Stimulation of sulphated glycosaminoglycan and decorin production in adult dermal fibroblasts by recombinant human interleukin-4

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Interleukin-4 (IL-4) is a pleiotropic cytokine expressed by inflammatory cells. Previous work from our laboratory has shown that it stimulates collagen synthesis in fibroblasts. Here we report the effects of recombinant human IL-4 on glycosaminoglycan (GAG) and proteoglycan synthesis in normal dermal fibroblasts from adult donors. IL-4 (10 and 100 units/ml) induced a dose-dependent increase of [^{3}H]glucosamine and [^{35}S]sulphate incorporation into total GAGs. The analysis of the different GAG fractions indicated the enhanced synthesis of dermatan/chondroitin sulphates. IL-4 had no effect on hyaluronan synthesis. The increase of sulphated GAG synthesis was correlated with an increase of proteoglycans in the culture medium. Decorin was identified as the major chondroitin/dermatan sulphate-containing proteoglycan in the culture medium of fibroblasts. Its synthesis was strongly stimulated by IL-4. Both the core-protein synthesis and mRNA expression were enhanced, indicating that the cytokine acted, at least in part, at the pre-translational level. These results indicate that IL-4 is able to modulate not only collagen, but also proteoglycan, production by human fibroblasts. Their implications in physiopathological processes such as wound healing or fibrosis is suggested.

INTRODUCTION

Interleukin-4 (IL-4) is a pleiotropic cytokine produced by stimulated T-cells, basophils and mast cells. It belongs to the modulatory cytokines (for a review, see [1]). In inflammatory cells, IL-4 functions via inositol phosphate [2] and tyrosine kinase [3] intracellular signalling pathways using a novel DNA-binding factor belonging to the STF family [4]. IL-4 has dramatic effects on T- and B-lymphocytes and mast cells, and is implicated in immunological response during inflammation. Fibroblasts express high affinity receptors for IL-4 under soluble and membrane bound forms [5,6]. IL-4 is a chemoattractant and, under some conditions, a mitogen for dermal and lung fibroblasts [7,8].

In previous papers, we showed that IL-4 stimulated collagen synthesis in dermal fibroblasts [9,10]. Fibronectin synthesis was also increased by the cytokine [11]. We and others suggested that elevated levels of IL-4 in inflammation and healing may contribute to the deposition of extracellular matrix in inflammation-induced fibrous-tissue formation [6,9,11].

Extracellular matrix is a complex structure composed of many types of macromolecules which interact with connective-tissue cells to maintain tissue integrity and functionality. Proteoglycans and hyaluronan are ubiquitous components of extracellular matrix. Their synthesis may be modulated by cytokines (for a review, see [12]). Dermal fibroblasts are able to synthesize hyaluronan, the major non-sulphated GAG, and at least three extracellular proteoglycans containing dermatan/chondroitin sulphate chains, namely decorin, biglycan and versican [13]. Decorin is the main proteoglycan secreted *in vitro* by cells into the culture medium. It interacts with type I collagen to control fibril diameter [14–16].

The present study demonstrated a stimulation of sulphated GAG synthesis by IL-4 in normal dermal fibroblasts and identified decorin as the major extracellular proteoglycan stimulated by the cytokine.

MATERIALS AND METHODS

Materials

Purified recombinant human IL-4 from *Escherichia coli* (specific activity 10^7 units/mg) was generously given by Schering–Plough Research (Bloomfield, NJ, U.S.A.). The radioactive precursors ([α-^{32}P]dCTP, sp. radioactivity 111 TBq/mmol; [^{3}H]leucine, sp. radioactivity 6.6 TBq/mmol; [^{3}H]glucosamine, sp. radioactivity 1.5 TBq/mmol; $H_2{}^{35}SO_4$, carrier-free) were from New England Nuclear (Paris, France). Pronase (from *Streptomyces griseus*) was from Boehringer-Mannheim (Meylan, France). Chondroitin ABC lyase (EC 4.2.2.4, from *Proteus vulgaris*) was from Seikagaku Kogyo (Tokyo, Japan). Glycosaminoglycan standards, reagents for Northern-blot analysis and immunoprecipitation were from Sigma (La Verpillière, France). The reverse transcriptase-PCR (RT-PCR) kit was from Perkin–Elmer/Cetus (Norwalk, CT, U.S.A). Reagents for cell cultures were from Gibco (Cergy-Pontoise, France), except sulphate and leucine-free minimal essential medium (MEM), from Institut Jacques Boy (Reims, France). Rabbit anti-(human decorin) polyclonal antiserum was from Chemicon (Temecula, CA, U.S.A.).

Cell cultures

Human dermal fibroblasts were obtained from normal adult skin or from child foreskin during plastic or reconstructive surgery, with the informed consent of the patients. Human embryo lung

Abbreviations used: DPBS, Dulbecco's PBS; GAG, glycosaminoglycan; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-4, interleukin-4; RT-, reverse transcriptase; SSC, standard saline/citrate; (D)MEM, (Dulbecco's) minimal essential medium; FCS, foetal-calf serum.

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MRC-5 fibroblasts were from Institut Merieux (Lyon, France). Cells were grown using routine techniques in Dulbecco's MEM (DMEM) supplemented with 10% (v/v)] foetal-calf serum (FCS). Only cultures between passages 4 and 6 were used. For metabolic labelling, a series of four confluent 25 cm^2 Costar culture flasks were incubated for 24 h with 740 kBq/ml [^{35}S]-sulphate and either 370 kBq/ml [^{3}H]glucosamine or 370 kBq/ml [^{3}H]leucine in sulphate- and leucine-free medium containing 2% (v/v) dialysed FCS and convenient concentrations of IL-4.

Glycosaminoglycan and proteoglycan characterization

The culture medium and cell layer were collected separately. The cell layer was washed twice with Dulbecco's PBS (DPBS). For GAG isolation, the culture medium was heated for 5 min at 95 °C. The cell layer was dissolved in 0.1 M NaOH, and an aliquot was taken for protein measurement [17]. The pH of the remainder was adjusted to 8.0 with 1 M acetic acid. The extracts were digested for 24 h at 48 °C with 0.4 mg/ml Pronase in 0.05 M Tris/HCl(pH 8.0)/0.02 M $CaCl_2$. Pronase was precipitated with 10% trichloroacetic acid and the solution centrifuged for 15 min at 5000 ***g***. The supernatant was exhaustively dialysed against distilled water at 4 °C. An aliquot was taken for measuring the radioactivity incorporated into GAGs. The remaining part was freeze-dried for electrophoretic analysis. Lyophilized samples were redissolved in water and deposited on the electrophoresis strips, together with standard GAGs in order to identify the position of the separated bands after migration. Cellulose-acetate-gel electrophoresis was performed on Cellogel membranes (Chemetron, Milan, Italy) at 40 V for 1 h [18]. After electrophoresis, the membranes were fixed in 100% ethanol and stained with 0.2% (w/v) Alcian Blue [19]. The GAG bands on the dried strips were cut off, dissolved in 0.4 ml of dioxan and the radioactivity measured with a liquid-scintillation counter. Duplicate strips were autoradiographed [20].

For proteoglycan analysis, culture media were supplemented with proteinase inhibitors (5 mM benzamidine/5 mM *N*-ethylmaleimide/20 mM 6-aminohexanoate/0.05% NaN_3/1 mM phenylmethanesulphonyl fluoride/2 mM EDTA/0.1% Na_2SO_4/0.1% Triton X-100). Urea was added to a final concentration of 6 M, and the samples were applied on to a Q-Sepharose column (1 cm × 5 cm) equilibrated with 6 M urea/0.05 M Tris/HCl (pH 7.6)/0.1% Na_2SO_4/0.1% Triton X-100. The column was washed with 8 vol. of the above buffer. The proteoglycans were then eluted with a NaCl gradient (0–1 M). Aliquots were collected and counted for radioactivity in a liquid-scintillation counter. The proteoglycan-containing fraction was dialysed against distilled water, freeze-dried and submitted to SDS/5%-PAGE as described by Laemmli [21].

For immunoprecipitation of decorin, 12 μl of anti-decorin antiserum was added to 1 ml of culture medium. The immune complex was adsorbed overnight at 4 °C on to Protein A–Sepharose with gentle agitation. Protein A–Sepharose beads were washed four times with 1% sodium deoxycholate, 0.5% Nonidet P40, containing proteinase inhibitors and 0.1% Na_2SO_4, then twice with DPBS.

The samples were treated with 100 munits of chondroitin ABC lyase in 0.2 ml of a 0.05 M Tris/HCl buffer, pH 8.0, containing proteinase inhibitors, for 5 h at 37 °C [22]. After digestion, urea and SDS were added to final concentrations of 4 M and 2% (w/v) respectively. The proteins were denatured at 100 °C for 3 min and submitted to SDS/PAGE. Protein bands on gels were revealed by autoradiography at −80 °C on hyperfilm-MP (Amersham) in a X-omatic cassette with an intensifying screen [23].

RNA analysis

Total RNA was extracted from cells by the guanidinium/phenol technique [24]. cDNA was prepared from 1 μg of total cellular RNA by reverse transcription at 42 °C for 45 min. The 20 μl reaction volume contained 200 units of Moloney-murine-leukaemia-virus reverse transcriptase, 2.5 μM random hexamers, 1 mM dATP, 1 mM dCTP, 1 mM dGTP, 1 mM dTTP, 20 units of RNasin (Promega), 5 mM $MgCl_2$ and 50 mM KCl in 10 mM Tris/HCl buffer, pH 8.3. The reaction product (10 μl) was amplified in a 100 μl PCR mixture containing 0.2 μM sense and 0.2 μM antisense primers, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 200 μM dTTP, 4 units of recombinant *Taq* DNA polymerase (Perkin–Elmer/Cetus), 2 mM $MgCl_2$ and 50 mM KCl in the above buffer and 185 kBq of [α-^{32}P]dCTP. The reaction was performed in a Hybaid (Teddington, Middx., U.K.) Omnigene temperature cycler by 32 cycles of denaturation at 95 °C for 20 s, primers annealing at 55 °C for 30 s and primer extension at 72 °C for 60 s. Amplification was controlled with ethidium bromide staining in 1.5%-(w/v)-agarose-gel electrophoresis [25]. After 15, 17, 19, 21, 23 and 25 cycles of amplification, 8 μl of PCR mixture was collected for analysis on an 8% (w/v) acrylamide gel [25], followed by autoradiography and densitometry on a Bio-Profile scanning system (Vilber-Loumat, Torcy, France). Human decorin sense and antisense primers were:

5′-ATCCTCCTTCTGCTTGCACA

and

5′-TGCTCCAGGACTAACTTTGCT

respectively. They were synthesized by Eurogentec (Liège, Belgium) according to the published sequence [26] and defined a 357-base target sequence. Human biglycan sense and antisense primers were:

5′-CGTGTCTCTGCTGGCCCTGA

and

5′-TGGAGTAGCGAAGCAGGTCTT

They were synthesized by Eurogentec and defined a 740-base target sequence [27]. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sense and antisense primers were:

5′-ACCACAGTCCATGCCATCAC

and

5′-TCCACCACCCTGTTGCTGTA

respectively. They were purchased from Clontech (Palo Alto, CA, U.S.A.) and defined a 452-base target sequence [28].

Northern blots

For Northern-blot analysis [29], RNAs were separated by 1%-(w/v)-agarose-gel electrophoresis, transferred by capillarity on a nylon membrane (Biodyne, Pall Ultrafine Filtration Corp., Glen Cove, NY, U.S.A.) and heated for 2 h at 80 °C. Membranes were prehybridized overnight at 42 °C in a mixture of 50% (v/v) formamide, 5 × standard saline/citrate (SSC), 5 × Denhardt's solution, 0.05% SDS and 100 μg/ml sonicated salmon sperm

DNA. Hybridization was carried out for 24 h at 42 °C in the same medium containing, in addition, specific random priming labelled (Gibco BRL kit) cDNA probes (3×10^6 c.p.m./ml). Membranes were then washed with SSC and subjected to autoradiography as described above. The probe for human decorin was a 1.6 kb cDNA fragment kindly provided by Dr. L. W. Fischer (Bone Research Branch, NIDR, Bethesda, MD, U.S.A.) [27]. The GAPDH 1.3 kb cDNA probe was provided by Dr. P. Fort (Université de Montpellier 2, Montpellier, France) [30].

RESULTS AND DISCUSSION

Glycosaminoglycan studies

Confluent dermal fibroblasts of adult healthy donors incorporated [^{3}H]glucosamine and [^{35}S]sulphate into GAGs secreted into the culture medium and associated with the cell layer. Addition of human recombinant IL-4 to the medium induced a dose-dependent increase in the incorporation of labelled precursors into the GAGs. The stimulation of the synthesis of GAGs by IL-4 was observed in three different strains of dermal fibroblasts from adult donors and in one strain of child foreskin fibroblasts. Results of a typical experiment are shown in Figure 1. No stimulation was observed in rat Swiss 3T3 cells (results not shown). The simultaneous increase of [^{3}H]glucosamine and [^{35}S]sulphate strongly suggested that the cytokine affected the synthesis of GAGs and not their specific radioactivity. Both the fraction secreted into the culture medium and associated with the cell layer were increased. The stimulation was, however, more intense for [^{35}S]sulphate-labelled GAGs, in particular GAGs secreted into the culture medium, than for [^{3}H]glucosamine, showing that the effects of IL-4 were preferential to sulphated GAG synthesis.

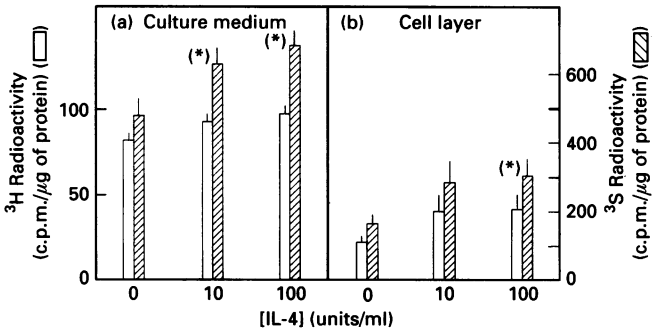

Figure 1 Effects of IL-4 on the synthesis of glycosaminoglycans

Confluent dermal fibroblasts were incubated for 24 h without (controls) or with IL-4, 10 or 100 units/ml, in the presence of [^{35}S]sulphate (▨) and (^{3}H)glucosamine (□). The GAGs of culture medium (**a**) and cell layer (**b**) were isolated by Pronase digestion and their radioactivity counted. Results are means ± S.D. for quadruplicate experiments. * $P < 0.01$ by Student's t test.

The characterization of the synthesized GAGs was performed by cellulose acetate electrophoresis, and the radioactivity of each separated band was determined in a liquid-scintillation counter (Table 1). In culture medium, about half of the [^{3}H]glucosamine incorporated into GAGs was found in the hyaluronan fraction, whereas 5% of [^{3}H]glucosamine and 17% of [^{35}S]sulphate were found in the heparan sulphate. Addition of IL-4 had no significant effect on these two fractions. In contrast, it enhanced, in a dose-dependent manner, the incorporation of both precursors into galactosaminoglycans (dermatan/chondroitin sulphate) or dermochondran sulphate [31]. In the cell layer, both dermatan/chondroitin sulphate and heparan sulphate were increased in cultures incubated with IL-4 (185 and 170% of controls respectively for the concentration 100 units/ml]. No detectable amount of hyaluronan was found. Taken together, these results confirmed that IL-4 stimulated the synthesis of sulphated GAGs only.

Proteoglycan studies

Fibroblast cultures incubated with [^{35}S]sulphate were used to identify the proteoglycans secreted into the culture medium. The radiolabelled material was fractionated by Q-Sepharose chromatography. Proteoglycans were eluted with a 0–1 M NaCl

Table 1 Effects of IL-4 on different glycosaminoglycan syntheses

GAGs were separated by cellulose acetate electrophoresis [18] and the radioactivity incorporated into hyaluronan, heparan sulphate and galactosaminoglycans (chondroitin/dermatan sulphate) was determined in a liquid-scintillation counter as described in the Materials and methods section. Results are means ± S.D. for quadruplicate experiments. * $P < 0.05$; ** $P < 0.01$ (by Student's t test).

	Radioactivity incorporated (c.p.m./μg of cell protein)				
	Culture medium			Cell layer	
[IL-4] (units/ml)	Hyaluronan	Heparan sulphate	Chondroitin/dermatan sulphate	Heparan sulphate	Chondroitin/heparan sulphate
0 (control)					
^{3}H	39.89 ± 5.19	4.33 ± 1.40	37.45 ± 3.10	13.12 ± 1.23	9.88 ± 2.22
^{35}S	–	84.31 ± 9.47	396.3 ± 52.6	56.47 ± 5.58	111.1 ± 22.3
10					
^{3}H	38.96 ± 3.81	5.93 ± 3.08	47.71 ± 5.55*	18.66 ± 4.02	19.59 ± 3.37**
^{35}S	–	97.97 ± 25.14	532.4 ± 48.2**	88.55 ± 21.44	199.4 ± 41.6*
100					
^{3}H	43.12 ± 2.83	4.73 ± 0.74	49.29 ± 1.41**	22.46 ± 5.64*	18.39 ± 4.20*
^{35}S	–	93.83 ± 15.33	590.2 ± 26.9**	97.46 ± 9.60**	207.2 ± 42.3**

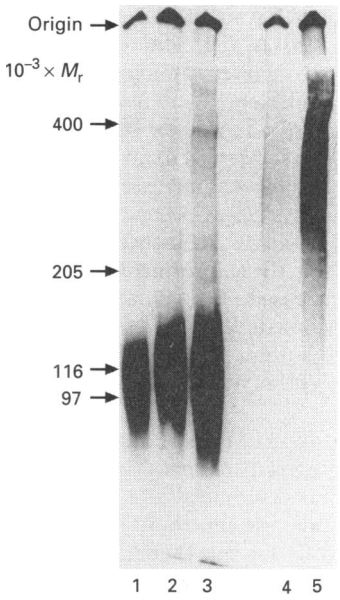

Figure 2 Effects of IL-4 on proteoglycan synthesis by dermal fibroblasts

The cultures were labelled for 24 h with [^{35}S]sulphate, and medium proteoglycans from control cells (lane 1) or cells treated with 10 units/ml IL-4 (lane 2) or 100 units/ml IL-4 (lane 3) were purified on a Q-Sepharose column, dialysed, freeze-dried and separated on SDS/5%-PAGE and autoradiographied. Lanes 4 and 5 represent the cell-layer proteoglycans from control cells and cells treated with 100 units/ml IL-4 respectively. The M_r values of the standard proteins are indicated on the left.

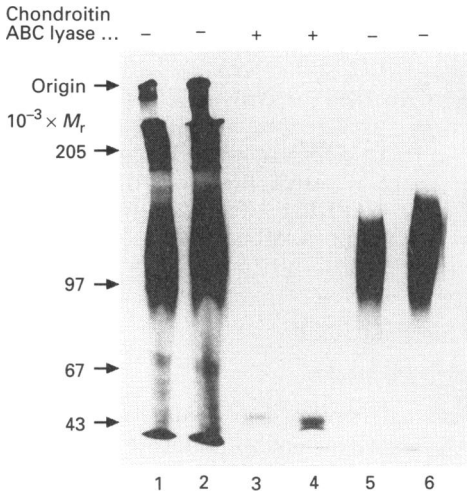

Figure 3 Effects of IL-4 on decorin synthesis

The cells were labelled with [^{3}H]leucine and [^{35}S]sulphate in the absence (odd-numbered lanes) or in the presence (even-numbered lanes) of 100 units/ml IL-4. The culture media (lanes 1 and 2) were incubated with anti-decorin antiserum and Protein A–Sepharose. Washed immunocomplexes (lanes 5 and 6) were digested with chrondroitin ABC lyase (lanes 3 and 4). All the samples were separated by SDS/5–10%-PAGE followed by autoradiography. Aliquots corresponding to 0.4 ml of the culture medium were deposited on the gel. The migration positions of the M_r markers are indicated on the left.

gradient. In IL-4-treated cultures, the incorporation of [^{35}S]sulphate into the proteoglycan-containing fraction was enhanced (results not shown). This fraction was analysed by SDS/PAGE and autoradiography. In the culture medium (Figure 2, lanes 1–3), SDS/PAGE of the labelled material showed two major proteoglycan fractions: a high-M_r band remained at the top of the gel and a second broad band migrated with an M_r of 110000–120000. An increase in the intensity of this band was observed upon incubation of the cells with IL-4 (Figure 2, lanes 2 and 3). It was not found in the cell layer, whether or not cells had been incubated with IL-4 (Figure 2, lanes 4 and 5). The migration of this band was characteristic of the small dermatan sulphate-containing proteoglycan decorin. No band was visible at the position of the M_r-200000 proteoglycan biglycan.

Decorin expression after stimulation with IL-4

To study the synthesis of decorin by fibroblasts stimulated with IL-4, the cells were incubated with [^{3}H]leucine and [^{35}S]sulphate, and decorin was precipitated by a specific anti-decorin antiserum. The GAG chains were digested by chondroitin ABC lyase and the core proteins separated by SDS/PAGE (Figure 3).

Anti-decorin antibodies immunoprecipitated a broad band of M_r 110000–120000 (Figure 3, lanes 5 and 6). Its synthesis, as measured by radioactivity counting or densitometry scanning, was enhanced over 2-fold after treatment of fibroblasts with 100 units/ml IL-4. No significant change in the whole proteoglycan M_r occurred. This proteoglycan gave two protein bands of M_r about 45000 after digestion with chondroitin ABC lyase (Figure 3, lanes 3 and 4), an aspect characteristic for decorin [33]. A second incubation of the culture medium with anti-decorin antibodies precipitated less than 10% of the radioactivity recovered by the first incubation, suggesting that the immunoprecipitation was complete.

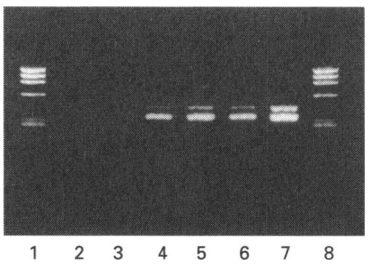

Figure 4 Decorin and GAPDH mRNA RT-PCR

RNAs from control cells (lanes 4 and 6) and cells treated with 100 units/ml IL-4 for 24 h (lanes 5 and 7) were used for reverse transcription and PCR with GAPDH and decorin primers as described in the Materials and methods section. A 4 μl sample of PCR products amplified through 30 cycles (lanes 4 and 5) and 32 cycles (lanes 6 and 7) was separated on 1.5% (w/v) agarose and stained with ethidium bromide. Lanes 2 and 3 represent PCR of non-transcribed RNAs from control and treated cells (negative PCR controls). Lanes 1 and 8, phage ϕX DNA digested with endonuclease *Hae*II.

RNA studies

The steady-state level of decorin mRNA was studied by semi-quantitative RT-PCR. To compare the levels of decorin mRNA in different samples, GAPDH mRNA was used as internal standard. The exhaustive amplification of cDNA fragments specific for decorin and GAPDH, performed in the same reaction tube, is shown in Figure 4. After 32 cycles, only two amplification products, of 357 bp (for decorin) and 452 bp (for GAPDH), were

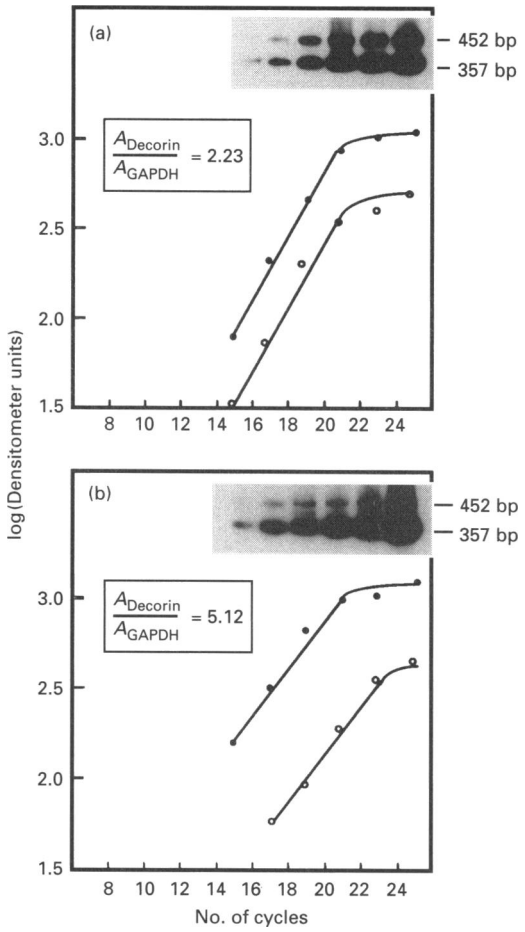

Figure 5 Semiquantitive RT-PCR

RNAs from control cells (**a**) and cells treated with IL-4 (100 units/ml for 24 h) (**b**) were used for reverse transcription and PCR in the presence of 185 kBq [α-^{32}P]dCTP. An 8 μl sample of PCR products was taken from the reaction mixture every two cycles, starting from cycle 15, separated by non-denaturing 8%-(w/v)-PAGE and autoradiographied. The ratios of decorin (●) to GAPDH (○) mRNAs were calculated by densitometric scanning as described by Chelly et al. [33].

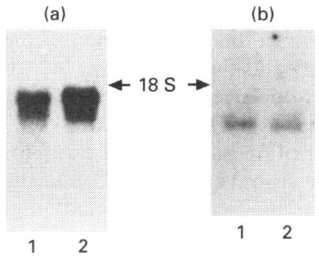

Figure 6 Northern-blot analysis of decorin mRNA

Total cellular RNA (10 μg/lane) from control cells (lane 1) or cells treated with 100 units/ml IL-4 (lane 2) were probed with random-primer-labelled cDNAs for decorin (**a**) and GAPDH (**b**) and autoradiographed. The migration position of the 18 S ribosomal RNA is indicated.

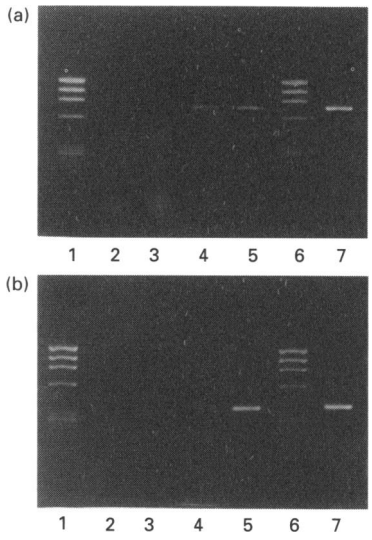

Figure 7 RT-PCR of biglycan and decorin mRNA

RNAs from control dermal fibroblasts (lanes 2 and 4), from dermal fibroblasts incubated with 100 units/mL IL-4 (lanes 3 and 5) or from MRC-5 fibroblasts (lane 7) were used for RT-PCR and amplified through 32 cycles. Lanes 2 and 3 represent negative control. Lanes 1 and 6, phage ϕX DNA digested with endonuclease *Hae*III (standard). (**a**) RT-PCR of biglycan mRNA; (**b**) RT-PCR of decorin mRNA.

detected with ethidium bromide staining on the 1.5%-agarose gel (Figure 4, lanes 4–7). The 357-bp product was further identified by digestion with endonuclease *Taq*1. It gave two bands of approx. 120 bp and 190 bp, corresponding to the predicted fragments from the human decorin sequence (results not shown).

To quantify the relative decorin mRNA steady-state level, the PCR was performed in the presence of [^{32}P]dCTP, and the amplification products were analysed after every two cycles, from cycles 15–25 (Figure 5). Up to 23 cycles the reaction was in exponential phase. The amplification curves were parallel, indicating a similar amplification rate for decorin and GAPDH cDNA fragments [33]. The relative ratio of decorin to GAPDH cDNAs increased over 2-fold in the cells treated with IL-4 (Figure 5b), indicating an increase in the decorin mRNA steady-state level. Similar results were obtained by Northern blotting with decorin and GADPH cDNA probes (Figure 6), which confirmed that IL-4 stimulated decorin synthesis at a pre-translational level.

To detect the presence of biglycan mRNA, the amplification of biglycan cDNA fragments was performed with RNAs extracted from human dermal fibroblasts. In control and IL-4-treated cultures, only a weak biglycan amplification product was detected with ethidium bromide staining (Figure 7a, lanes 4 and 5). In contrast, biglycan mRNA was strongly expressed in cell extracts of human embryo lung MRC-5 fibroblasts, as judged by the presence of a 740-bp amplification product (Figure 7a, lane 7). In the same experiments the decorin fragment was also amplified (Figure 7b). These results confirm that, under our culture conditions, adult skin fibroblasts express only a low amount of biglycan. The synthesis of biglycan is more active in embryonic cells [34,35].

Taken together, our results clearly demonstrate that IL-4 stimulates decorin production by human dermal fibroblasts at physiological concentrations (100 units/ml $\approx$ 0.7 nM). The effects of the cytokine occurred at a pre-translational level.

The control of decorin synthesis by cytokines is not well understood. For instance, depending on the cell type and culture conditions, transforming growth factor-β may stimulate [36] or

inhibit decorin expression [35,37,38]. Recently, Richards and Katz [39] characterized the IL-4 enhancer element of the murine CD 23 gene. Using the Fasta program [40], we have found a similar sequence of putative IL-4 response element in the decorin gene promoter. It was localized in the −308 to −293 region before the transcription start site of exon Ib, as reported by Santra et al. [41]. However, similar consensus sequences were also present on several transcription-factor genes (results not shown). Further studies are needed for complete identification of the mechanism of action of IL-4 on the decorin gene.

The extracellular matrix contains several types of proteoglycans which control the integrity of extracellular space and contribute to cell–matrix interactions [42,43]. Dermatan sulphate-containing proteoglycans are known to maintain spatial arrangements of collagen fibrils and are involved in the control of their lateral growth [44,45]. Decorin is the most abundant proteoglycan of this group capable of interacting with collagen. It is present at the surface of collagen fibrils and may control the fibril diameter [46]. The interaction between decorin and collagen is essential for the growth of the fibrillar network (for a review, see [16]).

Together with previous data from our laboratory [9,10] and others [6,11], which demonstrated a stimulation of collagen and fibronectin synthesis, the present results confirm that IL-4 is a potent activator of extracellular matrix synthesis by fibroblasts. This ability suggests that this cytokine may be one of the factors involved in the early events of wound healing and fibrosis.

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REFERENCES

1 Peyron, E. and Banchereau, J. (1994) Eur. J. Dermatol. **4**, 181–188
2 Gold, M. R., Duronio, V., Saxena, S. P., Schrader, J. W. and Aebersold, R. (1994) J. Biol. Chem. **269**, 5403–5412
3 Kotanides, H. and Reich, N. C. (1993) Science **262**, 1265–1267
4 Schindler, C., Kashleva, H., Pernis, A., Pine, R. and Rothman, P. (1994) EMBO J. **13**, 1350–1356
5 Lowenthal, J. W., Castle, B. E., Christiansen, J., Schreurs, J., Reunick, D., Arai, N., Hoy, P., Takebe, Y. and Howard, M. (1988) J. Immunol. **140**, 456–464
6 Stempowski, G. D., Beckmann, M. P., Derdak, S. and Phipps, R. P. (1994) J. Immunol. **152**, 3606–3614
7 Monroe, J. G., Haldar, S., Prystowsky, M. B. and Lammie, P. (1988) Clin. Immunol. Immunopathol. **49**, 292–298
8 Postlethwaite, A. E. and Seyer, J. M. (1991) J. Clin. Invest. **87**, 2147–2152
9 Fertin, C., Nicolas, J. F., Gillery, P., Kalis, B., Banchereau, J. and Maquart, F. X. (1991) Cell. Mol. Biol. **8**, 823–829
10 Gillery, P., Fertin, C., Nicolas, J. F., Chastang, F., Kalis, B., Banchereau, J. and Maquart, F. X. (1992a) FEBS Lett. **302**, 231–234
11 Postlethwaite, A. E., Holness, M. A., Katai, H. and Raghow, R. (1992) J. Clin. Invest. **90**, 1479–1485
12 Nietfeld, J. J. (1993) Experientia **49**, 456–469
13 Hardingham, T. E. and Fosang, A. J. (1992) FASEB J. **6**, 861–870
14 Vogel, K. G., Paulsson, M. and Heinegard, D. (1984) Biochem. J. **223**, 587–597
15 Scott, J. E. and Orford, C. R. (1981) Biochem. J. **197**, 213–216
16 Kresse, H., Hausser, H. and Schönherr, E. (1993) Experientia **49**, 403–415
17 Lowry, O. H., Rosenbrough, N. J., Farr, A. R. and Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
18 Wessler, E. (1971) Anal. Biochem. **41**, 67–69
19 Barthold, P. M., Wiebkin, O. W. and Thonard, J. C. (1982) Connect. Tissue Res. **9**, 165–172
20 Wegrowski, Y. (1993) FEBS Lett. **334**, 121–124
21 Laemmli, U. K. (1970) Nature (London) **227**, 680–685
22 Saito, H., Yamagota, T. and Suzuki, S. (1968) J. Biol. Chem. **243**, 1536–1542
23 Bonner, W. M. and Laskey, R. A. (1974) Eur. J. Biochem. **46**, 83–88
24 Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159
25 Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
26 Krusius, T. and Ruoslahti, E. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 7683–7687
27 Fischer, L. W., Termine, J. D. and Young, M. F. (1989) J. Biol. Chem. **260**, 4571–4576
28 Tokunaga, K., Nakamura, Y., Sakata, K., Fujimori, K., Ohkugo, M., Sawada, K. and Sakiyama, S. (1987) Cancer Res. **47**, 5616–5619
29 Gillery, P., Serpier, H., Polette, M., Bellon, G., Clavel, C., Wegrowski, Y., Birembaut, P., Kalis, B., Cariou, R. and Maquart, F. X. (1992) Eur. J. Cell Biol. **57**, 244–253
30 Fort, P., Marty, L., Piechaczyk, M., Sabrouty, E. E., Dani, C., Jeanteur, P. and Blanchard, J. M. (1985) Nucleic Acids Res. **13**, 1431–1442
31 Scott, J. E. (1993) Glycoconjugate J. **10**, 419–421
32 Glössl, J., Beck, M. and Kresse, H. (1984) J. Biol. Chem. **259**, 14144–14150
33 Chelly, J., Kaplan, J. C., Maire, P., Gautron, S. and Kahn, A. (1988) Nature (London) **333**, 858–860
34 Rauch, U., Glössl, J. and Kresse, H. (1986) Biochem. J. **238**, 465–474
35 Breuer, B., Schmidt, G. and Kresse, H. (1990) Biochem. J. **269**, 551–554
36 Bassol, A. and Massague, J. (1988) J. Biol. Chem. **263**, 3039–3045
37 Westergren-Thorsson, G., Antonsson, P., Malmström, A., Heinegård, D. and Oldberg, A. (1991) Matrix **11**, 177–183
38 Kähäri, V. M., Larjava, H. and Uitto, J. (1991) J. Biol. Chem. **266**, 10608–10615
39 Richards, M. L. and Katz, D. H. (1994) J. Immunol. **152**, 3453–3466
40 Dessen, P., Fondrat, C., Valancien, C. and Mugnier, C. (1990) Comput. Appl. Biosci. **6**, 355–356
41 Santra, M., Danielson, K. G. and Lozzo, R. V. (1994) J. Biol. Chem. **269**, 579–587
42 Ruoslahti, E. (1990) in Cell to Cell Interaction (Burger, M. M., Sordat, B. and Zinkernagel, R. M., eds.), pp. 88–98, Karger, Basel
43 Yanagishita, M. (1993) Acta Pathol. Jpn. **43**, 283–293
44 Scott, J. E. (1988) Biochem. J. **252**, 313–323
45 Scott, J. E. (1992) FASEB J. **6**, 2639–2645
46 Fleischmajer, R., Fisher, L. W., McDonald, E. D., Jacobs, L., Perlish, J. S. and Termine, J. D. (1990) J. Struct. Biol. **106**, 82–90

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