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# Interleukin 1β suppresses transforming growth factor-induced inorganic pyrophosphate ($PP_i$) production and expression of the $PP_i$-generating enzyme PC-1 in human chondrocytes

(cytokines/cartilage/mineralization)

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ABSTRACT **Articular cartilage chondrocytes have the unique ability to elaborate large amounts of extracellular pyrophosphate ($PP_i$), and transforming growth factor β (TGFβ) appears singular among cartilage regulatory factors in stimulating $PP_i$ production. TGFβ caused a time and dose-dependent increase in intracellular and extracellular $PP_i$ in human articular chondrocyte cultures. TGFβ and interleukin 1β (IL-1β) antagonistically regulate certain chondrocyte functions. IL-1β profoundly inhibited basal and TGFβ-induced $PP_i$ elaboration. To address mechanisms involved with the regulation of $PP_i$ synthesis by IL-1β and TGFβ, we analyzed the activity of the $PP_i$-generating enzyme NTP pyrophosphohydrolase (NTPPPH) and the $PP_i$-hydrolyzing enzyme alkaline phosphatase. Human chondrocyte NTPPPH activity was largely attributable to plasma cell membrane glycoprotein 1, PC-1. Furthermore, TGFβ induced comparable increases in the activity of extracellular $PP_i$, intracellular $PP_i$, and cellular NTPPPH and in the levels of PC-1 protein and mRNA in chondrocytes as well as a decrease in alkaline phosphatase. All of these TGFβ-induced responses were completely blocked by IL-1β. Thus, IL-1β may be an important regulator of mineralization in chondrocytes by inhibiting TGFβ-induced $PP_i$ production and PC-1 expression.**

In bone and growth plate cartilage, extracellular pyrophosphate ($PP_i$) provides a critical source of orthophosphate ($P_i$) for the physiologic deposition of basic calcium phosphate crystals in bone mineralization (1–3). Although $PP_i$ is required to induce calcification (4, 5), an excess of free $PP_i$ in relation to $P_i$ suppresses mineralization by inhibiting hydroxyapatite crystal nucleation from amorphous calcium phosphate (1–3). Chondrocytes of articular cartilage have the unique ability to constitutively elaborate extracellular $PP_i$ in large amounts (6, 7), which helps to suppress mineralization of the avascular cartilage matrix (8).

$PP_i$ elaboration is governed by the balance between $PP_i$ formation and degradation (9). $PP_i$ generation is a byproduct of many synthetic reactions in the cell (9) and a direct product of enzymes with NTP pyrophosphohydrolase (NTPPPH) activity (EC 3.6.1.8), which are concentrated in fractions enriched in plasma membranes (10) and cleave the phosphodiester I bond of purine and pyrimidine NTPs (2, 11, 12). $PP_i$ degradation is effected by several inorganic pyrophosphatases, including alkaline phosphatase (9, 13).

Regulation of NTPPPH and of other factors that modulate elaboration of extracellular $PP_i$ in cartilage and bone appears critical not only to physiologic mineralization but also to the development of certain disorders of pathologic mineralization (3). One example is a prevalent disease of the elderly, idiopathic chondrocalcinosis, where the deposition in articular cartilage of calcium pyrophosphate dihydrate (CPPD) crystals is strongly linked to substantial increases in NTPPPH activity and $PP_i$ concentration (14–16). A 2- to 3-fold increase in intracellular $PP_i$ has been found in cartilage cells, fibroblasts, and lymphoblasts cultured from chondrocalcinosis patients (17–19). The capacity of CPPD crystals to activate cells can promote acute and chronic inflammatory synovitis and cartilage degeneration (15, 20). In this regard, the presence of CPPD crystal deposition commonly complicates prior articular injury and is an adverse prognostic factor in osteoarthritis (15, 21). A second example is provided by hypophosphatasia (13, 16). This inherited deficiency of alkaline phosphatase is associated with increased accumulation of $PP_i$ in serum and urine, $PP_i$ supersaturation in bone and cartilage, chondrocalcinosis, and abnormalities in bone mineralization (13, 16).

Transforming growth factor β (TGFβ) is unique among growth factors and cytokines present in cartilage in its capacity to markedly up-regulate extracellular elaboration of $PP_i$ by porcine articular chondrocytes (22). It had been proposed that the elaborated $PP_i$ might be generated in part within the chondrocyte (23). Because $PP_i$ does not freely diffuse across membranes, it was hypothesized that the $PP_i$ might be released through an anion channel (23) or cosecreted with basic proteins such as collagen (24). Chondrocyte $PP_i$ elaboration is not dependent on *de novo* synthesis of glycosaminoglycans (25, 26) but appears to be partially mediated by *de novo* protein synthesis (24). In this regard, TGFβ treatment of porcine chondrocytes up-regulated membrane and extracellular NTPPPH activity by up to 36% (22). Recently, plasma cell membrane glycoprotein 1, designated PC-1, was identified as a species of human chondrocyte NTPPPH (27). Furthermore, *de novo* transcription of PC-1 in a variety of cells induced intracellular $PP_i$ generation proportional to the increment in NTPPPH activity (28).

TGFβ and the proinflammatory cytokine interleukin 1β (IL-1β) exhibit mutual antagonism of several functions in chondrocytes (29). This study shows that IL-1β profoundly reduced basal and TGFβ-induced $PP_i$ generation in chondrocytes. Furthermore, we demonstrate that the antagonism may be mediated by concordant effects on NTPPPH activity through modulation of PC-1 expression and alkaline phosphatase in human articular chondrocytes.

## MATERIALS AND METHODS

**Cells and Cell Culture.** Cartilage was obtained at autopsy from donors without history of joint disease as described (30).

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Abbreviations: $PP_i$, pyrophosphate; $P_i$, orthophosphate; IL-1, interleukin 1; TGF, transforming growth factor; NTPPPH, NTP pyrophosphohydrolase; CPPD, calcium pyrophosphate dihydrate; FBS, fetal bovine serum; RT–PCR, reverse transcription–PCR.

The age of the donors ranged from 19 to 74 years (mean = 44), and there were no apparent differences between male or female donors in the responses examined in the present study. Cartilage slices (1–3 mm thick) were removed from the femoral condyles and washed in Dulbecco's modified Eagle's medium (DMEM). Slices were then minced with a scalpel; transferred into a digestion buffer containing DMEM, 5% (vol/vol) fetal bovine serum (FBS), 584 mg of L-glutamine per liter, antibiotics (penicillin at 100 μg/mL and streptomycin at 100 μg/ml), and clostridial collagenase (Sigma) at 2 mg/ml; and incubated on a gyratory shaker at 37°C until the fragments were digested. Residual multicellular aggregates were removed by sedimentation, and the cells were washed three times in DMEM/5% FBS lacking the other ingredients before use in the experiments. Results presented in this paper were obtained with chondrocytes in primary or passage 1 culture. Cells were cultured at 500,000 cells per well in six-well plates in DMEM containing antibiotics and L-glutamine as above, and the indicated doses of FBS, recombinant human TGFβ1, and/or IL-1β (the last two from R & D Systems).

**$PP_i$ Assay.** $PP_i$ levels were measured in the culture media and cell lysates by a radiometric method (31, 32). The method uses a high specific activity UDP-D-[6-$^3$H]glucose (Amersham), which is separated from the reaction product 6-phospho-[6-$^3$H]gluconate by selective adsorption on charcoal activated with phosphoric and sulfuric acids (Sigma). NADP, UDP-glucose, and UTP-glucose pyrophosphorylase were products of Sigma, and the phosphoglucomutase and glucose-6-phosphate dehydrogenase were purchased from Boehringer Mannheim. $NaPP_i$ used to prepare the standard was purchased from Fisher Scientific. The standard concentrations ranged from 100 to 400 pmol of $PP_i$ and were included in each assay. To monitor recovery, $PP_i$ was added to the samples; recoveries ranged from 95% to 105% with a mean of 96%. Each sample was run in duplicate or triplicate. After adsorption of the reaction mixture on charcoal, it was centrifuged at 14,000 rpm for 10 min, and a 100-μl aliquot of the supernatant was carefully removed and assayed for radioactivity in 5 ml of Ecoscint (National Diagnostics).

**Protein and DNA Assay.** The Bio-Rad DC protein assay, based on the Bradford dye-binding procedure, was used to determine protein levels in the 0.1% Triton X-100 lysates of cells. $PP_i$ values were corrected for DNA content in all cell culture experiments. DNA concentrations were determined with a modified Burton method as described by Leyva and Kelley (33).

**NTPPPH and Alkaline Phosphatase Assays.** The 5′-nucleotide phosphodiesterase I assay for NTPPPH was performed with 1 mM *p*-nitrophenylthymidine 5′-monophosphate (PNTM) (Sigma) as substrate in 50 mM Hepes-buffered DMEM containing 1.6 mM $MgCl_2$ (pH 8.0) in a volume of 0.5 ml to which 0.05 ml of sample was added for 1 hr (27). The reaction was halted by addition of 4 vol of 100 mM NaOH, and absorbance at 410 nm was determined. Specific enzyme activity was measured against a standard curve of *p*-nitrophenol and expressed in units. Alkaline phosphatase was assayed as described by Oyajobi *et al.* (2). One unit of either enzyme activity was equivalent to 1 nmol of substrate hydrolyzed per hour. In selected experiments $PP_i$ formation was measured on cells pretreated for 4 days with TGFβ or IL-1 after a 1-hr incubation with 100 μM ATP (2).

**Metabolic Labeling and Immunoprecipitation of PC-1.** Chondrocytes were stimulated with IL-1β or TGFβ or both and lysed by treatment for 10 min in 1% Triton X-100. For metabolic labeling the cells were transferred to cysteine- and methionine-free media supplemented with dialyzed FBS and 1 μCi (37 kBq) of Trans$^{35}$S-label (containing [$^{35}$S]methionine and [$^{35}$S]cysteine) per ml for 48 hr. Cells were scraped into 0.5 ml of lysis buffer. Conditioned medium (1.0 ml) also was collected. Immunoprecipitates from the $^{35}$S-labeled samples were collected as described below, washed seven times, separated on SDS/PAGE, and visualized by autofluorography.

We used mouse monoclonal antibody 3E8 to native human PC-1 (12, 34). Immunoprecipitation was performed by a modification of a previously described method (27). Aliquots of protein (5 μg) from cell lysates were precleared with protein G-conjugated Sepharose followed by centrifugation and then were incubated with nonimmune ascites or the same volume of ascites containing monoclonal anti-human PC-1 (3E8) for 1 hr at 4°C with constant agitation. Then, 0.05 ml of 10% protein G-Sepharose was added for 1 hr at 4°C. The sample was centrifuged at 8700 × *g* for 2 min. The supernatant was collected and brought to a total volume of 0.07 ml in NTPPPH assay buffer containing 1% Triton X-100. The pellets were washed and resuspended to 0.07 ml in the same buffer.

**Western Blot Detection of PC-1.** Western blotting was performed as described (27) with a rabbit polyclonal antibody to PC-1 (designated 1769) as primary antibody (35). A 50% saturated ammonium sulfate cut of the primary antiserum was used at 1:2000 dilution in blocking buffer.

**Detection of PC-1 mRNA.** For reverse transcription–polymerase chain reaction (RT–PCR), a previously validated method for PC-1 was used (27) with PC-1 primers that spanned more than one intron (34). RT–PCR for IL-6 was performed as described (36). As a "housekeeping" gene control, we performed a single round of RT–PCR (30 cycles) on the same cDNA templates for the ribosomal protein L30 (27).

## RESULTS

**Human Articular Chondrocytes Release Increased Levels of $PP_i$ in Response to Serum and/or TGFβ.** The addition of serum caused primary human articular chondrocytes to increase the release of $PP_i$ by ≈2-fold above basal levels in serum-free medium after 48 hr and 5-fold after 96 hr. TGFβ1 increased $PP_i$ levels in the absence and presence of serum. TGFβ effects were dose-dependent and maximal between 10 and 20 ng/ml. The TGFβ effects were observed in confluent cultures, where normal chondrocytes do not divide. Furthermore, the greatest increase in $PP_i$ levels was observed in serum-free cultures, where chondrocytes do not proliferate or synthesize increased amounts of DNA in response to TGFβ (results not shown). This suggests that the observed increases in $PP_i$ levels were not a consequence of increased cell numbers in the TGFβ-stimulated cultures. During the course of these studies, chondrocytes from >20 different donors were stimulated with TGFβ. The increase in the $PP_i$ levels induced by TGFβ was variable, and in a small number of donors, no significant TGFβ effect was observed.

TGFβ increased cellular protein concentrations (Table 1). The levels of intracellular $PP_i$, although ≈50% lower as compared with the conditioned medium, changed concordantly with extracellular $PP_i$ in response to TGFβ. The in-

Table 1. TGFβ effects on intracellular protein and $PP_i$ levels in chondrocytes

| Stimulant | Protein, μg per total cells | $PP_i$ content, nmol: Total cells | $PP_i$ content, nmol: Medium |
|---|---|---|---|
| Control | 69 | 4.3 | 9.4 |
| TGFβ | 97 | 7.5 | 24.3 |
| IL-1β | 46 | 2.0 | 5.9 |
| TGFβ + IL-1β | 65 | 1.3 | 2.5 |

Chondrocytes were stimulated with TGFβ at 10 ng/ml or IL-1β at 5 ng/ml or both in medium containing 1% FBS. Conditioned media and Triton X-100 cell lysates were collected after 96 hr. $PP_i$ levels were determined in conditioned media and cell lysates from the identical cell cultures. Protein content in the cell lysates was quantified by the Bio-Rad protein dye reagent assay.

crease in extracellular $PP_i$ thus appeared to be the result of both TGFβ-induced $PP_i$ synthesis and release.

**Suppression of $PP_i$ Production by IL-1β.** TGFβ and IL-1β can antagonistically regulate certain chondrocyte functions (29). IL-1 reduced baseline and serum-induced $PP_i$ levels at 48 hr (data not shown) and 96 hr (Fig. 1), and it completely neutralized the $PP_i$-inducing activity of TGFβ, lowering $PP_i$ levels below baseline. Significant IL-1β effects occurred at low doses (0.1 ng/ml) and the suppressive effect of IL-1β on $PP_i$ elaboration was of greater magnitude than the inhibition of other chondrocyte responses. The effects of IL-1β were not due to the induction of chondrocyte necrosis or apoptosis as analyzed by erythrosin dye exclusion and 4,6-diamidino-2-phenylindole (DAPI) staining (not shown). However, IL-1β did reduce cellular protein concentrations and abrogated the TGFβ-induced increase (Table 1).

**$PP_i$ Elaboration, NTPPPH Activity, PC-1 Expression, and Alkaline Phosphatase.** To address mechanisms that may be responsible for the observed changes in $PP_i$ formation in response to extracellular regulators, the expression of NTPPPH and alkaline phosphatase was analyzed.

TGFβ treatment of chondrocytes increased NTPPPH activity with a similar time course and magnitude as the TGFβ effects on extracellular $PP_i$ levels (Fig. 2). In addition, TGFβ suppressed alkaline phosphatase activity. This TGFβ effect was also antagonized but not completely inhibited by IL-1β (Fig. 2).

Lysates from unstimulated and TGFβ-stimulated chondrocytes were treated with monoclonal antibody to native human PC-1 (3E8 ascites) and precipitated with protein G-Sepharose. Analysis of NTPPPH activity showed that precipitation with anti-PC-1 removed >50% of the NTPPPH activity from the cell lysates (Fig. 3). In contrast, cell lysates treated with normal mouse serum or nonimmune ascites, and protein G-Sepharose retained similar levels of NTPPPH activity. Western blots verified that

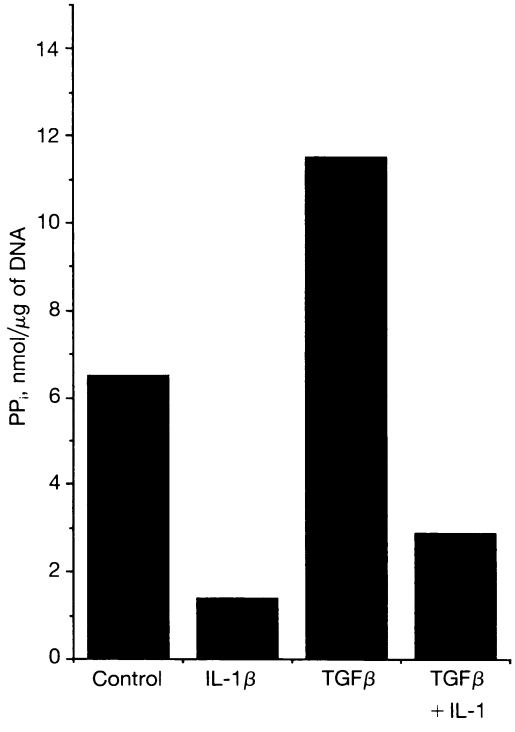


FIG. 1. IL-1β suppresses serum and TGFβ-induced $PP_i$ production. Chondrocytes cultured in DMEM containing 10% FBS were treated with IL-1β (1 ng/ml) or TGFβ (10 ng/ml) or both. $PP_i$ levels were determined in 96-hr conditioned medium. Results represent mean values of duplicate determinations with a standard deviation of <10%. Chondrocyte preparations from >11 different donors were tested, and in all experiments IL-1 suppressed $PP_i$ formation.

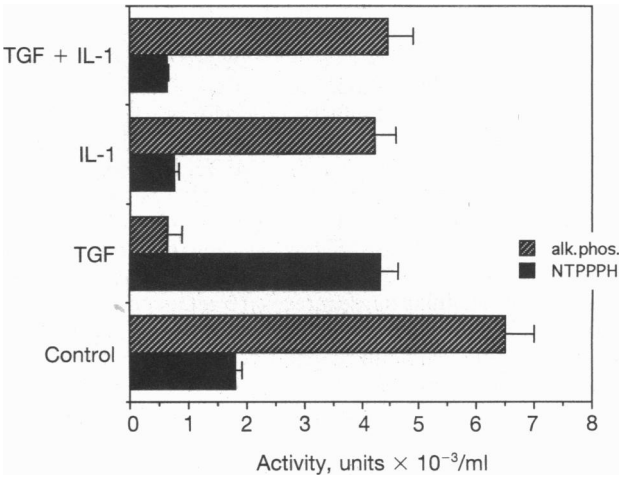


FIG. 2. Effects of TGFβ and IL-1β on NTPPPH and alkaline phosphatase. Chondrocytes were plated at 500,000 cells per well in six-well plates and incubated in the absence or presence of 10% heat-inactivated FBS with or without TGFβ1 at 10 ng/ml. Conditioned medium was collected at 96 hr and assayed for $PP_i$. Cell lysates were analyzed for the specific activities of NTPPPH and alkaline phosphatase (alk. phos.). Values are means ± SD.

most of PC-1 polypeptide (130 kDa) was adsorbed and precipitated by anti-PC-1 but not by nonimmune ascites under these conditions (not shown). Thus, a majority of the cellular NTPPPH activity in chondrocytes appeared attributable to PC-1.

**Suppression of TGFβ-Induced $PP_i$ Production by IL-1 Correlates with PC-1/NTPPPH Expression.** TGFβ increased PC-1 steady-state mRNA levels (Fig. 4). Furthermore, by 48 hr, TGFβ already detectably increased PC-1 protein synthesis and PC-1 release into conditioned medium, as assessed by immunoprecipitation of [$^{35}$S]methionine-labeled chondrocyte lysates and conditioned medium (Fig. 5). These findings

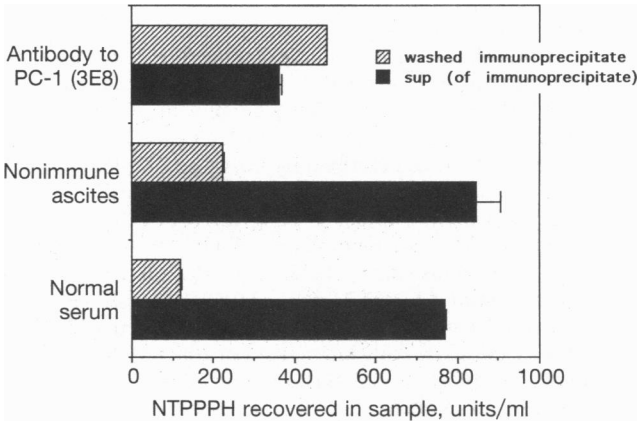


FIG. 3. Monoclonal anti-PC-1 antibody depletes the majority of NTPPPH activity from human chondrocyte lysates. Human chondrocytes were cultured for 96 hr with TGFβ at 10 ng/ml. Aliquots of protein (5 μg) from cells lysed in 1% Triton X-100 were precleared with protein G-Sepharose; this was followed by immunoprecipitation with 0.001 ml of normal mouse serum, nonimmune ascites (containing anti-apoE IgG), or ascites containing monoclonal anti-human PC-1 antibody (3E8) as described in text. The supernatant was collected and resuspended to a total volume of 0.070 ml in 1% Triton X-100 in NTPPPH assay buffer. The pelleted beads were washed once, and resuspended to 0.070 ml in the same buffer. Aliquots of supernatant and of washed and resuspended beads were assayed for NTPPPH. Results are expressed in units/ml recovered in supernatants or precipitates after incubation with the different antibodies. Specific depletion of PC-1 by immunoadsorption under these conditions was verified by immunoblotting (not shown).

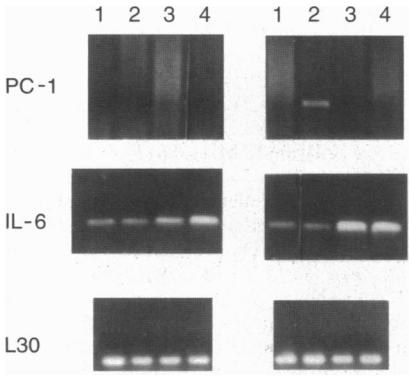


FIG. 4. Differential effects of IL-1 and TGFβ on PC-1 and IL-6 mRNA levels. Chondrocytes were stimulated with TGFβ1 at 10 ng/ml (lanes 2) or IL-1β at 1 ng/ml (lanes 3) or both (lanes 4) or neither (buffer alone; lanes 1) as indicated for 20 hr (*Left*) and 70 hr (*Right*). RNA was isolated and cDNA prepared. Aliquots of the cDNA were amplified with primers for PC-1 (*Top*), IL-6 (*Middle*), and L30 (*Bottom*). The PCR products were separated on 5% polyacrylamide gels and visualized after ethidium bromide staining.

showed that expression of PC-1/NTPPPH and $PP_i$ elaboration are concordantly up-regulated by TGFβ in chondrocytes, and this correlation suggested that regulation of PC-1/NTPPPH expression might be a mechanism involved in the IL-1 inhibition of $PP_i$ elaboration.

IL-1 treatment of chondrocytes for 70 hr reduced basal levels of NTPPPH activity by >50%, and it antagonized the effects of TGFβ on NTPPPH and alkaline phosphatase activity (Fig. 2). The effect of IL-1 on NTPPPH activity was associated with inhibition of both the expression of PC-1 protein and the release of newly synthesized PC-1 into the extracellular space in response to TGFβ (Fig. 5). The effect of IL-1 on NTPPPH activity also was associated with a complete inhibition of PC-1 mRNA expression (Fig. 4). The effects of IL-1 on PC-1 mRNA expression were evident not only at 70 hr, when PC-1 mRNA was most abundant, but also by 20 hr, when PC-1 mRNA expression was detectable by a more sensitive nested RT–PCR (27) (not shown).

To analyze whether the effect on PC-1 gene expression was selective, the levels of IL-6 mRNA, which is known to be IL-1 inducible (37), were analyzed in identical samples. IL-1 alone

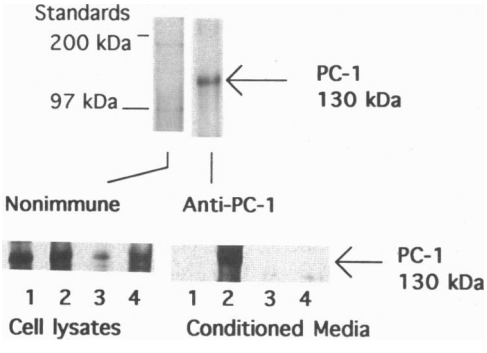


FIG. 5. IL-1β inhibits PC-1 protein expression by chondrocytes. [$^{35}$S]Methionine-labeled cell lysates and conditioned media were prepared from chondrocytes incubated for 48 hr with the agonists indicated and were immunoprecipitated as described in the text. Immunoprecipitates were analyzed by SDS/PAGE and autofluorography (exposure for 48 hr). (*Upper*) Monoclonal antibody 3E8 to native human PC-1, but not nonimmune ascites, immunoprecipitated PC-1 as the indicated 130-kDa polypeptide in chondrocyte lysates. (*Lower*) PC-1 expression was detected not only in cell lysates but also in conditioned media following stimulation with TGFβ, and IL-1β inhibited PC-1 expression and release. Stimulation in lanes: 1, none (control); 2, TGFβ; 3, IL-1; 4, TGFβ and IL-1.

increased IL-6 mRNA levels, and IL-1 in combination with TGFβ synergistically induced this cytokine gene (Fig. 4). Thus, IL-1-induced suppression of PC-1 expression was a selective effect of IL-1 in chondrocytes.

## DISCUSSION

This study shows that IL-1 suppressed basal, serum-induced, and TGFβ-induced $PP_i$ elaboration by human articular chondrocytes. The TGFβ-induced increase in extracellular $PP_i$ levels correlated with up-regulation of NTPPPH activity, which was attributable in large part to PC-1. A concurrent decrease in alkaline phosphatase was produced by TGFβ and this was prevented by the addition of IL-1. Furthermore, IL-1 suppression of $PP_i$ elaboration was associated with a reduction in NTPPPH activity and inhibition of PC-1 mRNA and protein expression. Collectively, these results provide new insight into the gene regulatory program associated with $PP_i$ formation and support a role of enhanced $PP_i$ synthesis by PC-1 and breakdown of $PP_i$ by alkaline phosphatase in this chondrocyte response.

Chondrocytes in primary or early-passage culture spontaneously released $PP_i$. This was increased by TGFβ or serum that contains at least two growth factors, transforming growth factor α and epidermal growth factor, known to be able to augment TGFβ-induced $PP_i$ release in porcine chondrocytes (22). The TGFβ effects on $PP_i$ elaboration occurred in the absence of serum in chondrocyte cultures that were completely confluent. Under serum-free conditions and in confluent cultures, TGFβ does not increase DNA synthesis in chondrocytes (38). These observations suggest that this chondrocyte secretory response to TGFβ is not dependent on its mitogenic activity on chondrocytes. Consistent with this, we observed no significant increase in DNA content in the TGFβ-treated confluent cultures. In contrast, the amount of cellular protein increased in TGFβ-treated cultures under conditions where $PP_i$ was elaborated but DNA content did not change. This suggested that the TGFβ effect on $PP_i$ formation may depend on the synthesis of proteins that are required for $PP_i$ formation.

Although many biosynthetic pathways have been reported to contribute to $PP_i$ elaboration (9), NTPPPH activity appears to be a major component in chondrocytes. More recently, insight into the molecular identity of NTPPPH has been provided by studies where overexpression of the PC-1 protein via cDNA transfection induced proportional increases in cellular NTPPPH activity and $PP_i$ levels (28). The present study shows a close correlation between PC-1 expression, NTPPPH activity, intracellular $PP_i$ levels, and extracellular $PP_i$ elaboration. Most of the NTPPPH activity associated with resting and TGFβ-stimulated human chondrocytes was attributable to PC-1, as it was removed by monoclonal antibody to human PC-1. However, we cannot exclude the possibility that other molecules with NTPPPH activity distinct from PC-1 exist.

Levels of $PP_i$ rose within TGFβ-stimulated chondrocytes as well as in their conditioned medium. It will be important to determine whether chondrocyte $PP_i$ elaboration into the extracellular space is due largely to release of *de novo* intracellularly formed $PP_i$ as previously suggested by results with the anion-channel inhibitor probenecid (23) or whether most of the $PP_i$ that is found extracellularly is produced there. Because $PP_i$ does not freely diffuse across the intact plasma membrane, chondrocytes might have a unique ability to actively export large amounts of $PP_i$. Alternatively, if chondrocytes are able to export NTPPPH substrates including ATP, then TGFβ-induced chondrocyte $PP_i$ elaboration could primarily be due to extracellular $PP_i$ generation. PC-1 is an ectoenzyme (12), and soluble PC-1 polypeptides also can be secreted from cells (34). Furthermore, extracellular ATP could be a quantitatively significant substrate for $PP_i$ generation in idiopathic CPPD deposition disease, where synovial fluid ATP (at ≈200 nM) is approximately twice that observed in joints that are normal or

have primary osteoarthritis (39). TGFβ-treated chondrocytes generated markedly increased levels of $PP_i$ when incubated in the presence of ATP as exogenous substrate (data not shown), compatible with previous evidence that a substantial amount of NTPPPH expressed by chondrocytes is accessible and active on the plasma membrane and elsewhere in the extracellular milieu (10). In this regard, we noted that TGFβ induced substantial release into the conditioned medium of newly expressed PC-1/NTPPPH by chondrocytes.

TGFβ and IL-1 are among the most important chondrocyte regulators, with qualitatively distinct effects on chondrocyte and cartilage and can be considered as prototypic stimuli for anabolic or catabolic responses, respectively (29). In several previous studies we analyzed the regulation of gene expression and secretory responses in chondrocytes in response to these cytokines. In human articular chondrocytes, TGFβ and IL-1 can have additive, synergistic, or antagonistic effects. TGFβ and IL-1 have qualitatively distinct and antagonistic effects on the formation of extracellular matrix. TGFβ, as a major anabolic agent, stimulates chondrocyte proliferation, extracellular matrix synthesis, and the synthesis of protease inhibitors. IL-1 as a major catabolic factor inhibits chondrocyte proliferation and matrix synthesis and stimulates the production of proteases (29). Other functions, such as the expression of certain chemotactic cytokines, can be additively or synergistically increased by IL-1 and TGFβ (40).

This study characterized $PP_i$ formation as a chondrocyte function that is particularly sensitive to suppression by IL-1. Spontaneous and serum-induced $PP_i$ production were significantly reduced and IL-1 completely blocked the TGFβ effects. Other TGFβ-induced chondrocyte functions are only partially reduced by IL-1 (30, 41). Suppression of $PP_i$ production is thus one of the most potent effects of IL-1 on chondrocytes. This IL-1 effect is related to a reduction of PC-1 rate of transcription and/or mRNA stability. The fact that increased levels of IL-1 are present in rheumatoid arthritis (42) may well account for the decrease in $PP_i$ observed in synovial fluids of patients with rheumatoid arthritis as compared with patients with osteoarthritis (43). Based on the results of this study, the functional antagonism of TGFβ and IL-1 may also extend to the regulation of mineralization. We also observed that tumor necrosis factor α shares the capacity of IL-1 to block TGFβ-induced $PP_i$ elaboration by chondrocytes and that IL-1 also inhibits TGFβ-induced PC-1/NTPPPH up-regulation in osteoblastoid cells (unpublished data).

In conclusion, this study suggests that an imbalance in the relative expression of TGFβ and IL-1 in cartilage and bone can influence cartilage CPPD crystal deposition and possibly bone mineralization.

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