Interleukin-1 β regulation of fibroblast proteoglycan synthesis involves a decrease in versican steady-state mRNA levels

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This study investigates the effects of interleukin (IL)-1 β on proteoglycan metabolism by fibroblasts surrounded by endogenous extracellular matrix. In both three-dimensional matrix cultures and long-term monolayer cultures IL-1 β caused a significant decrease in synthesis and deposition of sulphated proteoglycans, but had no effect on release of deposited material. The decrease in synthesis became successively more pronounced, and corresponded to 40–60 % of the control after 72 h incubation. The reduction was almost totally accounted for by an effect on the chondroitin ABC-lyase-sensitive proteoglycans. Gel electrophoresis showed a significant decrease in a high-molecular-mass chondroitin ABC-lyase-sensitive proteoglycan after incubation with IL-1 β . Northern-blot analyses of total RNA revealed a

pronounced decrease in the steady-state mRNA levels of versican, the large chondroitin sulphate, with levels corresponding to 10–30 % of controls. In comparison, the steady-state mRNA level for decorin, the major sulphated proteoglycan synthesized by the cells, was only slightly affected. The prominent decrease in synthesis of sulphated proteoglycans induced in long-term fibroblast cultures, including the pronounced decrease in versican steady-state mRNA levels, is likely to have a significant effect on the structure of the extracellular matrix. Induction of this type of change may constitute a significant mechanism whereby IL-1 β can affect the properties of connective tissue during inflammation and wound healing.

INTRODUCTION

Interleukin-1 (IL-1), one of the central mediators of inflammation, is known to modulate extracellular-matrix metabolism in a number of ways (Oppenheim et al., 1986). Thus IL-1 causes an increased synthesis of metalloproteases by chondrocytes (Di Pasquale et al., 1986; Pasternak et al., 1986) and an increased release of proteoglycans from cartilage (Saklatvala et al., 1985; Tyler, 1985a; Ratcliffe et al., 1986). Further, it has been shown to cause a decrease in collagen synthesis (Goldring et al., 1988) and a decrease in synthesis of sulphated glycosaminoglycans (Tyler, 1985b; Ikebe et al., 1986, 1988; Arner and Pratta, 1989; Yaron et al., 1989; Kolibas and Goldberg, 1989; Seckinger et al., 1990) by these cells.

Human diploid fibroblasts bear specific receptors for IL-1 (Dower et al., 1985; Qwarnström et al., 1988), and IL-1 is known to influence extracellular-matrix metabolism by these cells. Thus IL-1 induces release of collagenase (Mizel et al., 1981; Postlethwaite et al., 1983) and an increased synthesis of proteoglycan-degrading enzymes (Postlethwaite et al., 1983). Further, it causes an increase in synthesis of collagen (Postlethwaite et al., 1988) and of hyaluronic acid (Bocquet et al., 1985; Yaron et al., 1989; Postlethwaite et al., 1989; Seckinger et al., 1990). IL-1 has also been shown to induce an increased synthesis of sulphated glycosaminoglycans by fibroblasts (Yaron et al., 1987, 1989; Langris et al., 1987). Incubation with IL-1 β of fibroblasts growing in an endogenous matrix causes collapse of the matrix, and a decreased amount of sulphated proteoglycans, as indicated by decreased immunocytochemical staining (Qwarnström et al., 1989).

The present study examines the effects of IL-1 β on proteoglycan metabolism in long-term fibroblast cultures, which could

be responsible for the induced structural alterations. The results show that incubation with IL-1 of fibroblasts maintained in culture for an extended period of time, and surrounded by abundant extracellular matrix, results in a pronounced decrease in synthesis and deposition of sulphated proteoglycans, and further, that this affects in particular the chondroitinase-sensitive sulphated proteoglycans. The greatest relative effect is induced on the large chondroitin sulphate, versican (Zimmermann and Ruoslahti, 1989), involving a significant decrease in steady-state mRNA levels of its core protein.

MATERIALS AND METHODS

Cell cultures

Human fibroblasts, grown from gingival biopsies (Narayanan and Page, 1976) obtained from four different donors and designated HGF₅₁, HGF₅₂, HGF₅₄ and HGF₆₀, were used between passages 4 and 10, grown in endogenous matrix cultures or in monolayers. Cells were propagated in monolayer cultures in 75 cm² tissue-culture flasks (Corning Glassware, Corning, NY, U.S.A.) in medium containing 10 % fetal-calf serum (FCS; Gibco Laboratories, Grand Island, NY, U.S.A.). After harvest by a brief treatment with trypsin (0.05 %)/EDTA (0.02 %), cells were washed twice with Dulbecco–Vogt medium containing 10 % FCS, and seeded confluent (1700 cells/well) in Terasaki plates (Lux HLA plates; Miles Laboratories, Naperville, IL, U.S.A.). The wells had been previously coated with purified collagen type I derived from human placenta (gift from Dr. A. S. Narayanan, University of Washington, Seattle, WA, U.S.A., or purchased from Sigma, St. Louis, MO, U.S.A.) (1.5–2 mg/ml in 0.1 M acetic acid). Under these conditions, fibroblasts synthesize and inhabit a three-dimensional endogenous matrix, which after

Abbreviations used: IL-1, interleukin-1; FCS, fetal-calf serum; CPC, cetylpyridinium chloride; PDGF, platelet-derived growth factor; TGF- β , transforming growth factor- β .

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3 weeks in culture contains proteoglycans and fibronectin (Qvarnström et al., 1989). Cells were fed with fresh medium once every 48 or 72 h, and the matrix cultures were given ascorbic acid (50 µg/ml) daily. In addition, monolayer cultures were plated sub-confluently (10000 cells/well) in 96-well plates (Falcon; Becton Dickinson Labware, Oxnard, CA, U.S.A.), grown for 5–7 days, and fed as above without ascorbate added. For comparison, some cultures were maintained in DV medium containing 1% FCS for 5 days before incubation with IL-1β.

Cell proliferation

Cells were harvested and grown in monolayers (96-well plates; 8000 cells/well) in 10% FCS for 7 days, incubated with or without IL-1 for various times (0, 24 h, 48 h and 72 h) and harvested with trypsin (0.05%)/EDTA (0.02%). Cell number was determined for triplicate cultures by using a Coulter counter (Coulter Electronics, Hialeah, FL, U.S.A.) or a haemocytometer.

Total protein determination

For determination of total protein synthesis, cells were labelled for 24 h with [³⁵S]methionine (25 µCi/ml; NEN, Boston, MA, U.S.A.). Supernatant was collected and cell-layer protein extracted with NH₃ (0.5 M).

Trichloroacetic acid precipitates (5% trichloroacetic acid/0.5% tannic acid, 16 h, 4 °C) from medium and cell layer were washed (once with 5% trichloroacetic acid/0.5% tannic acid; once with acetone, –20 °C), and radioactivity incorporated was determined by liquid-scintillation counting (Packard Tri-Carb, model 1500).

[³⁵S]Sulphate incorporation into proteoglycans

Cells were incubated with various concentrations of IL-1β (0.1 pM–0.1 µM) for 72 h, or for various times (8, 16, 24, 48 or 72 h) with IL-1β (2 nM) and 10% FCS, or left in Dulbecco–Vogt medium containing 10% FCS. In addition, cultures grown for 5 days in 1% serum were incubated for various times (see above) with or without IL-1β (2 nM) in the presence of 1% serum. During the last 4 h of the incubation period, medium was replaced with Dulbecco–Vogt medium with or without IL-1, containing [³⁵S]sulphate (carrier-free, 200 µCi/ml) (ICN, Irvine, CA, U.S.A.). Subsequently supernatants were removed, the cultures rinsed with PBS, and supernatants and rinses pooled. Matrix and cell-layer proteoglycans were extracted in 8 M urea containing 0.25 M NaCl, 2 mM EDTA, 0.3% Triton X-100, and protease inhibitors (5 mM benzamidine, 100 mM 6-amino-hexanoic acid) in Tris/HCl (50 mM) buffer, pH 7.4 (urea extraction buffer). Quantitative changes in the rate of incorporation of label into proteoglycans were determined by precipitation with cetylpyridinium chloride (CPC) as described by Wasteson et al. (1973). Briefly, samples (50 µl) of medium, and extracted cell-layer samples from triplicate cultures, were spotted in duplicates on Whatman 3MM chromatography paper, dried and washed (5 × 1 h; 1% CPC/0.05 M NaCl). Radioactivity remaining was determined by liquid-scintillation counter (Packard Tri-Carb, Model 1500). Results of duplicate determinations of each triplicate were averaged, the total radioactivity per sample was normalized to cell number, and data were expressed as a percentage of control. Deposition was calculated by determining the incorporated radioactivity in the cell layer, and data are

expressed as a percentage of control. Control values for the various cell strains ranged from 16000 to 220000 c.p.m./100000 cells and 37000 to 230000 c.p.m./100000 cells in monolayers and matrix cultures respectively.

Pulse-chase experiments

After a 1 h incubation in sulphate-free medium, cells were incubated with [³⁵S]sulphate (100 µCi/ml) for 16 h to obtain maximal labelling of extracellular-matrix proteoglycans (Qvarnström et al., 1992). Subsequently, the cultures were rinsed extensively (four times with PBS and four times with Dulbecco–Vogt medium over 10 min), which decreased unincorporated label present in the cell layer at the beginning of the chase period to 1.5–2.5% of the remaining label as determined by PD-10 column chromatography of labelled matrices extracted with NaOH (0.25 M) (results not shown). Cultures were incubated with various concentrations of IL-1β (0.1 pM–0.1 µM) in the presence of 10% FCS for 48 h or for various times (0, 8, 16, 24 or 48 h) with or without IL-1β (2 nM). Medium and cell-layer samples were harvested as above, by using 8 M urea extraction buffer. Total radioactivity in both medium and cell layer was determined by liquid-scintillation counting. Background radioactivity present in the medium at zero time was subtracted for each time point and the data were expressed as percentages of radioactivity in the medium, and normalized to cell number.

SDS/PAGE

After radiolabelling of proteoglycans and extraction in 8 M urea (urea extraction buffer), samples were applied to a DEAE-Sephacel minicolumn (300 µl). Columns were washed with 8 M urea in 50 mM Tris/HCl buffer containing 2 mM EDTA, 0.3% Triton X-100 and 0.25 M NaCl (60 bed vol.). Material was eluted from the column with 2 M NH₄HCO₃ (Dower and Dwek, 1979) and subsequently with 8 M urea extraction buffer containing 3 M NaCl. Recovery from the column was greater than 90%. The NH₄HCO₃ eluate, subjected to vacuum centrifugation (model SVC 100E; Savant Instruments, Hicksville, NY, U.S.A.), and the ethanol precipitate (4 vol. of 1.3% potassium acetate in 95% ethanol, with 750 µg/ml chondroitin sulphate as carrier) of the salt (8 M urea/3 M NaCl) eluate, were pooled, before resolubilization in sample buffer (1% SDS/1.7% β-mercapto-ethanol). Samples (equal number of c.p.m./lane) were run on an SDS/PAGE gradient gel (3–10%) poured on Gelbond (FMC Bioproducts, Rockland, ME, U.S.A.) with a 2.5% discontinuous stacking gel, or run on a 4–10% SDS/PAGE gradient gel without gel bond by using a 3% discontinuous stacking gel (Laemmli, 1970). Gels were subsequently dried and placed on Kodak X-Omat AR-2 film for 3–6 weeks at –70 °C.

Selective digestion of the [³⁵S]sulphate-labelled material

Monolayers and three-dimensional matrix cultures were labelled for 4 h with [³⁵S]sulphate (200 or 100 µCi/ml) or for 24 h with [³⁵S]methionine (25 µCi/ml; ICN), harvested as described above with 8 M urea extraction buffer without protease inhibitors, applied on DEAE-Sephacel, and eluted with 8 M urea/3 M NaCl. Precipitate (4 vol. of 1.3% potassium acetate in 95% ethanol, with carrier 750 µg/ml chondroitin sulphate) from medium/rinse and cell-layer samples were subjected to Pronase digestion (0.3 mg/ml, 16 h, 55 °C) in enriched Tris buffer (0.3 M Tris, 0.06% BSA, 18 mM sodium acetate, pH 8). Subsequently, half of each sample from control and IL-1β-treated cultures was digested with chondroitin ABC lyase (2.5 units/ml) (ICN

Biochemicals, Cleveland, OH, U.S.A.) in enriched Tris buffer (Saito et al., 1968). To determine the relative effect on chondroitin and dermatan sulphates, separation of intact and degraded portions of digested and undigested [35 S]sulphate-labelled samples was done by column chromatography on Sephadex G-50 in 0.05 M Tris/HCl buffer. The IL-1-induced decrease in the enzyme-sensitive portion was determined and expressed as a percentage of the decrease in total incorporation. In addition, samples from some experiments using sulphate or methionine label were resolubilized in sample buffer, and separated by SDS/PAGE as above.

Immunoprecipitation of decorin

[35 S]Sulphated samples were reduced with 5 mM dithiothreitol for 5 min at 95 °C and incubated overnight at 4 °C in the dark with 35 mM iodoacetamide. After reduction and alkylation, the samples were dialysed with 3 changes over 24 h against Tris-buffered saline (TBS) with 0.1% Triton X-100 and protease inhibitors. Dialysed samples were pre-adsorbed against normal rabbit serum and immunoprecipitated by incubating the samples overnight with rabbit anti-(human skin fibroblast decorin) (provided by courtesy of Dr. Hans Kresse, University of Munster, Germany; Glossl et al., 1984), bound to Protein A-Sepharose 4B (Sigma), and washed five times in TBS, pH 7.2, containing 1% Triton X-100, 2.5 mM EDTA, 0.1% SDS, 1 mg/ml BSA and protease inhibitors as above and 1 mM phenylmethanesulphonyl fluoride. Samples were prepared for SDS/PAGE by extraction of the bead-bound antigen-antibody complexes with acetic acid (1 M), and extracted material was freeze-dried, boiled in sample buffer containing β -mercaptoethanol for 5 min and run on SDS gradient gels (3–10% acrylamide) as above.

RNA extraction and Northern transfer analysis

Total RNA was isolated from matrix and monolayer cultures of three different cell lines, by the single-step method as described by Chomczynski and Sacchi (1987). The yield was 1–10 μ g of RNA/ 10^5 cells. The A_{260}/A_{280} ratio of the RNA samples was 1.7 or higher.

RNAs (20 μ g/lane) were electrophoresed overnight (15–16 h) in 1% (w/v) agarose gels containing 16.7% (v/v) of 37% (w/w) formaldehyde (Fisher Laboratories) (Maniatis et al., 1982). After electrophoresis, RNAs were transferred from agarose gels to nitrocellulose filters (Bethesda Research Laboratories Life Technologies) as described by Thomas (1980). After transfer, the filters were baked at 80 °C for 2 h. Before hybridizing specific cDNA probes, the filters were prehybridized for at least 2 h at 42 °C in a solution containing 50% (v/v) formamide (Bethesda Research Laboratories Life Technologies), 6 $\times$ SSPE (1 $\times$ SSPE = 0.15 M NaCl, 0.2 M NaH₂PO₄ and 0.02 M Na₄EDTA), 5 $\times$ Denhardt's solution (1 $\times$ Denhardt's solution = 0.02% Ficoll, 0.02% polyvinylpyrrolidone and 0.02% BSA), 0.05% (w/v) SDS, 100 μ g/ml salmon testes DNA (Sigma) and 100 μ g/ml *Torula* yeast RNA (Calbiochem). Hybridizations of the filters with 32 P-labelled (nick-translation with the Nick Translation System from Bethesda Research Laboratories Life Technologies, and 5'-[α - 32 P]dCTP, from Amersham Corp.) cDNA probes [sp. radioactivity (0.2–1.0) $\times 10^8$ c.p.m./ μ g of DNA] were carried out at 42 °C in the same solution as above. The hybridization time was at least 16 h, whereafter the filters were washed twice with 2 $\times$ SSPE/0.1% SDS and twice with 0.1 $\times$ SSPE / 0.1% SDS for 15–20 min/time at 42–52 °C,

depending on the cDNA probe used for the analysis. After washing, the filters were air-dried and then autoradiographed at –70 °C by using Kodak XAR2 film for 8, 16 or 96 h. After autoradiography, fluorograms were scanned as described above with a densitometer (model GS 300; Hoefer Scientific Instruments, San Francisco, CA, U.S.A.). Hybridization of the filters with a cDNA probe for 28S rRNA was used for normalization of the loading of RNA on gels (Iruela-Arispe et al., 1991).

cDNA probes

The following cDNA probes were used: for large chondroitin sulphate proteoglycan, cDNAs encoding the C-terminal region and the middle region (corresponding to the sequences between base pairs 6412 and 8125 and 2607 and 6092, respectively) of the large human fibroblast proteoglycan, versican, kindly provided by Dr. E. Ruoslahti and Dr. R. LaBaron, La Jolla Cancer Research Foundation, Cancer Research Center, La Jolla, CA, U.S.A. (Zimmermann & Ruoslahti, 1989); for decorin (PG-II), a cDNA encoding the full-length core protein of bovine bone PG-II, kindly provided by Dr. M. Young, Bone Research Branch, National Institute of Dental Research, NIH, Bethesda, MD, U.S.A. (Day et al., 1987). For 28S rRNA, a cDNA fragment of 280 base pairs of bovine 28S ribosomal subunit was used, kindly provided by Dr. H. Sage, University of Washington, Seattle, WA, U.S.A.

RESULTS

Incubation of long-term fibroblast monolayers and matrix cultures with IL-1 β resulted in a concentration-dependent decrease in the rate of synthesis of sulphated proteoglycans (Figure 1). In contrast, pulse-chase experiments revealed no effect on release of deposited material after incubation with IL-1 β , over a range of concentrations (Figure 1). A concentration of IL-1 β of

Figure 1 Concentration-dependence of IL-1 β incubation on synthesis (●) and release (□) of sulphated proteoglycans

Synthesis experiments: cells in matrix and monolayer cultures were incubated with various concentrations of IL-1 β (0.1 pM–0.1 μ M) for 72 h. During the last 4 h of incubation, medium was replaced with fresh medium with and without IL-1 β containing [35 S]sulphate (200 μ Ci/ml). Pulse-chase experiments: cells were incubated with [35 S]sulphate (100 μ Ci/ml) for 16 h, rinsed and subsequently chased with medium containing various concentrations of IL-1 β (0, 1 mM–0.1 μ M) for 48 h. Medium and cell-layer samples were harvested, and radioactivity incorporated was determined by CPC precipitation and counted as described in the Materials and methods section. Data for synthesis experiments were calculated as total incorporated radioactivity and expressed relative to control. Data for pulse-chase experiments were calculated as radioactivity released in the medium as a percentage of total, related to cell number, and data are expressed relative to control.

Table 1 Effects of incubation with IL-1 β on proliferation and protein synthesis

Cells were plated in 96-well plates in 10% FCS as described in the Materials and methods section and grown for 7 days, and subsequently incubated for the indicated times with or without IL-1 β (1 nM) in the presence of 10% FCS. For proliferation experiments triplicate cultures were harvested by using trypsin (0.05%)/EDTA (0.02%), and cell number was determined with a Coulter counter or haemocytometer. Data shown are means $\pm$ S.E.M. for 2 experiments. For determination of total protein synthesis, cells were incubated with [35 S]methionine (25 μ Ci/ml) during the last 24 h of incubation, as indicated. Medium and cell-layer samples were harvested as described in the Materials and methods section, and radioactivity incorporated in triplicate cultures was determined by scintillation counting after precipitation with trichloroacetic acid.

Time (h)	Proliferation		Total protein synthesis (c.p.m./10 ³ cells)	
	Control	IL-1	Control	IL-1
0	17 355 $\pm$ 2028			
24	20 372 $\pm$ 912	20 938 $\pm$ 702	2749 $\pm$ 221	2097 $\pm$ 104
48	26 792 $\pm$ 2443	28 372 $\pm$ 670	2994 $\pm$ 339	2104 $\pm$ 87
72	31 140 $\pm$ 723	32 754 $\pm$ 670	2980 $\pm$ 250	2556 $\pm$ 400

2 nM, known to saturate receptor binding on these fibroblasts (Qvarnström et al., 1988), also gave maximal effects on proteoglycan synthesis and was chosen for subsequent experiments. This concentration of IL-1 had no significant effect on total protein synthesis, nor on cell proliferation in the presence of serum (Table 1). In addition, incubation had no effect on release of deposited proteoglycans, either on the rate of release (Figure 2a), or on the composition of the extracellular matrix (results not shown). The rate of synthesis of proteoglycans, however, decreased successively over a 72 h period of incubation (Figure 2b). In the monolayer cultures the rate of synthesis was slightly increased at early times, reaching about 115% after 4 h incubation with IL-1 β . Longer treatment resulted in a decrease which was most pronounced after 72 h incubation, corresponding to about 45% of control values. A similar effect was seen in the three-dimensional matrix cultures (Figure 2b). During the first 24 h of IL-1 β incubation, the effect on synthesis of sulphated glycosaminoglycans was slight, corresponding to 90–100% of control values. The continued presence of IL-1 β resulted in a decrease in sulphate incorporation to 75% and 40–50% of controls after 48 h and 72 h respectively. In both types of cultures, the relative rate of deposition of the labelled molecules into the cell-associated extracellular matrix paralleled the relative rate of synthesis (Figure 2b). In similar experiments carried out in the presence of 1% serum, IL-1 had no effect on proteoglycan synthesis (results not shown).

Enzyme treatment of synthesized material from medium and cell layer showed a pronounced decrease in the proportion of chondroitin ABC-lyase-sensitive sulphated proteoglycans in IL-1 β treated cultures, from 69 $\pm$ 4% to 21 $\pm$ 7% relative to control (results not shown). This decrease corresponded to 95 $\pm$ 10% of the decrease in total [35 S]sulphate incorporation, indicating a specific effect on chondroitin and dermatan sulphates.

SDS/PAGE of labelled material revealed a pronounced decrease in the relative amount of a high-molecular-mass band [Figures 3a and 3b, band (I)] after incubation with IL-1 β , whereas the minor proteoglycan with an apparent molecular mass of about 250 kDa was unaltered. The affected proteoglycan had an apparent molecular mass above 500 kDa. The pro-

Figure 2 Relative rates of release (a) and of synthesis and deposition (b) of sulphated proteoglycans incorporated into the cell layer

(a) Sulphated proteoglycans synthesized by cells grown in monolayers or in three-dimensional matrix cultures and incorporated into the cell layer were labelled with [35 S]sulphate (100 μ Ci/ml; 16 h). Subsequently cultures were rinsed and incubated with (■) or without (○) IL-1 β (2 nM) for various times as indicated. The radioactivity in medium and extracted cell-layer samples from triplicate cultures was determined, and data are expressed as percentage of total released into the medium. The graph represents the average $\pm$ S.E.M. of three separate experiments. The total radioactivity (medium + cell layer) in IL-1 β -treated cultures corresponded to 101 $\pm$ 15% and 103 $\pm$ 29% of control in monolayer and matrix cultures respectively. (b) Cells grown as above were incubated with or without IL-1 β (2 nM) for the indicated times, and, during the last 4 h of incubation, in the additional presence of [35 S]sulphate (200 μ Ci/ml). Triplicate media and cell-layer samples were harvested, and incorporation into proteoglycans was assayed by CPC precipitation as described in the Materials and methods section. Data, including total synthesis (●), are normalized to cell number and expressed as percentage of control. Deposition (□) is defined as the amount of radioactivity incorporated in the cell layer and expressed as percentage of control. The graph represents the average $\pm$ S.E.M. of three separate experiments.

portional decrease in this band was initially observed after 8 h of incubation [Figure 3a, band (I)] and became successively more pronounced. The relative decrease in synthesis caused a proportional decrease in the deposition of this high-molecular-mass material in the cell layer after 16 h [Figure 3b, band (I)]. The same effect was observed in medium and cell-layer samples from matrix cultures (results not shown). Incubation with IL- β also caused a slight decrease in the size of decorin, the major sulphated proteoglycan synthesized by the cells [Figure 3a, band (II)] (Qvarnström et al., 1992). This decrease in molecular mass was supported by immunoprecipitation of the 120 kDa molecule with a polyclonal antibody against decorin (Glossl et al., 1984) (Figure 3c).

Digestion of the sulphate- and methionine-labelled material showed that the high-molecular-mass proteoglycan, affected by incubation with IL-1 β , was chondroitin ABC-lyase-sensitive [Figures 4a and 4b, band (I)], and revealed core proteins of above 200 kDa, characteristic of versican (Zimmermann and Ruoslahti, 1989; Schonherr et al., 1991). Further, as expected, decorin was sensitive to chondroitin ABC lyase [Figures 4a and 4b, band (II)] and digestion showed core protein bands of about 45 kDa.

Northern analyses demonstrated that gingival fibroblasts express mRNA transcripts for versican (Figure 5a) (Zimmermann and Ruoslahti, 1989) and decorin (Krusius and Ruoslahti, 1986)

Figure 3 SDS/PAGE of proteoglycans in monolayer cultures

Media and cell-layer samples from monolayer cultures, untreated or treated with IL-1 β for various times and labelled, were subjected to DEAE-Sephacel column chromatography. The NH_4HCO_3 - and salt-eluted material was prepared as described in the Materials and methods section and separated on a 3–10% gradient gel with a 1.5% discontinuous stacking gel. In addition, some of the eluate was subjected to immunoprecipitation using an antibody against decorin, as described in the Materials and methods section, before gel electrophoresis. I, band above 500 kDa; II, band at 120 kDa (decorin). (a) Medium samples. (b) Cell-layer samples. (c) Immunoprecipitation of decorin.

(Figure 5b), the latter supporting our earlier immunoprecipitation studies (Qvarnström et al., 1992). After incubation with IL-1 β , there was a pronounced decrease in steady-state mRNA levels for versican (Table 2; Figure 5a). Thus in IL-1 β -treated monolayer cultures the steady-state level of versican mRNA corresponded to 10% of that of the control (Table 2; Figure 5a). Similar effects were seen with the matrix cultures, where versican mRNA levels decreased to 30% of control after incubation with IL-1 β (Table 2; Figure 5a). In comparison, IL-1 had much less effect on decorin mRNA, with levels corresponding to about 60% and 90% of controls in monolayers and matrix cultures respectively (Table 2; Figure 5b).

DISCUSSION

These studies show that IL-1 β has a prominent effect on synthesis of sulphated proteoglycans by fibroblasts maintained in long-term cultures and surrounded by endogenous extracellular matrix, but causes no changes in release of deposited material. The decrease in synthesis involved a decrease primarily in chondroitin and dermatan sulphates. The effect was partly caused by a pronounced decrease in mRNA levels for versican, the large chondroitin sulphate. In comparison, there was much less of a decrease in mRNA levels for decorin, the major sulphated proteoglycan synthesized by the cells.

The pronounced decrease in proteoglycan synthesis observed in this study, involving the decrease in mRNA levels for versican, is in contrast with a number of known effects of IL-1, induced after activation of general transcriptional enhancers such as c-jun (Alvarez et al., 1991). Indeed, IL-1 has been shown to cause an increase in synthesis of sulphated proteoglycans (Yaron et al., 1987, 1989; Langris et al., 1987) as well as augmented mRNA levels for PGII/decorin (Heino et al., 1988). The different response observed in the present study could be due to altered receptor levels, induced during a long time in culture (Terracio et

al., 1988). Alterations in receptor levels could also be caused by variations in serum concentration, and thus the amount of platelet-derived growth factor (PDGF) (Bonin and Singh, 1988) and transforming growth factor- β (TGF- β) (Dubois et al., 1990). In addition, PDGF and TGF- β in serum could affect the IL-1 response by regulating control levels of proteoglycan synthesis (Kahari et al., 1991; Schonherr et al., 1991). However, the level of serum alone cannot explain the differences in results, since IL-1 had no significant effect on proteoglycan synthesis in the low (1%) serum experiments. The pronounced decrease could in addition reflect an effect of accumulated extracellular matrix (Bade and Nitzgen, 1985; Roberts et al., 1981, 1983), as indicated by ongoing experiments revealing a reversal of the effect in cells plated for short times on tissue-culture plastic (E. E. Qvarnström, unpublished work). Since IL-1 receptors are localized at focal adhesions (Qvarnström et al., 1988) and IL-1 binding to its receptor causes rapid phosphorylation of talin (Qvarnström et al., 1991), a transmembrane protein linking the fibronectin receptor with the cytoskeleton, alterations in cell-matrix interactions may influence the response.

The lack of an effect on release of proteoglycans from the matrix differs from the documented effects of IL-1 in cartilage (Saklatvala et al., 1985), and could indicate that stromelysin synthesized (Mizel et al., 1981; Murphy et al., 1985; Okada et al., 1986; Saus et al., 1988) and released (MacNaut et al., 1990) during IL-1 incubation does not become activated. Further, potential degradation of proteoglycans by proteases released in the presence of IL-1 (Saklatvala, 1987; Ghosh et al., 1987; Pasternak et al., 1986) may be blocked by protease inhibitors in serum (Herman et al., 1987).

The pronounced relative decrease in versican is similar to the effects in cartilage, where incubation with IL-1 causes a significantly greater decrease in synthesis of the large chondroitin sulphate than in the small interstitial proteoglycans (Morales and Hascall, 1989). Since versican constitutes only between 15

Figure 4 SDS/PAGE of proteoglycans from cultures subjected to chondroitin ABC-lyase digestion and immunoprecipitation

(a) Medium samples from monolayer cultures were labelled with [35 S]sulphate, and prepared as described in Figure 3. Before suspension in solubilization buffer, half of each sample was subjected to chondroitin ABC-lyase (Ch. ABC) digestion and subsequently separated on a 4–12% gradient gel with a 3% discontinuous stacking gel. Band above 500 kDa (I); decorin (II). (b) Medium samples labelled with [35 S]methionine (25 μ Ci/ml) were harvested, and prepared as described above (Figure 3). Half of each sample was digested with chondroitin ABC-lyase as above, and subsequently analysed by SDS/PAGE (3–10%). Band above 500 kDa (I); high-molecular-mass core protein bands after digestion (arrowheads); decorin (II); core protein bands of about 45 kDa (arrow).

Table 2 Effects of incubation with IL-1 β on steady-state mRNA levels

Monolayer and matrix cultures of three different strains of fibroblasts were grown as described in the Materials and methods section and incubated with IL-1 β for 72 h. Subsequently, cell extracts were harvested and total RNA was prepared as described in the Materials and methods section. Data are normalized to cell number and expressed as ratios of IL-1 β -treated samples to controls. Samples (20 μ g/lane) were run on agarose gels and blots were incubated with labelled cDNAs for versican and decorin (PGII) respectively, dried, and films were exposed for 8, 15.5 and 96 h. Fluorograms were scanned and the values relative to control (IL-1 β /control) have been corrected for the signal from the 28S rRNA probe for each lane. Numbers represent averages $\pm$ S.E.M. for 2–4 experiments.

	Monolayer cultures	Matrix cultures
Versican	0.11 $\pm$ 0.03	0.30 $\pm$ 0.05
Decorin	0.60 $\pm$ 0.06	0.94 $\pm$ 0.18

Figure 5 Northern-blot analyses of steady-state mRNA levels for versican (a) and decorin (b)

Cells grown in monolayers (MI) or in three-dimensional matrix cultures (Mx) were incubated without (control) or with IL-1 β (2 nM) for 72 h, whereafter the cells were harvested for RNA isolation as described in the Materials and methods section. RNA samples (20 μ g/lane) were run overnight on a denaturing agarose gel and transferred to a nitrocellulose filter as described in the Materials and methods section. The filters were probed with a nick-translated cDNA fragment for versican (a) or decorin (b) and subsequently with a nick-translated cDNA fragment for 28S rRNA, the latter used for normalization of the loading of the RNA samples. The positions of the 28S and 18S rRNA markers were determined by ethidium bromide staining of the gel.

and 25% of the sulphated proteoglycans (E. E. Qvarnström, unpublished work), even a pronounced decrease of 70–90%, estimated from Northern analysis, is unlikely to account for the observed decrease in total sulphate incorporation. Additional changes most likely involve primarily decorin, the major chondroitin ABC-lyase-sensitive proteoglycan synthesized by the cells (Qvarnström et al., 1992), since the decrease could be accounted for by an effect on chondroitin and dermatan

sulphates. The decrease in sulphate incorporation into decorin, and the slight decrease in molecular mass of this molecule, could reflect alterations in the size of the glycosaminoglycan chains (Schonherr et al., 1991). Potential alterations in the core protein (Benton and Tyler, 1988) are thought to involve changes in the rate of translation or to be due to post-translational modifications, since Northern analysis revealed only minor effects on the steady-state mRNA levels of decorin.

The pronounced decrease in synthesis involving only the chondroitin ABC-lyase-sensitive proteoglycans, as well as the different alterations induced on versican and decorin, indicate specificity of the IL-1-induced effects for the various proteoglycans, as has been shown for PDGF and TGF- β (Kahari et al., 1991; Schonherr et al., 1991, 1993). The different responses could, in addition, in part be due to indirect effects. The delay in the decrease of total sulphate incorporation, likely involving changes in decorin, relative to the decrease in versican supports this notion. It has previously been shown that proliferation induced by IL-1 is due to PDGF secretion (Raines et al., 1989); and modulation of cartilage extracellular matrix by IL-1 has been suggested to be due in part to IL-6 release (Nietfeld et al., 1990).

We attribute the collapse of the fibroblast synthesized matrix, observed in our earlier study (Qwarnström et al., 1989), to the effect on sulphated proteoglycans. Although the change in matrix structure was accompanied by a decrease in immunocytochemical staining for chondroitin and dermatan sulphates, there was no change in staining of fibronectin, the other major component of the extracellular matrix (Qwarnström et al., 1989). Further, as shown in Table 1, IL-1 has no pronounced effect on total protein synthesis (Heino et al., 1988; Postlethwaite et al., 1988). Proteoglycans are known to participate in maintaining spatial arrangements in connective tissue (Hascall and Hascall, 1981). Both chondroitin and dermatan sulphates are known to interact with fibronectin (Yamagata et al., 1986; Schmidt et al., 1987; Lewanovska et al., 1987; Brennan et al., 1983). Decorin, the major sulphated proteoglycan deposited in the three-dimensional matrix (Qwarnström et al., 1992), is involved in collagen fibrillogenesis (Vogel et al., 1984). A pronounced general decrease in synthesis and deposition of these matrix components is likely to affect the three-dimensional structure of connective tissue. In addition, the relatively more extensive decrease in versican synthesis could play a major role in causing the matrix collapse induced by incubation with IL-1 β (Qwarnström et al., 1989). Versican has been suggested to participate in cell-extracellular-matrix interactions and/or in interactions between extracellular-matrix components (Zimmermann and Ruoslahti, 1989), and has been shown to bind hyaluronic acid (LeBaron et al., 1992). Assuming a similar function of versican in fibroblast-synthesized connective tissue to that of aggrecan in cartilage (Hardingham and Muir, 1972), a significant decrease in the level of this molecule, induced by IL-1, could have a profound effect on the hydrodynamic properties of the matrix.

In summary, these studies show that a major effect of IL-1 β on fibroblasts embedded in extracellular matrix is to cause a decrease in synthesis of sulphated proteoglycans. In addition, they demonstrate that the decrease is primarily due to a pronounced effect on the chondroitinase-sensitive proteoglycans, involving a marked decrease in mRNA levels for versican, the large chondroitin sulphate. Such changes could have a pronounced effect on the three-dimensional structure of soft connective tissue and may be a major mechanism of tissue destruction and resorption in areas of chronic inflammation. In addition, this type of alteration induced by IL-1 during inflammation and wound healing, resulting in collapse of the extracellular matrix,

could serve to facilitate cell migration and proliferation at a site of injury.

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