Human recombinant interleukin-1 regulates cellular mRNA levels of dermatan sulphate proteoglycan core protein

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Human skin fibroblasts were exposed to various concentrations (from 0.01 to 5.0 units/ml) of human recombinant interleukin-1 α and interleukin-1 β (IL-1 α and IL-1 β). Both IL-1 α and IL-1 β were found to increase dermatan-sulphate-proteoglycan (DSPG) core-protein mRNA levels. Maximal increase (3.0-fold) was seen at 48 h after addition of 1 unit of IL-1 β /ml. In spite of the elevated DSPG-core-protein mRNA only a slight increase (from 10 to 18%) could be seen in the production of DSPG to cell-culture medium. No changes in the molecular mass of DSPG could be detected.

INTRODUCTION

Interleukin-1 (IL-1) is a connective-tissue regulating factor produced in mononuclear phagocytes. The cDNA clones for two molecular forms of IL-1 have been described. (Auron *et al.*, 1984; March *et al.*, 1985). They code two structurally distinct peptides, termed 'IL-1 α ' and 'IL-1 β ', corresponding to anionic (pI 5.2/5.4) and neutral (pI 7.0) subtypes respectively. In spite of the fact that the homology these molecules is less than 30% (March *et al.*, 1985), they bind to a common fibroblast surface receptor (Chin *et al.*, 1987) and their biological effects are similar (Rupp *et al.*, 1986).

IL-1 is a potent regulator of connective-tissue metabolism. It increases the synthesis of neutral proteinases (Mizel *et al.*, 1981; Mochan *et al.*, 1986; Murphy *et al.*, 1986) and prostaglandin E₂ (Mizel *et al.*, 1981) by fibroblasts. Thus IL-1 acts as an initiative factor of extracellular-matrix degradation. Furthermore, IL-1 may also participate in connective-tissue remodelling. It is a mitogen for fibroblasts (Rupp *et al.*, 1986; Schmidt *et al.*, 1982) and regulates the synthesis of type I and type III collagen by increasing the corresponding mRNA levels (Kähäri *et al.*, 1987).

Mononuclear-cell- and macrophage-derived factors are known to increase glycosaminoglycan production by fibroblasts (Jalkanen, 1981; Bocquet *et al.*, 1985; Langris *et al.*, 1987). Recent studies have suggested that this is caused by IL-1 activity (Langris *et al.*, 1987; Hamerman & Wood, 1984; Yaron *et al.*, 1987). Recombinant IL-1 β has been shown to increase mainly the production of hyaluronic acid, but also that of sulphated proteoglycans (Yaron *et al.*, 1987). Here we show that recombinant IL-1 α and IL-1 β can also regulate the mRNA levels of dermatan sulphate proteoglycan (DSPG) core protein in normal skin fibroblasts.

MATERIALS AND METHODS

Fibroblasts obtained from an explanted skin biopsy of a healthy adult were grown in Dulbecco's modification

of Eagle's minimum essential medium (DMEM; GIBCO, Paisley, Renfrewshire, Scotland, U.K.) supplemented with antibiotics (100 i.u. of penicillin G/ml and 50 μ g of streptomycin sulphate/ml), buffer (23 mM-bicarbonate) and 10% (v/v) fetal-calf serum (FCS; GIBCO). Cells from the fourth to the eighth passage were used in the experiments.

Human recombinant IL-1 α and -1 β (1000 i.u./ml), prepared from *Escherichia coli*, were purchased from Genzyme Corporation, Boston, MA, U.S.A. [10⁸ units (mouse thymocyte assay) of IL-1 $\equiv$ 1 mg of protein].

Cells were grown to confluency and preincubated for 24 h in DMEM supplemented with 2% FCS, ascorbic acid (50 μ g/ml) and different concentrations of IL-1 α and -1 β (0.01, 0.1, 1.0 and 5.0 units/ml). After the 24 h preincubation period, similar fresh medium was added, but also containing β -aminopropionitrile (β -APN) (50 μ g/ml). After a 24 h incubation period cell layers were harvested for extraction of total cellular RNA.

In order to measure the kinetics of IL-1 action on DSPG-core-protein mRNA levels, confluent fibroblast cultures were first preincubated in DMEM supplemented with 2% FCS for 24 h. IL-1 β was added to the medium (final concentration 1 unit/ml) and the cells were harvested at different time points (2, 4, 6, 12, 24, 33 and 48 h) for extraction of total cellular RNA.

Total cellular RNA was isolated by using the proteinase-K-digestion-phenol/chloroform-extraction-ethanol-precipitation method as described by Rowe *et al.* (1978).

For Northern blots, 12 μ g portions of each RNA sample were fractionated by electrophoresis in 0.75%-agarose gel after denaturing the RNA with glyoxal (Thomas, 1980). RNA was transferred to Pall Biotryne A nylon membrane (Pall Corporation, Glen Cove, NY, U.S.A.) and immobilized on the membrane by baking at 80 °C for 2 h. Hybridizations were performed using cDNA probes nick-translated with [α -³²P]dCTP (800 Ci/mmol; Amersham International) to specific radioactivities of approx. 5 $\times$ 10⁷ c.p.m./ μ g. The cDNA probes used in hybridizations were PG40, specific for

Abbreviations used: β -APN, β -aminopropionitrile; DMEM, Dulbecco's modification of Eagle's medium; DSPG, dermatan sulphate proteoglycan; FCS, fetal-calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-1, interleukin-1.

Fig. 1. Effects of different concentrations of IL-1 α and IL-1 β on levels of DSPG core protein mRNA

Human skin fibroblasts were grown to confluency and exposed to various concentrations of IL-1 α or IL-1 β in the medium supplemented with 2% FCS. After 48 h the cells were harvested and total cellular RNA was isolated. Aliquots (12 μ g) of RNA were fractionated by electrophoresis on a 0.75% agarose gel and transferred to Pall Biotrans A nylon filter. The filter was subsequently hybridized with 32 P-labelled nick-translated cDNA probes specific for human-DSPG-core-protein mRNA. Abbreviation: C, control. The arrows indicate the positions of specific mRNAs for the cDNA probe. The positions of 28 S and 18 S rRNAs are indicated.

human DSPG-core-protein mRNA (Krusius & Ruoslahti, 1986), and pRGAPDH13, specific for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA (Fort *et al.*, 1985). Hybridization was revealed by autoradiography with Kodak X-Omat films at -70°C with the use of intensifying screens. The extent of hybridization was quantified with densitometric scans of X-ray films.

To measure the rate of DSPG production, fibroblast cultures were incubated 24 h in the presence of 2% FCS and with or without 1 unit of IL-1 β . Fresh medium was added, with or without 1 unit of IL-1 β /ml and 40 μ Ci of [35 S]methionine (sp. radioactivity > 800 Ci/mmol; New England Nuclear, Boston, MA, U.S.A.)/ml. In expt. I the medium was serum-free, and in expt. II it contained 2% FCS. After 24 h incubation, medium was collected, dialysed against water and freeze-dried. Immunoprecipitations were performed by using specific anti-DSPG antibodies (Krusius & Ruoslahti, 1986) and protein A-coated Sepharose (Pharmacia, Uppsala, Sweden). Immunoprecipitates were diluted in O'Farrell's (1975) electrophoresis sample buffer and analysed by SDS/polyacrylamide-gel electrophoresis (O'Farrell, 1975) and autoradiography (Pulleyblank & Booth, 1981).

RESULTS AND DISCUSSION

In cell culture, human skin fibroblasts synthesize several different proteoglycans. The major sulphated product is a small-sized DSPG (Krusius & Ruoslahti, 1986). This proteoglycan binds specifically to collagen and may be involved in the control of collagen fibrillogenesis (Scott, 1984; Vogel *et al.*, 1984). In the present study, we have measured the effects of IL-1 on

Fig. 2. Time-dependence of IL-1 β action

Human skin fibroblasts were grown to confluency and placed into medium supplemented with 1 unit of IL-1 β /ml and 2% FCS. The cells were harvested at the time points indicated in the Figure and total cellular RNA was extracted. Aliquots (12 μ g) of RNA were analysed by Northern blotting with subsequent hybridization to 32 P-labelled nick-translated cDNA probes specific for human DSPG-core-protein mRNA. The arrows indicate the positions of specific mRNAs for the cDNA probe. The positions of 28 S and 18 S rRNAs are indicated.

mRNA levels of DSPG core protein by using a cDNA probe specific for small-sized DSPG (Krusius & Ruoslahti, 1986).

In the concentration-dependency experiment, fibro-

Table 1. Time-dependency of IL-1 β action

Human skin fibroblasts were cultured in medium containing 2% FCS. IL-1 β was added and cellular RNA extracted after different incubation times. DSPG and GAPDH mRNA levels were measured with Northern-blot hybridizations and densitometric scannings of autoradiograms.

	DSPG mRNA levels (arbitrary densitometric units/ 12 μ g of RNA)							
	C	Incubation time (h)...2	4	6	12	24	33	48
Raw value	4.2	5.2	4.3	4.8	8.1	8.2	7.7	7.7
Value when correlated with GAPDH mRNA levels	4.2	5.6	4.7	4.8	6.5	8.2	8.1	12.4

Table 2. Effect of human recombinant IL-1 β on dermatan sulphate proteoglycan (DSPG) production in culture skin fibroblasts

Fibroblast cultures were preincubated 24 h with medium containing 2% FCS, with or without 1 unit of IL-1 β /ml. After preincubation cells were labelled in fresh medium with 40 μ Ci of [³⁵S]methionine/ml in the presence (1 unit/ml) or absence of IL-1 β . In expt. I (a) the incubation medium was serum-free, whereas in expt. II (b) it contained 2% FCS. DSPG was immunoprecipitated with specific antibodies, and the radioactivity in precipitates was counted. The amount of DSPG in the immunoprecipitates was also estimated by using SDS/polyacrylamide-gel electrophoresis, autoradiography and densitometric scans. Results are means $\pm$ S.E.M. for four parallel samples.

(a) Expt. I

Conditions	10 ⁻³ $\times$ Immunoprecipitation with anti-DSPG antibodies [radioactivity (c.p.m.)]	Densitometric analysis of electrophoresis (densitometric units)
Control	21.8 $\pm$ 1.0 (100 %)	9.5 $\pm$ 0.8 (100 %)
IL-1 β (1 unit/ml)	25.8 $\pm$ 2.1 (118 %)	11.1 $\pm$ 0.4 (117 %)

(b) Expt. II

Conditions	10 ⁻³ $\times$ No. of cells/cm ²	10 ⁻³ $\times$ Total trichloroacetic acid-precipitated radioactivity (c.p.m.)	10 ⁻³ $\times$ Immunoprecipitation with anti-DSPG antibodies [radioactivity (c.p.m.)]
Control	40.3 $\pm$ 1.5	936.4 $\pm$ 32.0 (100 %)	22.4 $\pm$ 1.0 (100 %)
IL-1 β (1 unit/ml)	39.4 $\pm$ 2.9	974.6 $\pm$ 35.6 (104 %)	24.6 $\pm$ 1.2 (110 %)

blasts were cultured 48 h in the presence of IL-1 α or IL-1 β (Fig. 1). We have previously shown that IL-1 can increase procollagen mRNA levels (Kähäri *et al.*, 1987). To study the effects of IL-1 on DSPG mRNA levels under the same cell-culture conditions, we used, in the first experiment, ascorbic acid and β -APN in the culture medium. IL-1 α increased DSPG-core-protein mRNA levels from 1.4- to 1.7-fold (1.0 unit and 5.0 units/ml); a similar increase was also seen with IL-1 β (1.4-, 1.3- and 1.1-fold increases with 0.1 unit, 1.0 unit and 5.0 units/ml respectively). These results suggest that the effects of IL-1 α and IL-1 β are similar. In previous studies also the biological activities of IL-1 subtypes have been identical (Rupp *et al.*, 1986). To avoid potential mistakes in loading the agarose gels and to indicate the specificity of IL-1 action, cellular DSPG-core-protein mRNA levels were, in all experiments, correlated with GAPDH mRNA levels. The GAPDH enzyme is known to be a 'housekeeping protein' that is not under hormonal

regulation, and for that reason is a suitable control for mRNA measurements (Fort *et al.*, 1985).

The action of IL-1 β was studied further by measuring DSPG-core-protein mRNA levels at different time points after IL-1 was added (Fig. 2; Table 1). In this experiment neither ascorbic acid nor β -APN was present. In 12 h IL-1 β increased DSPG-core-protein mRNA levels 1.6-fold, and a maximal increase (3.0-fold) was seen after 24 h (Table 1). Our previous experiments suggest that the increase seen in pro- α 1(I) and pro- α 1(III) collagen as well as in fibronectin mRNA levels is larger and also faster (Kähäri *et al.*, 1987; A. Mauviel, unpublished work).

Immunoprecipitation assays suggested that about 2.5% of all ³⁵S-containing trichloroacetic acid-insoluble material is DSPG (Table 2). This is in accordance with previous studies (Krusius & Ruoslahti 1986). Under cell-culture conditions used in the present study, IL-1 had no effect on the cell number (Table 2). In spite of the

Fig. 3. SDS/polyacrylamide-gel electrophoresis of immunoprecipitated DSPG

Fibroblast cultures were preincubated 24 h with medium containing 2% FCS and IL-1 β (1 unit/ml). After preincubation, cells were labelled with 40 μ Ci of [35 S]-methionine/ml in the serum-free and methionine-free medium, which contained also IL-1 β (1 unit/ml). DSPG was immunoprecipitated with specific antibodies, and the molecular size of DSPG in the immunoprecipitates was estimated by using SDS/polyacrylamide-gel electrophoresis and autoradiography. Lane 1, control sample; lane 2, IL-1 treated sample; lane 3, second precipitation of sample 1. The sizes of standard proteins are indicated.

elevated mRNA levels, only a very slight increase (from 10 to 18%) could be seen in the DSPG production (Table 2). In a previous study, Yaron *et al.*, (1987) proposed that human recombinant IL-1 β slightly stimulates the synthesis of sulphated glycosaminoglycans. The stimulation was much smaller than the IL-1-induced increase in hyaluronic acid production. In their study the rate of DSPG-core-protein synthesis was not estimated. Langris *et al.* (1987) used a gel-filtration fraction of mononuclear-cell medium containing IL-1 activity and described an enhancement in the incorporation of radioactive sulphate into proteoglycan fraction of the fibroblast culture medium. They proposed that this was mainly due to increased length of glycosaminoglycan side chains, the increase in core-protein synthesis being of less significance. In the present study, however, the results of SDS/polyacrylamide-gel electrophoresis suggested that human recombinant IL-1 β does not change the molecular size of DSPG (Fig. 3).

To conclude, our results confirm the previous data suggesting that IL-1 stimulates DSPG production only slightly. However, we did not observe any change in the

molecular mass of DSPG. In our previous studies IL-1 enhanced DSPG-core-protein mRNA levels less than it did those of procollagen and fibronectin mRNA. However, the increase in DSPG mRNA levels was much greater than that observed in DSPG production.

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