Differential modulation of degradative and repair responses of interleukin-1-treated chondrocytes by platelet-derived growth factor

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Interleukin 1 (IL-1) plays a dual role in cartilage matrix degeneration by promoting extracellular proteinase action such as the matrix metalloproteinases (increased degradation) and by suppressing the synthesis of extracellular matrix molecules (inhibition of repair). Platelet-derived growth factor (PDGF) is a wound-healing hormone which is released along with IL-1 during the inflammatory response. Since previous studies have shown that PDGF enhances IL-1 α effects on metalloproteinase activity, in this report, we have examined whether PDGF modifies IL-1 β effects on cartilage proteoglycan synthesis. Initially, we confirmed that rabbit articular chondrocytes treated with IL-1 β +PDGF induced higher proteinase activity, in comparison with IL-1-treated cells. We further observed that the increased proteinase activity correlated with an increase in the synthesis of collagenase/stromelysin proteins and a corresponding increase in the steady-state mRNA levels for both the enzymes. Studies

on IL-1 receptor expression suggested that PDGF caused an increase in IL-1 receptor expression which, by augmenting the IL-1 response, may have led to the increase in proteinase induction. Analysis of proteoglycan synthesis confirmed that IL-1 reduced the incorporation of sulphated proteoglycan, aggrecan, into the extracellular matrix of chondrocytes, whereas PDGF stimulated it. However, cells treated with IL-1+PDGF synthesized normal levels of aggrecan. This is in contrast with cells treated with IL-1+fibroblast growth factor, in which case only proteinase activity was potentiated. The results allow us to conclude that (a) the two effector functions that play a role in matrix remodelling, namely matrix lysis (proteinase induction) and matrix repair (proteoglycan synthesis), occur via distinct pathways and (b) PDGF may play a crucial role in cartilage repair by initially causing matrix degradation followed by promoting new matrix synthesis.

INTRODUCTION

Articular cartilage plays a crucial function in the synovial joints, by providing an articulating surface that resists compression. This function is accomplished through the unique architecture of the tissue in which the collagen network and proteoglycan aggregates interact and maintain a proper balance of bio-mechanical function [1–3]. A critical aspect of the structural integrity of cartilage is the ability of chondrocytes to synthesize and maintain a functional matrix. It is well recognized that a constant turnover of some matrix components occurs in cartilage and that the homeostasis is maintained through a balance of degradation and new synthesis [4–6]. Although the exact mechanisms of turnover of matrix molecules in normal cartilage are not known, exaggerated degradation is commonly observed in inflammatory conditions such as rheumatoid arthritis and osteoarthritis [7–11]. In general, tissue response to trauma is associated with inflammation and degradation of extracellular matrix, followed by synthesis and assembly of new matrix molecules. As a consequence of trauma, neutrophils and monocytes accumulate at the site of injury and secrete cytokines such as interleukin 1 (IL-1) [12,13]. IL-1, in turn, induces the production of matrix-degrading proteinases amongst which are metalloproteinases such as collagenase, gelatinase and stromelysin, which are believed to cause matrix lysis [14–19]. In addition, IL-1 also suppresses the synthesis of cartilage matrix molecules such as type II collagen and aggrecan [20–22]. The resultant increase in degradation coupled with reduced matrix molecular synthesis are believed to be important aspects of pathological cartilage degradation.

For the past several years, we have been interested in

identifying factors that can potentially modulate IL-1 activity on cartilage degradation and repair. Several polypeptide growth factors have been shown to play a role in the repair of soft-tissue damage by promoting cell recruitment to the site of injury, cell proliferation and increased matrix synthesis, all leading to accelerated repair [23]. Recent studies suggest that different growth factors may play either a pro-inflammatory [fibroblast growth factor (FGF), platelet-derived growth factor (PDGF)] or anti-inflammatory [transforming growth factor- β (TGF- β)] role by modifying IL-1 activity on cartilage [24–28]. In general, these studies primarily examined the effects of growth factors on neutral proteinase induction. Since IL-1 plays a dual role by causing proteinase induction and proteoglycan suppression, in this report, we have investigated whether or not these two effects are related. We have specifically used PDGF to determine if it modifies one or both the aspects of IL-1 activities.

MATERIALS AND METHODS

Materials

Recombinant human interleukin 1 β was isolated by cloning and expressing a synthetic gene [29]. The following products were purchased from R&D Systems, Minneapolis, MN, U.S.A.: recombinant and natural human PDGF AB, AA, BB, basic FGF, TGF- β and neutralizing antibody to PDGF AB. ^{125}I -labelled IL-1 β (specific radioactivity 94.3 mCi/mg) and $\text{Na}_2^{35}\text{SO}_4$ (specific radioactivity 43 Ci/mg) were from New England Nuclear, Boston, MA, U.S.A. Trans ^{35}S -label (specific radioactivity 1096 Ci/mmol) was from ICN, Irvine, CA, U.S.A.

Abbreviations used: DMEM, Dulbecco's modified Eagle's medium; FGF, fibroblast growth factor; GdmCl, guanidinium chloride; IL-1, interleukin 1; PDGF, platelet-derived growth factor; TGF- β , transforming growth factor - β .

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Cell culture

Articular chondrocytes were prepared from 1–1.5 kg male New Zealand White rabbits [13,24]. The tibial cartilage was cut into small pieces and was treated for 20 min each in sequence with 2 mg/ml hyaluronidase (Sigma) and Tos-Phe-CH₂Cl (TPCK)-treated trypsin (Worthington), followed by a 4–5 h treatment with collagenase (Worthington). All incubations were carried out at 37 °C in an atmosphere of 5% CO₂+95% air. The cells released after collagenase digestion were seeded at a density of 2×10^4 cells/cm² in Ham's F-12 medium containing 10% (v/v) fetal bovine serum plus 2 mM L-glutamine and 50 µg/ml gentamicin. The cells were maintained in a humidified chamber of 5% CO₂+95% air and fed on days 3 and 6 after plating. Only confluent primary cultures (5×10^4 cells/cm²) were used. For all treatments, chondrocytes were washed three times with Dulbecco's PBS. Treatments were then added in serum-free Dulbecco's modified Eagle's medium (DMEM) containing 2 mM L-glutamine and 50 µg/ml gentamicin. For biosynthetic studies, ascorbic acid (50 µg/ml) was included in the treatment.

Neutral proteinase assay

The neutral metalloproteinase activity was determined as described previously, utilizing [³H]casein as the substrate [13,24]. Briefly, cells were treated with various factors as described above. Unless mentioned, the duration of the treatments was 48 h. The latent proteinases released into the conditioned media were activated by incubation with Tos-Phe-CH₂Cl-treated trypsin (100 µg/ml) for 10 min at 25 °C, followed by incubation with soya-bean trypsin inhibitor (150 µg/ml) for 30 min at 25 °C to inactivate the trypsin. The media were further incubated with [³H]casein (1200 d.p.m./mg) at 37 °C for 2 h. The reaction was stopped by the addition of trichloroacetic acid (10%, w/v). The undigested substrate was removed by centrifugation (500 g), and the radioactivity in the supernatant fraction was determined. One enzyme unit is defined as the amount of enzyme required to digest 1 µg of substrate/min at 37 °C.

Determination of collagenase/stromelysin steady-state mRNA levels

Chondrocytes were treated with IL-1 (10 ng/ml), PDGF (30 ng/ml) or a combination thereof in serum-free DMEM for the indicated time intervals. At this concentration, IL-1 exhibited a half-maximal effect on neutral proteinase activity (results not shown). The total cellular RNA was extracted with guanidinium chloride (GdmCl), then with chloroform plus butan-1-ol, followed by two ethanol precipitations [22,30]. The specific mRNAs were analysed by slot-blotting equal amounts of RNA (2 µg/treatment) on to 'Genescreen' (NEN), followed by hybridization for 18 h with oligonucleotide probes for rabbit procollagenase (nucleotide sequence 842–886 [31]) and stromelysin (877–921 [32]). The oligonucleotides were 5'-end-labelled with ³²P using T4 polynucleotide kinase, and the signal intensity was standardized by probing the filters with 5'-end-labelled oligo(dT) [33].

Biosynthesis of collagenase/stromelysin

For studies on the synthesis of neutral proteinases, chondrocytes were treated with various factors in serum-free DMEM for 48 h, in the presence of Tran³⁵S-label (40 µCi/ml). The labelled media were dialysed against distilled water, and the radioactivity in the non-dialysable fraction was determined. An equal volume of each treatment mixture (corresponding to 10⁵ cells/treatment)

was lyophilized, solubilized in sample buffer containing dithiothreitol (7.5 mg/ml), denatured and electrophoresed in an SDS/polyacrylamide gel, consisting of a 3–15% linear gradient resolving gel with a 3% stacking gel [24,34]. To determine the relative electrophoretic mobility, protein M_r standards from Bio-Rad were included. For fluorography, the gel was immersed in 'Entensify' (NEN), dried and exposed to X-ray film (Kodak X-omat AR).

IL-1-receptor-binding studies

The receptor-binding studies were carried out as described before [24,27]. In brief, chondrocytes were treated for 18 h with the growth factors, rinsed extensively with binding buffer [PBS, BSA (0.1%, w/v), Hepes (20 mM), CaCl₂ and MgSO₄ (0.01 mg/ml each)], and incubated with ¹²⁵I-labelled IL-1β (10 ng/ml) in binding buffer at 25 °C with rocking for 4 h. Unbound label was removed by washing with a solution containing PBS (pH 7.2), Hepes (20 mM) and fetal bovine serum (1%, v/v). Bound material was extracted with a lysing buffer [10% glycerol (v/v)+0.1% Triton X-100 (v/v) in binding buffer]. The radioactivity in a portion of the extract was determined by an LKB Compugamma counter. Specific binding was defined as the difference between total binding and that which occurred in the presence of a 1000-fold excess of unlabelled IL-1β. All treatments were done in quadruplicate.

Biosynthesis of proteoglycans (aggrecan)

The effect of PDGF and IL-1 on proteoglycan synthesis was studied by determination of ³⁵[S]sulphate incorporation into the matrix of chondrocytes and also into that of cartilage explants. Cells in monolayer were treated for 20 or 44 h with IL-1 and various growth factors, the media were removed, fresh media containing the same set of growth factors plus Na₂³⁵SO₄ (40 µCi/ml) were added and the incubation was continued for another 4 h. For cartilage, rabbit tibial condylar cartilages were cut into small pieces and 5–10 mg (wet wt.) of explants (DMEM) were similarly treated, except that the growth factor/cytokine treatments were carried out for only 20 h. The media were removed and the cell layer plus matrix (or the cartilage) were extracted with a solution containing 4 M GdmCl, 10 mM CHAPS and proteinase inhibitors (100 mM sodium acetate, pH 6, 10 mM EDTA, 25 mM benzamidine hydrochloride, 100 mM ε-amino-hexanoic acid and 2 mM phenylmethanesulphonyl fluoride) at 4 °C for 48 h [35]. The extracts were dialysed against distilled water and radioactive incorporation was determined.

The identity of the proteoglycan was established by the following procedures.

Composite agarose/acrylamide electrophoresis

The [³⁵S]sulphate-labelled matrix extracts of chondrocytes were analysed on a composite gel using a modification of previously published procedures [36–38]. The samples representing radioactive counts from cells (10⁵ cells/treatment) subjected to various treatments (see above) were dialysed against water, lyophilized and solubilized in 25 µl of buffer containing 0.5 mM sodium acetate, 0.125 mM Na₂SO₄ and 8 M urea (pH 6.8), and were electrophoresed using a horizontal gel apparatus (BRL). The large aggregating proteoglycan monomer, aggrecan, was used for comparison of electrophoretic mobility. The monomer was prepared from rat chondrosarcoma using established procedures involving associative and dissociative CsCl₂ centrifugations, followed by column chromatography on DEAE-cellulose and on

CL6B Sepharose [2,39]. After electrophoresis, the gel was fixed and stained with Toluidine Blue [0.02 % (w/v) in 3 % (v/v) acetic acid], destained overnight in distilled water, then equilibrated with 'Entensify' for 30 min, dried gently using in-house vacuum, and exposed to X-ray film.

Chondroitinase ABC digestion

The [35 S]sulphate-labelled extracts were treated for 40 min for chondroitinase ABC (0.125 unit/ml) in a buffer containing 50 mM Tris/HCl (pH 8.0), 60 mM sodium acetate, 5 mM phenylmethanesulphonyl fluoride, 10 mM *N*-ethylmaleimide and 10 mM EDTA. The digested products were examined by SDS/PAGE/fluorography [39].

Immunoprecipitation

The [35 S]sulphate-labelled matrix plus cell extracts were analysed by immunoprecipitation using chicken antiserum to rabbit aggregating proteoglycan monomer. This antibody showed no cross-reactivity against link protein [39]. For immunoprecipitation, preimmune serum or antiserum (10 μ l) was allowed to bind to Protein A-Sepharose for 4 h, in the presence of BSA (0.1 %, w/v) at 4 °C. The unbound antiserum was removed by extensive washing with a solution containing PBS (pH 7.2), Tween 20 (0.5 %, v/v) and BSA (0.1 %, w/v). The antibody bound to Protein A-Sepharose beads was further mixed with labelled cartilage extracts with constant rotation at 4 °C for 18 h; the unbound material was removed by extensive washing and the bound material was solubilized in SDS sample buffer and analysed by 3–15 % linear gradient SDS/PAGE after reduction with dithiothreitol. The bands were visualized by fluorography.

RESULTS

PDGF potentiates IL-1-induced collagenase/stromelysin synthesis

Previous studies have shown that PDGF enhances IL-1 α -induced neutral proteinase activity. In order to determine if the increase in neutral proteinase activity was due to an increase in the synthesis of the proteinases, rabbit articular chondrocytes were treated with IL-1 β (10 ng/ml) with or without PDGF AB (30 ng/ml) for 48 h in the presence of [35 S]methionine. The media were dialysed, electrophoresed on an SDS/polyacrylamide gel (3–15 % linear gradient), and the bands were visualized by fluorography. The results (Figure 1) indicate several differences among various treatments. The media of cells treated with PDGF alone contained at least two major proteins, of M_r 220 000 and 43 000, that are not detected in control cells. Of particular relevance to this study, cells treated with IL-1 synthesized two bands of M_r 57 000 and 53 000. Using an immunoprecipitation technique, we have previously identified the 57 000- M_r band as procollagenase, and the 53 000- M_r band is likely to be prostromelysin [24]. Cells treated with IL-1 + PDGF exhibited a 2–3-fold increase in these two bands. Control and PDGF-treated cells contained no discernible levels of these proteinases. These studies suggest that the increase in the synthesis of these enzymes may account for at least part of the increase in the proteinase activity.

PDGF potentiates IL-1-induced steady-state mRNA levels for collagenase and stromelysin

We next asked if the increased metalloproteinase synthesis correlated with an increase in the mRNA levels for these enzymes.

Cells were treated with IL-1 or IL-1 + PDGF for 4, 8, 18, 24 or 48 h. Total cellular RNA was then prepared by GdmCl extraction [30], and equal amounts of the RNA (2 μ g/slot) were probed with [32 P]oligonucleotides for collagenase and stromelysin. These probes have been shown previously by Northern-blot analysis to hybridize specifically with the mRNA of these enzymes [40]. The results confirm that mRNA levels for either enzyme were not detectable in control [40] and PDGF-treated cells (results not shown). A time-dependent increase in the mRNA levels was found for both stromelysin and collagenase in response to IL-1 (Figure 2). The peak levels were found by 24 h, but were greatly reduced by 48 h. In IL-1 + PDGF-treated cells, 3–4-fold higher levels of collagenase and stromelysin mRNA levels were found 24 h after treatment. The differences were not apparent at 48 h. These observations further suggest that PDGF potentiation of IL-1 activity on neutral proteinase synthesis is, at least in part, due to an increase in the steady-state mRNA levels for the enzymes. The reduction in mRNA levels in control cells at 48 h is likely to be because of prolonged maintenance in serum-free medium.

Figure 1 Collagenase/stromelysin synthesis and PDGF

Cells (10^5 /treatment) were treated with IL-1 β (10 ng/ml), PDGF AB (30 ng/ml) or both for 48 h in serum-free DMEM containing [35 S]methionine (40 μ Ci/ml). The conditioned media were dialysed, lyophilized and subjected to electrophoresis in an SDS/polyacrylamide (3% stacking and 3–15% running) gel, after reduction. The bands were visualized by fluorography. M_r values were determined on the basis of mobilities of high- M_r standards from Bio-Rad. Each lane contains counts from an equal number of cells. Lane 1, control; lane 2, cells treated with IL-1 β ; lane 3, cells treated with PDGF and IL-1 β ; lane 4, cells treated with PDGF.

Figure 2 PDGF and collagenase/stromelysin mRNA levels

Cells (5×10^6) were treated with 10 ng/ml IL-1 (tracks 1), 30 ng/ml PDGF (not shown) or IL-1 + PDGF (tracks 2) for 4, 8, 18, 24 and 48 h. Total RNA was extracted, equal amounts of RNA ($2 \mu\text{g/slot}$) were applied to 'Genescreen' and specific mRNAs were determined by Northern-blot hybridization using oligonucleotide probes for stromelysin and collagenase. A similarly prepared filter was also probed with oligo(dT) to verify the signal intensity in each slot.

Figure 3 PDGF induces ^{125}I -labelled IL-1 binding

Chondrocytes (2×10^5 per treatment) were treated with increasing concentrations of PDGF AB (0–30 ng/ml) for 18 h, washed and incubated with ^{125}I -labelled IL-1 (10 ng/ml) at 25°C for 4 h. A parallel set of cells was treated with TGF- β (5 ng/ml) + PDGF (0–30 ng/ml). The binding medium was removed, cells were extensively washed, the bound material was extracted with a lysing buffer (see the Materials and methods section), and the radioactivity was determined. Specific binding was determined by including a 1000-fold excess of unlabelled IL-1 β during the course of binding of the radioligand to the cells. All values are the average of four determinations.

PDGF Induces IL-1 receptors on chondrocytes

Since the activity of IL-1 requires an initial binding to a cell surface receptor (reviewed in ref. [38]), and IL-1 receptor number has been shown to be regulated by other growth factors [25,27], we wanted to determine if PDGF potentiation of IL-1 activity was due to its effects on IL-1 receptors. Chondrocytes were treated for 18 h with various concentrations PDGF AB, and specific binding of ^{125}I -labelled IL-1 β (10 ng/ml) was determined. We have established previously that, under the conditions described here, IL-1 binding represents true functional receptors [24,27]. In confirmation of previous studies with IL-1 α binding [28], our results (Figure 3) also show that PDGF increases IL-1 β binding in a concentration-dependent fashion. Since TGF- β , a

natural IL-1 antagonist, abolishes the expression of constitutive IL-1 receptors on chondrocytes [24], we also examined whether the PDGF-induced IL-1 receptors were also down-regulated by TGF- β . The results (Figure 3) show that PDGF induction of IL-1 receptors was inhibited by TGF- β at all concentrations of PDGF tested. This suggests that PDGF may enhance IL-1 activity by inducing additional receptors on chondrocytes and that TGF- β , a growth factor that inhibits IL-1 activity, also blocks PDGF induction of IL-1 receptors.

PDGF blocks IL-1-induced suppression of [^{35}S]sulphated macromolecular synthesis

Since IL-1 activity on cartilage involves not only the induction of destructive enzymes, but also inhibition of extracellular matrix proteins, we next questioned whether PDGF modifies another activity of IL-1, namely the inhibition of proteoglycan synthesis. This was initially assessed by determination of radioactive sulphate incorporation. Chondrocytes were treated with IL-1 (10 ng/ml), PDGF AB (30 ng/ml) or a combination of the two for 24 h. This concentration of IL-1 caused a maximal response on proteinase induction [24]. The PDGF concentration was based on the amount of growth factor needed to cause proteinase enhancement at suboptimal IL-1 (0.1 ng/ml) levels (A. K. Harvey and S. Chandrasekhar, unpublished work). During the last 4 h, cells were also incubated with $\text{Na}_2^{35}\text{SO}_4$ (40 $\mu\text{Ci/ml}$), the matrix plus cell layer was extracted, and the radioactive incorporation was determined. The results (Table 1) confirm that, in comparison with the control, IL-1 treatment resulted in a marked reduction of sulphate incorporation (about 30 % of control values). Cells treated with PDGF alone or those treated with a combination of IL-1 + PDGF incorporated about 120 % of the control values. These results indicate that PDGF alone can stimulate proteoglycan synthesis in chondrocytes and that it restores proteoglycan synthesis in IL-1-treated cells to that of control levels. These results were further confirmed using explants of rabbit articular cartilage (Table 1). These data suggest that PDGF, in contrast with its potentiating effects on neutral proteinase activity (Figures 1 and 2), was able to inhibit IL-1 suppression of proteoglycan.

The specificity of PDGF effects was examined by including a neutralizing antibody to PDGF AB in the assay. Chondrocytes were treated with various PDGF concentrations (0–30 ng/ml), and constant amounts of IL-1 β (10 ng/ml) plus PDGF antibody

Table 1 PDGF blocks IL-1 suppression of proteoglycan synthesis

Chondrocytes (10^5 /treatment) or rabbit tibial cartilage (5–10 mg wet wt.) were treated with DMEM, IL-1 (10 ng/ml), PDGF AB (30 ng/ml) or PDGF + IL-1. The treatment times were 44 h and 20 h for chondrocytes and cartilage respectively. The media were removed and the cells were subjected for an additional 4 h to the same treatments plus $\text{Na}_2^{35}\text{SO}_4$ (40 $\mu\text{Ci}/\text{ml}$). The cell layers (or explants) were rinsed, extracted with 4 M GdmCl + 0.01 M CHAPS and the radioactive incorporation was determined. The results are expressed as non-dialysable counts (d.p.m.) per 10^5 cells (chondrocytes; three experiments in triplicate $\pm$ S.D.) or per mg wet wt. (cartilage, one experiment).

Treatments	Sulphate incorporation (d.p.m.)	
	Chondrocytes	Cartilage
DMEM	1080 $\pm$ 170	3827
IL-1	320 $\pm$ 85	1756
PDGF	1653 $\pm$ 151	4846
IL-1 + PDGF	1487 $\pm$ 114	3305

Figure 4 Effect of anti-PDGF serum on $[^{35}\text{S}]$ sulphate incorporation in PDGF-induced chondrocytes

Rabbit articular chondrocytes (2×10^5 cells/treatment) were treated with IL-1 (10 ng/ml), PDGF AB (0–30 ng/ml) or both in serum-free DMEM. In a parallel treatment, PDGF with (b) or without (a) IL-1 was premixed with a neutralizing antibody to PDGF (30 $\mu\text{g}/\text{ml}$). After a 44 h treatment, the media were removed, and cells were further subjected to the same treatments in the presence of $\text{Na}_2^{35}\text{SO}_4$ (40 $\mu\text{Ci}/\text{ml}$) for 4 h. The cells were washed, extracted with 4 M GdmCl + 0.01 M CHAPS, and the radioactive incorporation was determined. The values represent the average of three determinations $\pm$ S.D. ●, PDGF; ○, PDGF + antibody; ■, IL-1 + PDGF; □, IL-1 + PDGF + antibody.

(30 $\mu\text{g}/\text{ml}$). The results show that the antibody to PDGF was able to inhibit PDGF-stimulated sulphate incorporation (Figure 4a) and the ability of PDGF to reverse IL-1-induced inhibition of sulphate incorporation (Figure 4b).

Identification of $[^{35}\text{S}]$ sulphated molecules as proteoglycans (aggrecan)

We next confirmed the proteoglycan nature of the $[^{35}\text{S}]$ sulphated molecules by (a) analysing them in a composite polyacrylamide gel, followed by fluorography, (b) treating with chondroitinase ABC, and (c) subjecting them to immunoprecipitation using an antiserum against intact rabbit proteoglycan. Analysis of cell layer plus matrix on agarose/acrylamide gels confirms that a band co-migrating with standard rat aggregating proteoglycan

Figure 5 Composite gel electrophoresis of proteoglycans

GdmCl extracts from an equal number of $[^{35}\text{S}]$ sulphate-labelled cells (Table 1) were subjected to electrophoresis on a composite gel composed of agarose and acrylamide. The radioactivity was visualized by fluorography after emersion of the gel in 'Entensify' and exposure of the dried gel to X-ray. The standard rat aggrecan was prepared from chondrosarcoma and the mobility was determined by staining the gel with Toluidine Blue.

Figure 6 Characterization of aggrecan

Chondrocytes were treated with IL-1, PDGF or both for 44 h and then labelled with $[^{35}\text{S}]$ sulphate for 4 h as in Table 1. The radiolabelled extracts were further digested with chondroitinase ABC (a) or immunoprecipitated with a polyclonal antiserum against rabbit proteoglycan raised in chickens (b and c). All samples were analysed by SDS/PAGE (3–15% linear gradient resolving and 3% stacking gel). The radioactive bands were visualized by fluorography using 'Entensify'. The M_r of the major band is $> 350\,000$, and was determined by reference to Bio-Rad high- M_r standards (not indicated).

(aggrecan) was present in control, but not in IL-1-treated, cells (Figure 5). Cells treated with PDGF alone contained higher levels of the same band than the control cells. Cells treated with IL-1 + PDGF synthesized approx. 80% of the control values. These results further confirm that PDGF was able to block IL-1 activity on proteoglycan loss from chondrocyte extracellular matrix. Interestingly, cells treated with IL-1 or IL-1 + PDGF also contained a faster migrating band that was not present (or present at very low levels) in control or PDGF-treated cells. It is not clear whether it represents a degradation product of the large aggregating proteoglycan or the lower- M_r proteoglycans.

In order to establish the proteoglycan nature of these molecules further, the samples were also treated with chondroitinase ABC

and were analysed in an SDS/polyacrylamide gel. More than 95% of the sulphate label was associated with a band migrating at $M_r > 350000$ and was susceptible to chondroitinase ABC digestion (Figure 6a). In IL-1-treated cells, this band was virtually absent or present at greatly reduced levels. Cells treated with PDGF contained two to three times more of this band than the controls. The [35 S]sulphate-labelled molecules, after immunoprecipitation with anti-(rabbit aggrecan), were analysed by SDS/PAGE/fluorography (Figures 6b and 6c). We have previously shown that this antibody recognized the core protein of the large aggregating proteoglycan [36]. In both control and PDGF-treated cells, but not in IL-1-treated cells, a band of $M_r > 350000$ was immunoprecipitable. The results confirm that the sulphated molecule is the aggregating proteoglycan, namely aggrecan. The results further establish that (a) PDGF alone can induce the synthesis of aggrecan and (b) PDGF is able to block the ability of IL-1 to suppress proteoglycan synthesis.

Relative effects of PDGF and FGF on IL-1-suppressed aggrecan synthesis

Since PDGF potentiated proteinase synthesis, but antagonized IL-1-induced aggrecan suppression, we next asked whether this differential activity is unique to PDGF. Previous studies have demonstrated that another growth factor, FGF, also potentiated IL-1-induced collagenase and stromelysin synthesis [24,26]. Therefore we compared the two growth factors with respect to neutral proteinase activity and aggrecan synthesis. This was tested at a constant concentration of IL-1 β (10 ng/ml) and various concentrations of PDGF or FGF (0–1 pM). The results show that, at any given concentration, both PDGF and FGF were effective in potentiating IL-1-induced metalloproteinase synthesis, with FGF being slightly more effective than PDGF (Table 2). With respect to proteoglycan synthesis, however, PDGF, but not FGF, was able to reverse the inhibition of sulphate incorporation induced by IL-1 and induce proteoglycan synthesis in the absence of IL-1 (Table 3). These results suggest that the two functions affected by IL-1, namely, proteinase induction and proteoglycan synthesis inhibition, occur via distinct pathways. Whereas PDGF and FGF affect the IL-1-induced proteinase activity similarly, they display distinctly different activities with respect to proteoglycan synthesis.

Distinct modulation of PDGF effects by TGF- β

The differential regulation of proteinase induction and proteoglycan synthesis was further confirmed by testing the ability of TGF- β to transmodulate PDGF effects on the two aspects of IL-1 activity. Our previous studies have shown that TGF- β blocks IL-1 effects on proteinase induction and proteoglycan suppression [24]. For this experiment, chondrocytes were treated with IL-1 with or without PDGF in the presence or absence of TGF- β , and neutral proteinase activity and sulphate incorporation were determined. The results show that TGF- β inhibits both IL-1- and PDGF-potentiated neutral proteinase activity (Table 4). In contrast, TGF- β stimulates proteoglycan synthesis either alone or in combination with IL-1 (Table 4). Further, the sulphate incorporation in cells treated with TGF- β + PDGF + IL-1 was higher than that of PDGF + IL-1-treated cells, suggesting that TGF- β effects can be additive to PDGF effects on sulphate incorporation. These results suggest that TGF- β will block proteinase production irrespective of the method of induction (IL-1 versus IL-1 + PDGF), but, under identical conditions,

Table 2 Relative potentiation effects of PDGF and FGF on IL-1-induced neutral proteinase activity

Rabbit chondrocytes (2×10^5 cells/treatment) were treated with a constant concentration of IL-1 (10 ng/ml) and various concentrations of PDGF AB or FGF for 48 h in serum-free DMEM. The neutral proteinase activity released into the media was assayed using [3 H]casein as substrate (see the Materials and methods section). The values represent the average of four determinations $\pm$ S.D.

Growth factor concn. (nM)	Neutral proteinase activity	
	IL-1 + PDGF	IL-1 + FGF
0 (IL-1 alone, 10 ng/ml)	2.8 $\pm$ 0.5	2.8 $\pm$ 0.5
0.10	3.2 $\pm$ 0.5	4.0 $\pm$ 0.5
0.50	4.4 $\pm$ 0.6	5.7 $\pm$ 0.6
1.0	4.9 $\pm$ 0.5	6.4 $\pm$ 0.5

Table 3 Relative effects of PDGF and FGF on IL-1 suppression of aggrecan synthesis

Chondrocytes (2×10^3 /treatment) were treated with a constant concentration of IL-1 (3 ng/ml) and various concentrations (0–1 nM) of either PDGF or FGF (as in Table 1); the media were removed after 44 h, washed and the cells were incubated with fresh treatments of the same factors + Na $_2^{35}$ SO $_4$ (40 μ Ci/ml) for an additional 4 h period (Table 1). The matrix plus cell layer was extracted as before, and the radioactivity determined. The values represent an average of three determinations $\pm$ S.D.

Growth factor concn. (nM)	10 $^{-3} \times$ [35 S]Sulphate incorporation (d.p.m.)			
	PDGF		FGF	
	– IL-1	+ IL-1	– IL-1	+ IL-1
0	4.2 $\pm$ 0.5	1.8 $\pm$ 0.2	4.2 $\pm$ 0.5	1.8 $\pm$ 0.2
0.1	3.9 $\pm$ 1.2	1.6 $\pm$ 0.7	4.1 $\pm$ 0.7	1.9 $\pm$ 0.7
0.5	7.0 $\pm$ 0.8	3.4 $\pm$ 0.7	4.2 $\pm$ 0.3	1.3 $\pm$ 0.1
1.0	10.1 $\pm$ 1.1	4.9 $\pm$ 0.4	2.9 $\pm$ 0.8	1.2 $\pm$ 0.4

Table 4 TGF- β modulation of PDGF effects on neutral proteinase and proteoglycan synthesis

Chondrocytes grown to confluency in a 96-well microtitre plate (neutral proteinase assay) or 24-well plate (sulphate incorporation) were washed free of serum with PBS and subjected to indicated treatments. All dilutions were made in DMEM. For proteinase assay, the media were collected after 48 h and assayed for total enzyme activity using [3 H]casein as substrate (see the Materials and methods section). For sulphate incorporation, the media were removed after 44 h, washed and the cells were incubated with fresh treatments of the same factors + Na $_2^{35}$ SO $_4$ (40 μ Ci/ml) for an additional 4 h period (Table 1). The matrix plus cell layer was extracted as before, and the radioactivity determined. The results are the average of four determinations and the variations were within 10% of the mean.

Treatments	Neutral proteinase (units)	10 $^{-3} \times$ sulphate incorporation (d.p.m.)
DMEM	0.01	10.1
IL-1 β (10 ng/ml)	0.78	3.3
PDGF-AB (30 ng/ml)	0.01	22.0
IL-1 + PDGF	2.80	12.5
TGF- β (10 ng/ml)	0.01	48.0
IL-1 + TGF- β	0.01	36.0
IL-1 + PDGF + TGF- β	0.01	34.2
PDGF + TGF- β	0.01	54.1

TGF- β effects on proteoglycan synthesis are additive to those of PDGF on IL-1-treated cells. These observations further establish that the pathways leading to the production of proteinase and suppression of proteoglycan synthesis by IL-1 are likely to involve distinct post-receptor mechanisms.

DISCUSSION

Extracellular matrix remodelling occurs during normal turnover, development and in response to injury [12,23,41,42]. Excessive cartilage matrix turnover is a feature of degenerative joint diseases such as osteoarthritis and rheumatoid arthritis, leading to the eventual erosion of cartilage [7–11,41]. Homeostasis of cartilage must involve a balance between the synthesis of the matrix components and their degradation [4–6]. Although the normal turnover of matrix molecules is not understood, IL-1, a pro-inflammatory cytokine, is believed to play an important role in cartilage remodelling and destruction associated with pathological conditions [14–22]. In this report, we provide evidence that PDGF, a growth factor that is a product of several cell types and released during wound healing in other soft tissues, could play an important role in moderating IL-1-induced cartilage degradation. We show that PDGF enhances IL-1-induced proteinase activity by inducing higher levels of the steady-state mRNA levels for both stromelysin and collagenase and a similar increase in the biosynthesis of the proteins. However, in contrast with its effects on proteinases, PDGF reverses the inhibitory effect of IL-1 on cartilage aggregating proteoglycan (aggrecan) synthesis. These results are consistent with the suggestion that PDGF may play a critical role in cartilage remodelling during inflammatory conditions by promoting matrix lysis and matrix regeneration.

The ability of PDGF to potentiate IL-1 activity was established by three approaches: (a) determination of enzyme activity using [3 H]casein as the substrate; (b) analysis of [35 S]methionine-labelled proteins by SDS/PAGE; and (c) determination of steady-state mRNA levels for collagenase and stromelysin, using oligonucleotide probes specific for these enzymes. The potentiation of proteinase activity by PDGF has been previously demonstrated, using IL-1 α as the inducer [28]. Our present study, using IL-1 β , confirms that observation and further extends it to show that the increased activity was due to an increase in the synthesis of the enzyme, as measured by an increase in the steady-state mRNA levels and biosynthetic protein products. IL-1 activity was potentiated by all three isoforms of PDGF, namely PDGF AB, PDGF BB and PDGF AA, with PDGF AB being the most active (results not shown). Further, PDGF alone does not induce any detectable metalloproteinase in rabbit articular chondrocytes; instead, it potentiates IL-1-induced enzyme synthesis. Our previous studies with other growth factors have also indicated that only IL-1 was able to induce metalloproteinases, whereas FGF and TGF- β modulated IL-1 activity [24,25,27]. PDGF appears to follow a similar trend and enhances IL-1 activity. In comparison with FGF, however, PDGF appears to be less potent (Table 2). Finally, the PDGF potentiation of IL-1 effects were antagonized by TGF- β , suggesting that PDGF and FGF are likely to act similarly in inducing proteinase production and can be inhibited by agents such as TGF- β .

Since IL-1 inhibits the synthesis of the large aggregating proteoglycan aggrecan, we also examined whether PDGF could influence IL-1-mediated suppression of aggrecan synthesis. The results show that PDGF alone increased sulphated macromolecular synthesis in a concentration-dependent fashion. PDGF+IL-1 co-treatment resulted in the reversal of the inhibitory effects of IL-1. The identity of the proteoglycan as the

aggregating proteoglycan, aggrecan, was established by several criteria: (1) [35 S]sulphate incorporation into chondrocyte and cartilage matrix and its digestibility with chondroitinase ABC; (2) electrophoretic co-migration with purified rat aggrecan in a composite agarose/acrylamide gel; (3) immunoprecipitation of the [35 S]sulphated molecules using an antiserum that primarily recognizes the core protein of aggrecan, and its relative migration position of $M_r > 350\,000$ [39]; (4) by immunoblotting experiments using composite agarose/acrylamide electrophoresis, using 2-B-6 monoclonal antibody against the terminal chondroitin 4-sulphate [43] (results not presented); (5) finally, our previous studies have established that these molecules bound strongly to hyaluronate-Sepharose affinity columns and were dissociable only by a strong chaotropic agent, 4 M GdmCl [39]. These results establish that PDGF stimulated proteoglycan synthesis independently and that it restored proteoglycan synthesis in IL-1-treated chondrocytes (monolayer) and cartilage (explant) cultures.

The mechanism of PDGF antagonism of IL-1-induced proteoglycan suppression remains to be established. This could be (a) a direct over-riding effect of PDGF on proteoglycan synthesis or (b) an actual interference in the IL-1 pathway or both. We have previously shown that TGF- β inhibited IL-1-induced neutral proteinase synthesis while enhancing proteoglycan synthesis [24,25,27]. These studies suggested that IL-1 activity on metalloproteinase induction and matrix protein suppression could share common post-receptor pathways. However, several observations now suggest that these two effector functions (proteinase production and matrix synthesis) may occur via independent pathways. (1) PDGF (this study) and TGF- β [25] can directly stimulate proteoglycan synthesis in the absence of any exogenous IL-1 but have no direct effect on proteinase production. (2) FGF potentiates IL-1-induced proteinase activity and promotes IL-1 receptor induction [24], but has no effect on proteoglycan synthesis (Table 3). (3) TGF- β antagonizes IL-1 effects by down-regulating proteinase synthesis and IL-1 receptor expression and up-regulating proteoglycan synthesis [24,27]. (4) PDGF potentiates IL-1-induced proteinases, up-regulates IL-1 receptors [28], but reverses IL-1-induced proteoglycan synthesis (this study). (5) Insulin-like growth factors I and II promote proteoglycan synthesis, but have no effect on proteinase induction by IL-1 [44–47]. These results collectively imply that (a) IL-1 receptor modulation correlates only with proteinase induction and not with proteoglycan synthesis and (b) the two aspects of matrix remodelling can occur independently of each other. Thus a dichotomy in activity appears to exist that sets PDGF apart from other growth factors (FGF and TGF- β) that modulate IL-1 activity. It is the only example where an induction of IL-1 receptors allows for continued response to IL-1 with respect to proteinase activity, but not the other aspect of IL-1 activity, namely suppression of proteoglycan synthesis. These results also highlight the complex interplay of factors present during inflammation and further emphasize the importance of a balance in their expression for cartilage homeostasis.

The relevance and the proposed role of PDGF in cartilage remains to be established. PDGF is a wound-healing growth factor that is present in circulating blood, and therefore can become available to cartilage [48–51]. It is also synthesized locally by a variety of cells, including chondrocytes [52]. Human PDGF exists in three isoforms, namely PDGF AA, PDGF BB and PDGF AB [48–51]. The response of a given cell is likely to be determined both by the availability of a specific PDGF and by the type of PDGF receptor expressed. Although chondrocytes have recently been shown to synthesize PDGF AA [52], the observation that PDGF AA is the least active form with respect

to both proteinase and proteoglycan synthesis ([28]; A. K. Harvey and S. Chandrasekhar, unpublished work) would suggest a paracrine, rather than an autocrine, role for PDGF in cartilage. This, however, is only speculative. Irrespective of the type of activity, PDGF could function as a wound-healing hormone in cartilage metabolism by increasing the clearance of damaged matrix and at the same time initiating an early repair response that will lead to eventual matrix synthesis and assembly, as proposed for other soft tissues [41,53,54].

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