely abolished iNOS mRNA expression (Fig. 2A) and led to a 65% decrease in COX-2 mRNA (Fig. 2B). In contrast to 15d-$PGJ_2$, troglitazone, at 10 μM, was much less effective because it only led to a 18% reduction in IL-1β-induced iNOS mRNA expression and did not modulate COX-2 mRNA. No effect was observed with 1 μM troglitazone, whereas 100 μM troglitazone was highly toxic for the cells, as shown by a strong increase in

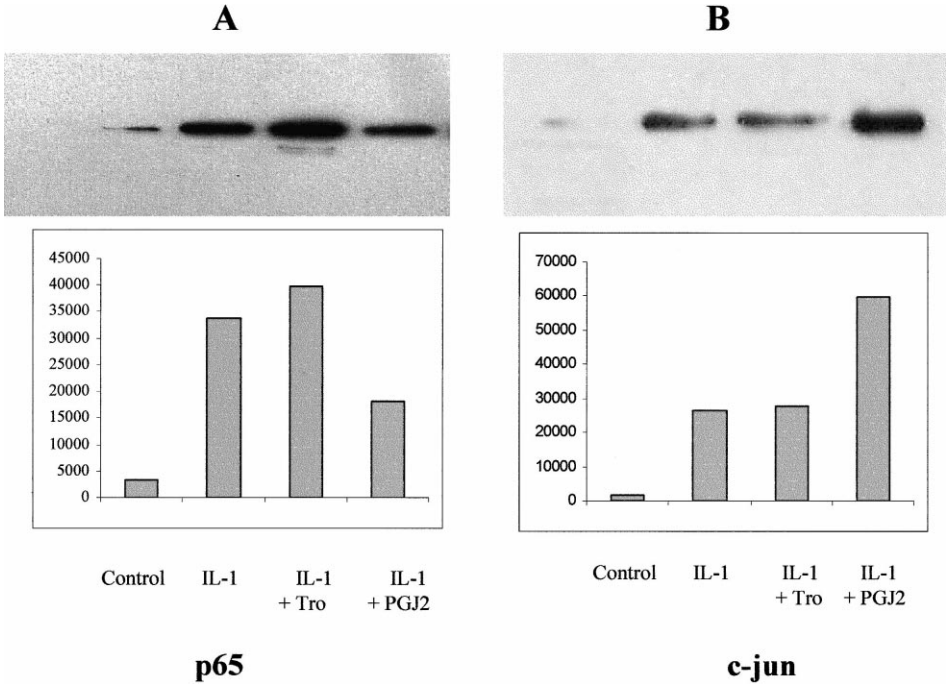


Fig. 4. Effect of troglitazone and 15d-$PGJ_2$ on IL-1β-induced translocation of p65 and c-jun in the nucleus. Top: Western blot analysis of p65 (A) and c-jun (B) in chondrocyte nuclei after treatment of chondrocyte cultures with IL-1β (50 U $ml^{-1}$) in the presence or absence of PPARγ ligands. Nuclear proteins were extracted and immunoblot analysis was performed with specific antibodies. Bottom: the intensity of the bands was determined by densitometric analysis. Quantifications are the result of three independent experiments.

the extracellular lactate dehydrogenase activity (data not shown).

### 3.3. Effects of PPARγ ligands on IL-1β-induced PGs synthesis decrease and NO production

The chondroprotective potentiality of PPARγ ligands was thereafter tested on chondrocytes cultured in alginate beads by measuring the PGs synthesis and the NO production. Treatment of chondrocytes with IL-1β (50 U $ml^{-1}$) for 24 h induced a 40% inhibition of radiolabeled sulfate incorporation (Fig. 3A) and a significant increase in nitrite production (Fig. 3B). When chondrocyte cultures were pretreated with 10 μM 15d-$PGJ_2$, a 50% restoration of the PGs synthesis was observed, whereas no effect was obtained with troglitazone at 10 μM (Fig. 3A). Moreover, 15d-$PGJ_2$ completely abolished the IL-1β-induced NO production, which is coherent with its effects on iNOS mRNA, whereas troglitazone led to a weaker inhibition of NO release (−34%; $P < 0.05$ versus IL-1, Fig. 3B).

### 3.4. 15d-$PGJ_2$ inhibits both NF-κB and AP-1 activation pathways in IL-1β-treated chondrocytes

NF-κB and AP-1 are two important IL-1β-inducible transcription factors that drive the expression of many genes involved in inflammation or in cartilage degradation, such as iNOS, COX-2, IL-1β, tumor necrosis factor-α and metalloproteinases. To test if PPARγ ligands could antagonize NF-κB or AP-1 activation pathways, chondrocytes were pre-incubated for 4 h in the presence of 10 μM 15d-$PGJ_2$ or 10 μM troglitazone before being stimulated with IL-1β (50 U $ml^{-1}$) for 1 h. Western blot and gel-shift analyses were performed on nuclear extracts to visualize the translocation of p65 and c-jun proteins in the nucleus and the binding of NF-κB and AP-1 on the DNA, respectively. Fig. 4A shows that 15d-$PGJ_2$ led to a strong decrease in IL-1β-induced p65 translocation in the nucleus (−48%). In contrast, 15d-$PGJ_2$ was shown to potentiate the effects of IL-1β on the translocation of c-jun in the nucleus (+114%) (Fig. 4B). For both proteins, no effect was observed after a pre-incubation with troglitazone at 10 μM.

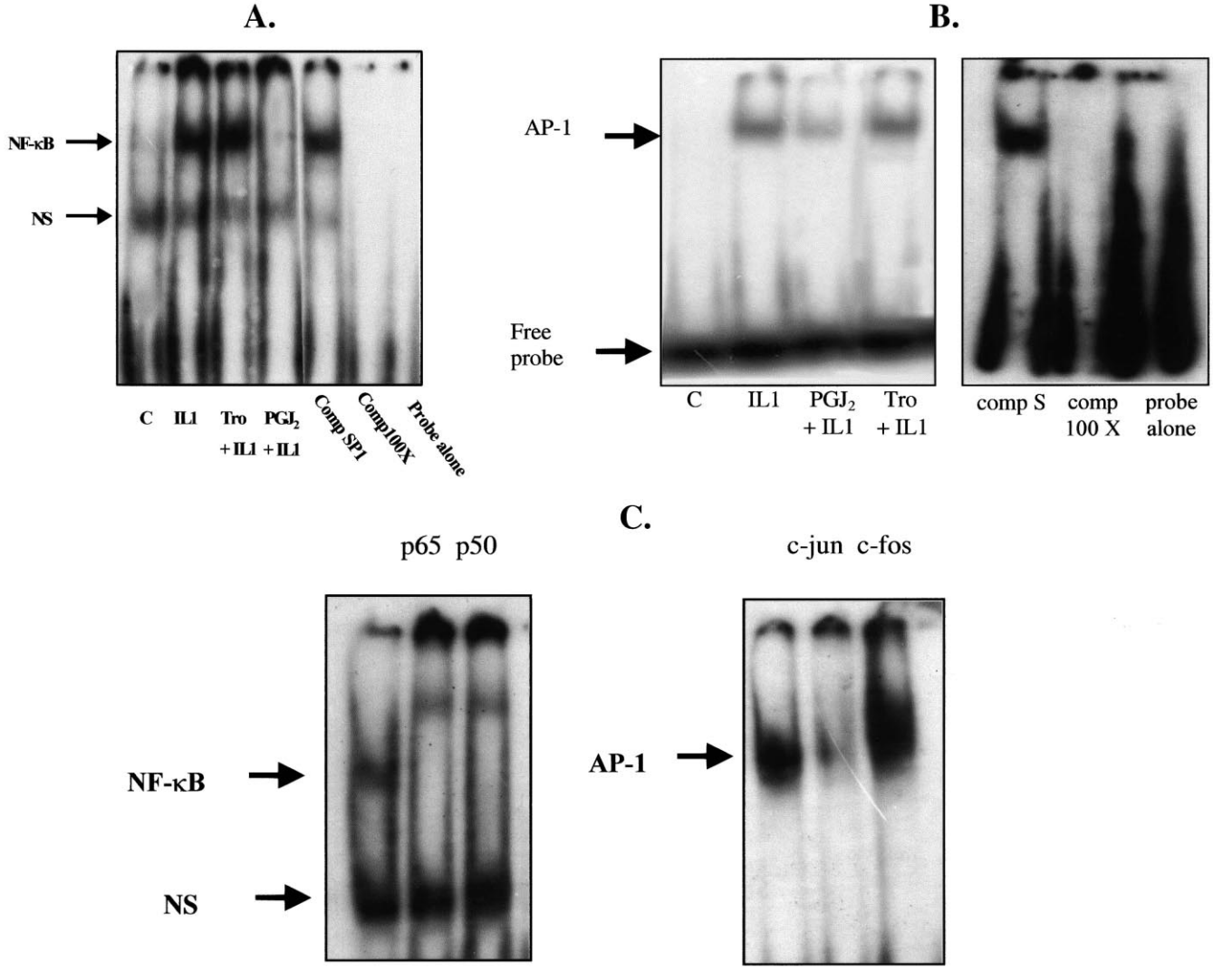


Fig. 5. 15d-$PGJ_2$, but not troglitazone, reduces IL-1β-induced NF-κB and AP-1 DNA binding activities in cultured human chondrocytes. Chondrocytes were cultured in 1% FCS-containing medium with 15d-$PGJ_2$ (10 μM), troglitazone (10 μM) or vehicle (DMSO 0.1%) for 12 h and subsequently treated with IL-1β (50 U $ml^{-1}$) for 1 h. Nuclear proteins were extracted and 5 μg of each sample were subjected to EMSA using NF-κB (A) or AP-1 (B) consensus site radiolabeled probes. Complexes were visualized by autoradiography. NS = non-specific complex. 100-fold concentrated unlabeled probe. C: EMSA 'supershift' assays identifying the subunit components for NF-κB and AP-1 dimers The experiments were performed three times; representative results are shown.

EMSA allowed us to visualize the IL-1β-induced binding of NF-κB and AP-1 on oligonucleotide probes containing their specific response element (Fig. 5). After 4 h of pre-incubation, 15d-$PGJ_2$ prevented NF-κB and AP-1 activation by IL-1β, whereas troglitazone was ineffective in both cases. Incubation of nuclear proteins with a 100-fold concentrated unlabeled probe was performed to indicate the specificity of binding of NF-κB or AP-1 to the DNA. Moreover, a pre-incubation in the presence of specific antibodies made it possible to identify the components of the protein complex as being p65/p50 heterodimer for NF-κB and c-jun/c-fos for AP-1.

## 4. Discussion

Besides its key roles in energy metabolism and cell differentiation, PPARγ has been shown to be involved in the anti-inflammatory action of specific agonists. These effects have been observed in different inflammatory models, such as in a mouse model of colonic inflammation [15], in a vascular inflammation model using smooth muscle cell cultures [7] and in Aβ-stimulated microglial activation experiments [12]. These studies suggested that PPARγ ligands would be efficient for the treatment of Alzheimer's disease, inflammatory bowel disease or atherosclerosis. Moreover, both PPARα and PPARγ ligands have been reported to inhibit the production and the effects of proinflammatory cytokines by monocytes/macrophages in vitro [8,9]. These data indicate that PPARα and/or PPARγ could constitute potential targets in the field of inflammatory or degenerative arthropathies. However, the PPARs have been poorly described in the context of arthritic diseases [27], and especially in cartilage.

We have recently reported the constitutive expression of PPARα and PPARγ in normal rat cartilage at both mRNA and protein levels [16]. The aim of the present work was to characterize PPARγ in human normal chondrocytes and to evaluate the effects of ligands on IL-1β-stimulated cells. The action of the ligands was first tested on iNOS and COX-2 mRNA expression, two major enzymes expressed in chondrocytes after IL-1β treatment. Our results show that 15d-$PGJ_2$ is much more efficient than troglitazone. It is worth noting that 15d-$PGJ_2$ completely abolished IL-1β-induced iNOS mRNA expression. In the same way, troglitazone only led to a slight inhibition of NO production without any chondroprotective effect.

15d-$PGJ_2$ is described as a ligand for PPARγ. However, the absence or the low effect of troglitazone in the present work raises the question of the real involvement of the receptor in the action of this prostaglandin in human chondrocytes. In activated monocyte or macrophage cell lines, cyclopentenone prostaglandins have been shown to modulate cellular activation through the transcriptional inhibition exerted via PPARγ [8,9]. On the other side, the contribution of other PPARγ-independent mechanisms on the anti-inflammatory action of 15d-$PGJ_2$ have been demonstrated, in part, because of the lack of effect of synthetic PPARγ ligands [11,17–19]. Thus, other intracellular targets have been identified, especially in the NF-κB signalling pathway. The most reported one is I-κB kinase (IKK), which is inhibited by direct phosphorylation of its catalytic subunit, IKKβ [18]. Other lines of evidence suggested that the cyclopentenone ring system of 15d-$PGJ_2$ could also directly inhibit the DNA binding activity of NF-κB through direct alkylation of a cysteine residue located in the DNA binding domain of the p65 subunit [19]. These two mechanisms have been shown to act in combination to inhibit transactivation of the NF-κB target gene COX-2 in HeLa cells and RAW264.7 cells. It is well established that NF-κB and AP-1 are two important IL-1β-inducible transcription factors involved in the expression of many genes central to arthritic diseases and cartilage degradation [28,29]. Our results clearly show that 15d-$PGJ_2$ significantly inhibited p65 translocation in the nucleus, but was also very potent to inhibit DNA binding activity of NF-κB. In comparison, troglitazone was completely ineffective on both steps. Some papers suggested that the involvement of PPARγ, in the action of 15d-$PGJ_2$, varied among different cell types. The lack of effect of troglitazone in the present work would suggest a PPARγ-independent mechanism of action of 15d-$PGJ_2$, perhaps because the expression level of the receptor in chondrocytes was too low [16]. However, the inefficiency of troglitazone may also be attributed to the decrease in PPARγ expression upon IL-1β stimulation. To verify this hypothesis, we have measured the expression of PPARγ in cells treated with IL-1 β in the presence of one of the ligands. We found that both 15d-$PGJ_2$ and troglitazone restored the expression of the receptor at the level of control cultures (data not shown). One must also take into account the relative low potency of troglitazone as a PPARγ ligand. Numerous other agonists have been recently identified as being much more potent and selective towards PPARγ [30].

Concerning AP-1 activation, our results clearly show that both ligands were ineffective to counteract c-jun translocation in the nucleus, and 15d-$PGJ_2$ was even shown to potentiate IL-1β effects. However, the DNA binding of AP-1 was reduced by 15d-$PGJ_2$. These two opposite effects on the AP-1 pathway in chondrocytes are somewhat surprising. In PPARγ-negative HeLa cells, 15d-$PGJ_2$ was shown to strengthen the activating effect of phorbol 12-tetradecanoate 13-acetate on AP-1 [19]. This effect was believed to be attributable to the activity of 15d-$PGJ_2$ to stimulate jun N-terminal kinase (JNK). This would explain the results of the present work, since p65 is phosphorylated by JNK in response to IL-1β. In HeLa cells, 15d-$PGJ_2$ was also shown to induce the phosphorylation of cysteine residues located in the DNA binding domain of NF-κB subunits. The action of 15d-$PGJ_2$ on the AP-1 signalling pathway has been much less studied and, to our knowledge, such a mechanism has not been described. However, it could therefore explain the present observations.

In conclusion, the present work characterizes, for the first time, the potentiality of PPARγ-ligands to modulate the IL-1β effects on human chondrocytes. By taking into account the low effects obtained with troglitazone, the action of the 15d-$PGJ_2$ in human chondrocytes seems to be mainly PPARγ-independent, via the inhibition of both NF-κB and AP-1 pathways.

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