Identification of a Bimodal Regulatory Element Encompassing a Canonical AP-1 Binding Site in the Proximal Promoter Region of the Human Decorin Gene

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We have previously described a tumor necrosis factor α (TNF-α) response element, located between residues −188 and −140 of the human decorin promoter, that mediates the inhibitory effect of TNF-α on decorin gene expression (Mauviel, A., Santra, M., Chen, Y.-Q., Uitto, J., and Iozzo, R. V. (1995) J. Biol. Chem. 270, 11692–11700). In this report, we demonstrate that interleukin 1 (IL-1), a pleiotropic cytokine that shares a wide variety of biological properties with TNF-α, is capable of reversing the response to TNF-α. Supershift assays with the promoter, this promoter fragment requires the AP-1 sequence to be responsive to IL-1. Transient transfection experiments using decorin promoter-chloramphenicol acetyl transferase reporter gene constructs. Additional transfection experiments with various 5′-deletion promoter-chloramphenicol acetyl transferase reporter gene constructs demonstrate that both the inhibitory effect of TNF-α and the stimulatory effect of IL-1 are mediated by activation of the corresponding promoter, as shown in transient cell transfection experiments using decorin promoter-chloramphenicol acetyl transferase reporter gene constructs. Overexpression of c-jun, an oncogene encoding the c-Jun/AP-1 transcription factor, reduces the basal activity of both decorin and −188/−140 thymidine kinase promoter constructs. In contrast, blockage of c-jun expression with an antisense c-jun construct potentiates the stimulatory effect of IL-1 and reverses the response to TNF-α. These data indicate that the region between residues −188 and −140 of the human decorin promoter functions as a bimodal regulatory element and allows transcriptional repression by c-Jun/AP-1 complexes.

Decorin, a member of the small leucine-rich proteoglycan gene family with ubiquitous tissue distribution, is thought to play essential biological roles during inflammation and cancer invasion (reviewed in Ref. 1). In particular, decorin has been postulated to play an important regulatory role in collagen fibril formation through its ability to bind type I collagen (2). Also, decorin binds growth factors, such as transforming growth factor β (3). The latter property implies that decorin may remove transforming growth factor β from the cellular microenvironment and consequently block its activity. In fact, in an experimental animal model of glomerulonephritis, infusion of decorin prevents transforming growth factor β-induced fibrosis of renal glomeruli (4). More recently, it has been shown that de novo expression of decorin suppresses the malignant phenotype of colon cancer cells, by blocking the tumor cells in the G0-G1 phase of the cell cycle (5). All these observations point to the central role of decorin in the control of cell division and proliferation.

A complex cytokine network controls a variety of physiological and pathological conditions and allows maintenance or recovery of tissue homeostasis (reviewed in Refs. 6 and 7). Among these cytokines, interleukin 1 (IL-1) and tumor necrosis factor α (TNF-α), two mononuclear cell products with a broad spectrum of activities in the fields of immunology, tissue repair, and inflammation, have been shown to alter the expression of a variety of matrix-related genes (reviewed in Refs. 8 and 9). Also, despite their distinct molecular structures and receptor and signal transduction pathways, these two cytokines can induce a common set of transcription factors, such as NFκB and Jun/AP-1 (10–13), thereby leading to numerous overlapping biological activities. These cytokines have the potential to control the deposition of extracellular matrix components through their ability to regulate the expression of various proteases or through a direct action on the synthesis and gene expression of various matrix constituents. For example, proinflammatory cytokines are potent inducers of collagenase and stromelysin gene expression, acting as factors promoting connective tissue destruction and remodeling (6). Also, these cytokines can directly modulate the expression of a variety of extracellular matrix components, such as type I and type III collagen, fibronectin, elastin, and certain proteoglycans, and they sometimes exhibit opposite regulatory activities on certain matrix genes (7). For example, IL-1 up-regulates elastin gene

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¶ The abbreviations used are: IL-1, interleukin 1; bp, base pair; CAT, chloramphenicol acetyl transferase; TK, thymidine kinase; TNF-α, tumor necrosis factor α; RSV, Rous sarcoma virus; DEC, decorin; PCR, polymerase chain reaction; AS, antisense; WT, wild type.
expression and promoter activity in dermal fibroblasts (14), whereas TNF-α transcriptionally inhibits elastin expression through an AP-1-mediated mechanism (15). Similarly, the expression of type I collagen is enhanced in fibroblasts on IL-1 stimulation (16, 17), whereas it is reduced by TNF-α (17–19).

We and others have shown previously that IL-1 induces decorin mRNA levels in dermal fibroblasts (20, 21), whereas TNF-α is a potent transcriptional inhibitor of decorin gene expression (22). Recent cloning of both human (23) and mouse (24) decorin genes allowed us to characterize some of the functional elements that reside within the 5’-flanking DNA sequences (22, 25). In particular, we demonstrated that inhibition of decorin gene expression by TNF-α is mediated by two independent cis elements, one located within the decorin promoter between residues −188 and −140, and the other within the untranslated exon 1B of the decorin gene (22).

In this study, we demonstrate that the 48-bp region of the decorin promoter located between residues −188 and −140 and functions as a functional, and AP-1 binding site, is a bimodal regulator of decorin gene expression, allowing both its down-regulation by TNF-α and its up-regulation by IL-1β.

MATERIALS AND METHODS

Cell Cultures—Human dermal fibroblast cultures, established from tissue specimens obtained from adult individuals during surgical procedures or from neonatal foreskins, were used in passages 3–8. The cell cultures were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, and 50 μg/ml streptomycin.

Cytokines and Growth Factors—Human recombinant IL-1β and TNF-α were purchased from Boehringer Mannheim. Human recombinant IL-1α and IL-1β were a generous gift of Dr. David R. Olsen (Celtrix Laboratories, Santa Clara, CA).

Northern Analyses—Total RNA was isolated using a standard procedure and analyzed by Northern hybridization with 32P-labeled cDNA probes, as described previously (22). The 32P-labeled cDNA-mRNA hybrids were visualized by autoradiography, and the steady-state levels of mRNA were quantitated by scanning densitometry using a He-Ne laser scanner at 633 nm (LKB Produkter, Bromma, Sweden). The following cDNAs were used for Northern hybridizations to detect specific mRNA transcripts: for cjun, a full-length human cDNA in pRSV e expression vector pRSV-A, a full-length cDNA in pRSV e expression vector pRSV-B, a full-length cDNA in pRSV e expression vector pRSV-C (both kindly provided by Dr. Michael Karin, University of California San Diego, La Jolla, CA). The 1.3-kilobase rat glyceraldehyde-3-phosphate dehydrogenase was used as a control (27).

Plasmid Constructs—To study the transcription regulation of decorin gene expression, transient transfection experiments were performed with various 5’-deletion constructs derived from pDEC-983/CAT, a plasmid containing −1 kilobase of decorin promoter linked to the CAT gene, with high homology with cjun sequences from various species, was amplified by polymerase chain reaction (PCR). The PCR amplifier was cloned into a PCRII plasmid vector (Invitrogen, Portland, OR), and the clones containing the inserts were sequenced to ensure the fidelity of the PCR amplification. The PCR product was then inserted in an antisense orientation as an Xhol-HindIII fragment into the expression vector pRSV to generate the construct pRSV-ASC-cjun (5). The efficacy and specificity of the construct were tested in stable transfection experiments, as described under “Results and Discussion.”

Transient Cell Transfections and CAT Assays—Transient transfections of human foreskin fibroblasts were performed by the calcium phosphate/DNA co-precipitation method, as described previously (22). Briefly, the cells were transfected with 10 μg of DNA, unless stated otherwise. After glycerol shock, the cells were plated in Dulbecco’s modified Eagle’s medium containing 1% fetal calf serum, 4 h prior to the addition of cytokines. After an additional 40 h of incubation, the cells were rinsed twice with PBS, harvested by scraping, and lysed in Reporter Lysis Buffer (Promega, Madison, WI). Aliquots corresponding to identical amounts of protein, as measured with a commercial assay kit (Bio-Rad), were used for each CAT assay with [14C]chloramphenicol as substrate using thin layer chromatography. Following autoradiography, the plates were cut and counted by liquid scintillation to quantify the percentage of acetylated [14C]chloramphenicol (28).

Gel Mobility Shift Assays—For gel retardation assays, nuclear extracts were prepared as described previously (26). Briefly, the nuclear extracts were incubated with 5–10 μg of protein extracts for 30 min on ice in 20 μl of binding reaction buffer (12 mM HEPES, pH 7.9, 4 mM Tris, 60 mM KCl, 1 mM EDTA, 12% glycerol), in the presence of 2 μg of poly(dI-dC). For competition experiments, a 60-fold molar excess of DNA was added 20 min prior to the binding reaction. DNA-protein complexes were separated from unbound oligomers on 4 or 5% acrylamide gels in 0.4 × Tris borate-EDTA. The gels were fixed for 30 min in 30% methanol and 10% acetic acid, dried, and exposed to x-ray films at −70°C. Details of the competition assays are provided in the legends to the figures and in the corresponding text under “Results and Discussion.” In some experiments, nuclear proteins were incubated overnight at 4°C with 2 μl of antibody for c-Jun, Jun-B, Jun-D, c-Fos, Fos-B, Fra-1, or ATF2 (TransCruzTM gel supershift reagents; Santa Cruz Biotechnology, Santa Cruz, CA) prior to binding reaction with the labeled oligonucleotide, as described above.

RESULTS AND DISCUSSION

Interleukin 1β Enhances Decorin Gene Expression by Activation of the Promoter in Skin Fibroblasts—We have previously demonstrated that the two forms of human IL-1, namely IL-1α and IL-1β, are both capable of increasing dermatan-sulfate proteoglycan/decorin mRNA levels in dermal fibroblasts (20) in a time- and dose-dependent manner. To investigate further the mechanisms by which IL-1 up-regulates decorin gene expression, we performed transient cell transfection experiments with pDEC-983/CAT, a construct that contains −1 kilobase of human decorin promoter linked to the CAT gene. Confluent fibroblast cultures were transfected with pDEC-983/CAT, and the cultures were incubated with 10 units/ml IL-1β, a concentration previously shown to elevate decorin mRNA levels (29) above the controls (20). Assay of CAT activity after 40 h of incubation indicated that IL-1β enhanced the promoter activity (Fig. 1A) 3-fold above the control values (Fig. 1B). The extent of stimulation is very similar to that observed at the mRNA level (20), suggesting that enhancement of decorin gene expression by IL-1 is mediated by enhancement of transcription of the
decorin gene at the promoter level. These results are in contrast with our recent observations that TNF-α is a potent inhibitor of decorin gene expression (22). This is particularly interesting since these cytokines, IL-1 and TNF-α, are known for their pleiotropic, often similar, activities in a variety of biological and immunological situations (8).

Mapping of the IL-1-responsive Sequences in the Decorin Promoter—To characterize the putative IL-1-responsive sequences of the decorin promoter, we used a battery of 5′-deletions of the decorin promoter linked to the CAT reporter gene (22, 25). The constructs were used in transient cell transfection experiments, and their responsiveness to IL-1β was examined. Deletion of the 5′-sequences from positions −983 to −188 did not alter IL-1β responsiveness (Fig. 2). Specifically, IL-1β enhanced the promoter activity from 2.5- to 4-fold with all constructs tested. Further deletion at the 5′-end of the promoter from −188 to position −140 totally abolished IL-1β responsiveness, indicating that the sequences located between residues −188 and −140 are essential for IL-1 response (Fig. 2). Interestingly, we have previously shown that this fragment of the decorin promoter also mediates down-regulation by TNF-α (22). Therefore, these findings suggest a bimodal regulatory role for this short (48 bp) segment of the decorin promoter, capable of acting as a negative or positive element, depending on the stimulus.

The potential for the −188 to −140 promoter region to confer both inducibility and repression of decorin gene expression by cytokines led us to examine the interactions of IL-1 and TNF-α in modulating decorin promoter activity. To this end, confluent fibroblast cultures were transfected with pDEC−188CAT as described under “Materials and Methods.” After glycerol shock, the cultures were incubated for 3 h in medium containing 1% fetal calf serum and incubated for another 40 h without or with IL-1β (10 units/ml) prior to CAT assay. The relative promoter activity is expressed as the percentage of the corresponding control, mean ± S.D. of five separate experiments with overlapping sets of promoter constructs. The position of the 5′-end of each construct is indicated.

FIG. 2. Effect of IL-1β on the activity of 5′-deletions of the decorin gene promoter in transient cell transfection assays. Confluent fibroblast cultures were transfected with various 5′-deletion constructs of the decorin gene promoter linked to the CAT gene, as described under “Materials and Methods.” After glycerol shock, the cultures were incubated for 3 h in medium containing 1% fetal calf serum and incubated for another 40 h without or with IL-1β (10 units/ml) prior to CAT assay. The relative promoter activity is expressed as the percentage of the corresponding control, mean ± S.D. of five separate experiments with overlapping sets of promoter constructs. The position of the 5′-end of each construct is indicated.

FIG. 3. Effect of IL-1β in combination with TNF-α on the decorin promoter activity. Confluent fibroblast cultures were transfected with DEC−188/CAT. After glycerol shock, the cultures were incubated for 3 h in medium containing 1% fetal calf serum and incubated for another 40 h without (CTL) or with IL-1β (10 units/ml) in the presence or absence of TNF-α (25 ng/ml) prior to CAT assay. A, autoradiogram of a representative experiment. B, graphic representation of the mean ± S.D. of four separate experiments, each performed with duplicate samples. In each experiment, the controls were set as 100%. AC, acetylated [3H]chloramphenicol; C, [3H]chloramphenicol.

The −188/−140 Region of the Decorin Promoter Confers IL-1 Responsiveness to the Heterologous Thymidine Kinase Promoter—To analyze the role of the −188/−140 region of the human decorin promoter in further detail, a plasmid construct that contains a synthetic oligonucleotide spanning that region of promoter cloned upstream of the TK promoter and driving the expression of the CAT gene, pDEC−188−140TK/CAT, was generated (22). This construct was used in transient transfections of fibroblasts, followed by addition of either IL-1β or TNF-α to the culture medium. IL-1β markedly elevated the pDEC−188−140TK/CAT construct activity (3-fold above control values), whereas TNF-α had little, if any, effect. These results indicate that the −188/−140 element, which exerts a bimodal regulatory role in the context of the decorin promoter, only responds as a positive element after IL-1 stimulation when cloned upstream of a heterologous promoter.

Characterization of Nuclear Factor Binding to the −188/−140 Region of the Decorin Promoter—To further understand the mechanisms by which IL-1 and TNF-α transcriptionally regulate decorin gene expression, we mapped the nuclear factor binding sequences within the responsive region located between residues −188 and −140 of the promoter. For this purpose, we designed a series of overlapping oligonucleotides spanning the entire −188/−140 sequence (Fig. 4A). First, gel mobility shift assays were performed with radiolabeled wild type (WT) probe. Nuclear proteins from control fibroblast cultures exhibited low binding activity to the WT probe (Fig. 4B, lane 1). The binding activity was strongly enhanced in nuclear extracts from fibroblast cultures treated with either IL-1 or TNF-α for 6 h (Fig. 4B, lanes 2 and 3). The binding was...
A series of oligonucleotides spanning the sequence between residues 188 and 140 of the human decorin gene was synthesized. These synthetic oligonucleotides were end labeled with [γ-32P]ATP and used in gel electrophoresis mobility shift assays to study their ability to bind transcrip-
tive factors. A, schematic representation of the various oligonucleo-
tides used in gel mobility shift assays. In addition to a wild type fragment spanning the entire region from 188 to 140, two oligonu-
cleotides, ΔAP-1 and ΔNF-IL-6, encompassing the entire wild type se-
quence but lacking the 8 bp corresponding to either the AP-1 or NF-IL-6 binding sites, respectively, were synthesized. Also, oligonucleotides A, B, and C, spanning the entire sequence but without any overlap, were synthesized, as well as oligonucleotide C, covering the 3’-end of A and the 5’-end of B and containing the complete AP-1 binding site identified in this segment of promoter (22). B, mapping of nuclear protein binding to the WT oligonucleotide. The nuclear extracts used were as follows: 1. control, untreated confluent fibroblast control cultures; 2. nuclear extracts from IL-1β-treated confluent fibroblast cultures; and 3. nuclear extracts from TNF-α-treated confluent fibroblast cultures. Competi-
tions were performed with a 100-fold molar excess of unlabeled oligo-
nucleotides, WT, A, B, and C, as indicated. C, gel mobility shift assays with radiolabeled WT, ΔAP-1, and ΔNF-IL-6 probes. Nuclear extracts are the same as in B. Note the absence of binding to the ΔAP-1 probe. N.S., nonspecific binding.

competed by a 100-fold molar excess of unlabeled oligonucleo-
tides WT and C but not by oligonucleotides A and B in the same molar excess, suggesting that binding of nuclear proteins in-
volved the central sequences of the WT probe, contained within oligonucleotide C and spanning the AP-1 binding site. In fact, this result was further confirmed in gel shift assays using radiolabeled A, B, and C oligonucleotides, demonstrating no binding to either A or B oligonucleotides (not shown). Computer sequence analysis revealed the presence of a potential NF-IL-6 binding site, 5’-TGTTGCAAG-3’, within the 3’-end of that segment, in addition to the previously identified AP-1 binding site (22). To investigate the respective role of these putative cis elements, gel mobility shift assays with radiolabeled WT, ΔAP-1, and ΔNF-IL-6 probes were performed in parallel (Fig. 4C). The binding activity observed with the WT probe was not seen with the radiolabeled ΔAP-1 probe but was identical with the ΔNF-IL-6 probe, indicating that the potential NF-IL-6 element was not functional. These results confirm that both IL-1 and TNF-α induce strong binding activity to the AP-1 binding sequence located between residues 174 and 168 of the human decorin promoter. Interestingly, we have recently shown that TNF-α-induced binding to the 188/140 region of the decorin promoter is weakly competed by a 23-bp oligonucleo-
tide overlapping the AP-1 binding site located at position 70 of the collagenase promoter, suggesting that the binding affinity to the decorin AP-1 sequence is higher than that to the collagenase sequence (22).

The ΔAP-1 oligonucleotide was then cloned into pBLCAT5, upstream of the TK promoter. This construct was used in transient transfections of fibroblasts, followed by incubation with IL-1β (10 units/ml) for 48 h, and its activity was compared with that of pDEC–188–140TK/CAT. Removal of the 8-bp sequence corresponding to the AP-1 binding site within the region 188/140 led to a strong reduction of the basal activity of the chimeric TK construct and to a complete loss of responsiv-

eseness to IL-1 (not shown), indicating that the AP-1 site is essential to mediate the IL-1 effect, in correlation with the results of DNA binding assays presented in Fig. 4.

Analysis of the Dual Function of the Cytokine Response Ele-
ment Located Between Residues 188 and 140 of the Decorin Promoter: Role of the AP-1 Trans-activation Pathway—The AP-1 transcription factors are formed of dimers of gene prod-

ucts of the Fos and Jun families of oncogenes, with closely related recog-
nition sites but with different transcriptional activities and DNA binding affinities (reviewed in Ref. 29). Because both IL-1 and TNF-α are potent inducers of c-jun expres-
sion in dermal fibroblasts in culture (10–12), we examined the specific role of c-Jun in the regulation of decorin promoter activity. To this end, pDEC–188/CAT was cotransfected with an expression vector for c-Jun, pRSVe-jun, and the empty expres-
sion vector pRSVe was used as a control. As shown in Fig. 5A, overexpression of c-Jun led to a dramatic reduction in decorin promoter activity, by as much as 85%, when compared with the control values. Similar results were obtained when pDEC–188–140TK/CAT was used instead of pDEC–188/CAT (Fig. 5B). The inhibitory effect of c-Jun was specific for decorin, since we have previously demonstrated, using the same expres-
sion vector, that c-Jun trans-activates both the human collagenase promoter and a chimeric promoter construct containing three collagenase AP-1 binding sites cloned upstream of the TK promoter (25). Interestingly, inhibition of elastin gene expres-
sion by TNF-α is also mediated by an AP-1 binding sequence within the corresponding promoter (15). Collectively, our present data suggest that: (a) AP-1/c-Jun is a potent inhibitor of decorin gene expression; and (b) the inhibitory effect of c-Jun transcription factor is mediated by the region 188/140 of the promoter. Also, our findings suggest that other transcriptional mechanisms, with a stimulatory activity on the decorin prom-

erator, are likely to be involved in the case of IL-1, but not

FIG. 4. Binding of nuclear proteins to a DNA fragment span-
ning the region from 188 to 140 of the decorin promoter. A series of oligonucleotides spanning the sequence between residues 188 and 140 of the human decorin was generated. These synthetic oligonucleotides were end labeled with [γ-32P]ATP and used in gel electrophoresis mobility shift assays to study their ability to bind transcrip-
tion factors. A, schematic representation of the various oligonu-
cleotides used in gel mobility shift assays. In addition to a wild type fragment spanning the entire region from 188 to 140, two oligonu-
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tions were performed with a 100-fold molar excess of unlabeled oligo-
nucleotides, WT, A, B, and C, as indicated. C, gel mobility shift assays with radiolabeled WT, ΔAP-1, and ΔNF-IL-6 probes. Nuclear extracts are the same as in B. Note the absence of binding to the ΔAP-1 probe. N.S., nonspecific binding.
TNF-α, whereas both cytokines induce the inhibitory factor c-Jun.

Blockage of c-jun Expression Potentiates IL-1 Effect on Decorin Promoter Activity and Reverses TNF-α Response—To further understand the function of the −188/−140 fragment of decorin promoter in AP-1-mediated inhibition of promoter activity, we used an antisense c-Jun expression vector to block the expression of c-jun in transfection experiments. The efficacy and specificity of the antisense construct have been described recently (30). Specifically, we showed that transfection of pRSV-ASc-jun results in an almost complete loss of c-jun expression without alteration in the expression of junB. In addition, blockage of c-jun expression results in prevention of TNF-α-induced, c-Jun/AP-1-mediated up-regulation of collagenase gene expression, indicating that this construct provides a convenient tool to study the regulation of gene expression by cytokines in the absence of c-Jun.

To investigate the role played by the c-jun proto-oncogene in the regulation of decorin gene expression by IL-1 and TNF-α, we performed co-transfection experiments of pDEC−188/CAT and pDEC−188−140TK/CAT with either pRSVe or pRSV-ASc-jun. Four h after glycerol shock, IL-1 or TNF-α was added to the cultures, and CAT activity was determined 40 h later. As shown in Fig. 6, co-transfection of pRSVe with either promoter-CAT reporter construct did not modify their response to IL-1 and TNF-α, compared with experiments in which the reporter constructs were transfected alone (see above). Specifically, the extent of stimulation on IL-1 treatment was −3-fold for both pDEC−188/CAT (Fig. 6, A, left, and C) and pDEC−188−140TK/CAT (Fig. 6, B, left, and D). At the same time, TNF-α reduced pDEC−188/CAT activity by ~50%, whereas it had no effect on pDEC−188−140TK/CAT activity. Co-transfection of pRSV-ASc-jun resulted in enhanced response to IL-1, approximately 6- to 9-fold above control for both pDEC−188/CAT (Fig. 6, A, right, and C) and pDEC−188−140TK/CAT (Fig. 6, B, right, and D). Interestingly, blocking of c-jun expression reversed the normal inhibitory response of pDEC−188/CAT to TNF-α, resulting in CAT activity ~3-fold above control values (Fig. 6, A, right).
right). Similarly, in these same experimental conditions, TNF-α stimulated pDEC – 188–140TK/CAT, normally unresponsive to the cytokine (see above and Ref. 22), by about 3–5-fold above control levels (Fig. 6, B, right, and D).

Collectively, these results indicate that TNF-α can stimulate both promoter constructs, as IL-1 does, when c-jun expression is blocked. Furthermore, our findings show that the sequence between residues –188 and –140 of the decorin promoter is sufficient to confer a stimulatory response by both TNF-α and IL-1, whether in the context of the decorin promoter or not, when c-jun expression is blocked. It appears, therefore, that IL-1, but not TNF-α, most likely activates a transcription factor capable of reversing the inhibitory effect of c-Jun on decorin gene expression, and that this thus far uncharacterized factor stimulates decorin gene transcription by activation of the corresponding promoter through the sequences located between nucleotides –188 and –140. Also, because TNF-α exerts a stimulatory effect on decorin promoter activity in the absence of c-Jun, our data suggest that TNF-α may also activate this unknown factor, but to levels lower than achieved by IL-1, that is, to levels that are unable to counteract the inhibitory effect of c-Jun.

To characterize further the protein complex binding to the AP-1 binding site within the –188/-140 region of the human decorin gene, nuclear extracts from IL-1- or TNF-α-treated fibroblast cultures were incubated with antibodies against c-Jun, Jun-B, Jun-D, c-Fos, Fos-B, and Fra-1 prior to detection of DNA-protein interactions by gel mobility shift assay. The antibodies against c-Jun, Jun-B, and Fra-1, but not those against Jun-D, c-Fos, and Fos-B, induced a supershift of the labeled DNA probe incubated with nuclear extracts from control and Jun-D, c-Fos, and Fos-B, induced a supershift of the labeled antibodies against c-Jun, Jun-B, and Fra-1, but not those against Jun-D, c-Fos, and Fos-B, induced a supershift of the labeled DNA-protein interactions by gel mobility shift assay. The addition of c-Jun antibodies against c-Jun, Jun-B, and Fra-1, but not those against Jun-D, c-Fos, and Fos-B, induced a supershift of the labeled DNA-protein interactions by gel mobility shift assay. The addition of c-Jun to the reaction competed for the labeled DNA-protein interactions.

**Conclusions**—Using a battery of 5′-deletion constructs of the human decorin promoter, we have mapped the element(s) necessary for an IL-1 response between nucleotides –188 and –140. This region of the decorin promoter was previously shown to mediate the inhibitory effect of TNF-α (22) and is, in fact, sufficient to allow antagonism between these two cytokines, thereby allowing fine regulation of decorin gene expression. Sequence analysis of this short fragment of the decorin promoter indicates that it contains a consensus AP-1 binding site, TGAGTCA. Overexpression of c-jun inhibits decorin promoter activity, whereas blockage of c-jun expression with a specific antisense construct results in enhanced responsiveness to IL-1 and reversed responsiveness to TNF-α. These data indicate that the c-Jun/AP-1 transcription factor is a potent inhibitor of decorin gene expression. In addition, our data provide the first evidence of intrinsic differences between IL-1 and TNF-α, often viewed as proinflammatory cytokines with similar molecular pathways of gene activation, in their ability to modulate c-Jun/AP-1-dependent gene expression.

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