Journal of Cellular Biochemistry 101:753–766 (2007)

Interleukin-1β Selectively Decreases the Synthesis of Versican by Arterial Smooth Muscle Cells

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Abstract Proteoglycans accumulate in lesions of atherosclerosis but little is known as to which factors regulate the synthesis of these molecules. Interleukin-1β (IL-1β) is a cytokine involved in vascular lesion development but it is not clear whether it has specific effects on proteoglycan synthesis by arterial smooth muscle cells (ASMC). Monkey ASMC were treated with IL-1β and proteoglycan synthesis assessed using [^{35}S]-sulfate and [^{35}S]-Trans amino acid labeling. Four prominent size populations of proteoglycans, as determined by SDS–PAGE gradient gel electrophoresis, were observed in the culture medium and identified as versican, biglycan, decorin, and an unknown population that migrated to the gel interface. IL-1β treatment decreased significantly the synthesis of versican, while increasing the synthesis of decorin, but having no effect on biglycan synthesis. Northern blot analyses confirmed this selective effect on versican and decorin mRNA transcripts. Nuclear run-on and RNA inhibition studies showed that decreased mRNA for versican was due to increased mRNA degradation and not to changes in transcription. In addition, IL-1β increased the synthesis of the population of proteoglycans that separated at the SDS–PAGE gel interface. Chondroitinase ABC lyase digestion of this population revealed a complex of proteins composed of versican (350 kDa), an unidentified protein (215 kDa), and a 23 kDa protein identified by sequence analyses as serglycin. These data demonstrate that IL-1β selectively downregulates versican synthesis by ASMC, while positively regulating the synthesis of other proteoglycans. J. Cell. Biochem. 101: 753–766, 2007.

Key words: versican; interleukin-1β; decorin; serglycin; smooth muscle cells

Versican is an extracellular matrix proteoglycan that accumulates in vascular diseases such as atherosclerosis and restenosis [Geary et al., 1996; Halpert et al., 1996; Matsuura et al., 1996; Lin et al., 1996a; Gutierrez et al., 1997; Wight et al., 1997; Evanko et al., 1998; O'Brien et al., 1998; Geary et al., 2002]. The principal source of vascular versican in these lesions is the arterial smooth muscle cell (ASMC) [Chang et al., 1983; Schönherr et al., 1991; Yao et al., 1994; Schönherr et al., 1997; Lemire et al., 1999]. Versican influences the phenotype of ASMCs [Evanko et al., 1999; Lemire et al., 2002; Merrilees et al., 2002] and endothelial cells [Cattaruzza et al., 2002] and may regulate key events associated with lipid retention [Camejo et al., 1998; Williams and Tabas, 1998; Chait and Wight, 2000] and plaque thrombosis [Kolodgie et al., 2002; Mazzucato et al., 2002; McGee and Wagner, 2003]. The inhibition of versican synthesis by antisense modulates tropoelastin synthesis and elastic fiber formation [Huang et al., 2006], possibly affecting intima or plaque stability. For these reasons, it becomes important to understand those factors that regulate versican synthesis by ASMC.

Studies to date have identified a number of growth factors such as platelet-derived growth factor (PDGF) and transforming growth

Grant sponsor: National Institutes of Health; Grant number: HL 18645.

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Received 17 August 2006; Accepted 16 November 2006
DOI 10.1002/jcb.21235

factor-β1 (TGFβ1) that positively regulate the synthesis of versican by ASMC [Schönherr et al., 1991; Schönherr et al., 1997; Evanko et al., 2001]. However, there has been only one report of a cytokine negatively regulating versican synthesis by ASMC and that involved IL-1β [Ostberg et al., 1995]. We found that IL-1β decreased mRNA for versican in both fibroblasts and ASMC [Ostberg et al., 1995] but no information was available as to the mechanism(s) by which IL-1β decreased versican mRNA transcript in these cells, nor was it clear whether the IL-1β effect was specific for versican. Furthermore, it was not known whether these changes in mRNA were translated into differences in the accumulation of versican in these cultures.

We have now extended our earlier studies and demonstrate that IL-1β decreases mRNA stability for versican in ASMC and these changes are reflected in decreased levels of versican synthesized by these cells. This is the first evidence of negative regulation of versican synthesis controlled at the post-transcriptional level in ASMCs. Furthermore, we show this negative regulation of versican synthesis by IL-1β is accompanied by positive regulation of other proteoglycans such as decorin. Finally we identify serglycin, a previously unidentified proteoglycan in these cultures as part of an aggregate that is synthesized by ASMC and positively regulated by IL-1β. These studies illustrate the complex activity of specific inflammatory cytokines on the production of proteoglycans by ASMCs.

MATERIALS AND METHODS

Materials

DMEM, trypsin, non-essential amino acids, sodium pyruvate, penicillin, and streptomycin were from Irvine Scientific. Calf serum was from Intergen. DMEM minus methionine, cysteine, and glutamate, chondroitin ABC lyase, [^{35}S]-sulfate, and Trans-label ([^{35}S]-labeled amino acids) were from ICN. Fibronectin, agarose, and Trizol were from Invitrogen. Urea was from Fisher Scientific. Tris-Base, Triton X-100, and bovine serum albumin were from Boehringer Mannheim. Electrophoresis reagents including acrylamide, and Zetaprobe membrane were from Biorad. Formaldehyde was from J.T. Baker. Protran nitrocellulose (0.2 μm) and the Elutrap system were from Schleicher and Schuell. Vitronectin was from Collaborative Biomedical Products. DECAprime II DNA labeling kit was from Ambion. DEAE Sephacel, phenylmethylsulfonic fluoride, L-glutamine, and some of the IL-1β were from Sigma. X-ray film and benzamidine hydrochloride were from Eastman-Kodak. 6-Aminohexanoic acid was from ACROS. The Enhanced Chemiluminescence kit was from Tropix. α[^{32}P]-dCTP was from Andotech. Enlightening was from Dupont.

The IL-1β for some experiments was a gift of Dr. Steven Dower, Immunex, Seattle, WA. Experiments showed that IL-1β from Immunex and from Sigma had the same effect on ASMC proteoglycan synthesis. Antibody to recombinant versican (V1) was prepared as described [LeBaron et al., 1992; du Cros et al., 1995]. Anti-biglycan (LF-51) and anti-decorin (LF-30) [Fisher et al., 1995] antibodies were gifts from Dr. Larry Fisher, National Institutes of Dental Research, Craniofacial and Skeletal Diseases Branch, National Institutes of Health.

Cell Culture

Monkey ASMC were a gift from Elaine Raines, University of Washington. Cells were maintained in DMEM 5% calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin sulfate, 1 mM sodium pyruvate, and non-essential amino acids. They were used between passage 8 and 13. For most experiments, 1×10^6 cells were plated on 60-mm tissue culture plastic dishes. Cells were cultured for 24 h before the addition of fresh medium containing or lacking IL-1β (10^{-10}M). To label the glycosaminoglycan chains of the proteoglycans, [^{35}S]-sulfate (80 μCi/ml) was added to some cultures 4 or 6 h prior to harvest. For labeling of protein cores 6 h prior to harvest, the medium was changed to medium lacking methionine and cysteine, and 40 μCi/ml [^{35}S]-Trans-label was added. For cultures previously treated with IL-1β, 10^{-10} M IL-1β was added to the labeling medium in order to replace the previous dose. This treatment had a similar effect to the response of the [^{35}S]-sulfate-labeled cultures in which IL-1β was only added once because the medium was not changed (data not shown). Protease inhibitors (1 mM phenylmethylsulfonic fluoride, 5 mM benzamidine hydrochloride, 100 mM 6-aminohexanoic acid) were added to media at harvest and samples were stored at −70°C until proteoglycan isolation.

Plates were coated, respectively, with fibronectin (5 μg/ml), Type I collagen (1 mg/ml), and

vitronectin (10 μg/ml) for 3 h prior to plating. Cells were then plated on bare tissue culture plastic and the above different substrates for 24 h. Subsequently, cultures were incubated with or without IL-1β (10^{-10} M) for 24 h and labeled with [^{35}S]-sulfate (80 μCi/ml) for 6 h prior to harvest. Media samples were harvested with protease inhibitors and isolated by DEAE-Sephacel chromatography, precipitated with ethanol, and electrophoresed on 4%–12% gradient SDS–PAGE with 3.5% stacking gel.

RNA Isolation, Northern Blotting, Hybridization

RNA was isolated using Trizol reagent (Invitrogen). Ten micrograms of RNA was electrophoresed in 0.8% agarose formaldehyde gels, transferred to Zetaprobe membrane, and immobilized by UV cross-linking [Järveläinen et al., 1991; Sambrook et al., 1998]. Blots were probed with [^{32}P]-labeled DNA probes and washed as previously described [Schönherr et al., 1997]. The DNA probes were as follows: human versican, a 2.4 kb fragment from the βGAG region [Zimmermann and Ruoslahti, 1989], decorin [Day et al., 1987], human biglycan [Fisher et al., 1989], 28S rRNA [Iruela-Arispe et al., 1991].

Nuclear Run-on Transcription Assay

Cells were plated at near confluence for 24 h and incubated for 6 h in the presence or absence of 10^{-10} M IL-1β. Cells were scraped with PBS and lysed with 0.5% Triton X-100 on ice for 2 min. They were then layered over a 5 ml sucrose pad (0.33 N Sorbitol, 59 mM HEPES, 1 mM $MgCl_2 \cdot 6H_2O$, 2% BSA, 59.9% Sucrose, pH 7.5) and spun at 3,900 × *g* for 1 h to pellet the nuclei. The supernatant was carefully removed and the nuclei pellets were resuspended in a glycerol storage buffer (50 mM Tris HCl, pH 8.3, 5 mM $MgCl_2 \cdot 6H_2O$, 0.1 mM Na_2EDTA, 40% glycerol). To radiolabel newly synthesized RNA, an equal volume of reaction buffer {10 mM Tris, pH 8.0, 5 mM $MgCl_2$, 300 mM KCl, 0.5 mM each of ATP, CTP, and GTP, 2 mM DTT, 1 U/ml RNAsin, and 0.1 mCi [^{32}P]UTP (3,000 Ci/mmol)} was added to the nuclei and incubated at 30°C for 30 min. RNA was isolated using the Trizol reagent. The newly synthesized radiolabeled RNA (3×10^6 dpm) was hybridized to slot blots containing 0.8 μg of linearized human versican cDNA insert and 28S plasmid on Zetaprobe membranes (Biorad) in 50% formamide, 5% Denhardt's reagent, 6× SSPE, 0.5% SDS, 100 μg/ml denatured salmon sperm DNA at 42°C for 72 h. Filters were washed twice in 2× SSPE, 0.1% SDS 20 min at 42°C and once in 0.3× SSPE, 0.1% SDS 20 min at 55°C. Filters were then processed for autoradiography.

mRNA Stability Assay

Cells were plated at confluent stage for 24 h and were treated in the presence or absence of 10^{-10} M IL-1β for 6 h and subsequently for 0,1, 2, 5, and 10 h in the presence of 10 μg/ml Actinomycin D, an inhibitor of RNA synthesis. Total RNA was isolated at the various time points and Northern analysis was performed. The membrane was probed with human versican cDNA. For normalization, the membrane was stripped and rehybridized with a probe for 18S rRNA.

Proteoglycan Isolation, PAGE, and Western Blotting

Proteoglycans in the culture medium were isolated by DEAE Sephacel chromatography [Schönherr et al., 1997], precipitated with ethanol, and electrophoresed on 4%–12% gradient SDS–PAGE gels with 3% stacking gels [Laemmli, 1970] unless otherwise noted. Some samples were subjected to chondroitin ABC lyase digestion [Schönherr et al., 1991, 1997] prior to electrophoresis. The gels were treated with Enlightening solution as directed by the manufacturer and exposed to film. Proteins were transferred from some gels to nitrocellulose and probed with antibodies as previously described [Schönherr et al., 1997], except that the primary antibody was diluted in buffer containing 2% calf serum. In some cases the chemiluminescent signal from the Western analysis was allowed to decay for at least 2 days and blots were autoradiographed for 2 or more days to detect [^{35}S]-labeled molecules.

Electroelution from PAGE Gels

Individual proteoglycan bands were cut from 4%–12% PAGE gels with 3% stacking gels and recovered from the gels by electroelution (18 h, 100 volts) in PAGE gel buffer lacking SDS, using the Elutrap system. Following ethanol precipitation, the recovered proteins were analyzed by PAGE gels followed by autoradiography or Western blotting as above.

Protein Core Isolation and Peptide Sequencing

Monkey ASMC were plated at an initial density of 6×10^6 cells per 150-mm dish using the same culture conditions as described in the text. A total of 22 dishes were cultured. At 24 h post plating, 10^{-10} M IL-1β was added to each culture. To obtain a radiolabeled preparation to be used as a tracer, four dishes were treated with the same concentration of IL-1β plus ^{35}S-labeled amino acids (40 mCi/ml). All cultures were maintained for an additional 24 h after which the conditioned media (CM) was collected. Proteoglycans were isolated from the CM by ion-exchange chromatography (DEAE-Sephacel) and subsequently concentrated by ethanol precipitation. The preparation was then electrophoresed on a 4%–12% gradient gel as previously described [Schönherr et al., 1991]. The region of the gel flanking the interface was then excised from the gel for subsequent electroelution.

To facilitate elution of the interface band, the gel slice was incubated in 1.5 ml of elution buffer (25 mM Tris-HCl, 192 mM glycine, 0.1% SDS w/v, pH 8.3) for 48 h at 4°C. The band (as well as the 1.5 ml of elution buffer) were placed in the sample trap of an Elutrap electroeluter (Schleicher and Schuell) and electroeluted at 100V/20mA (constant voltage) for 48 h. Eluted sample was collected and the elution buffer changed every 12 h. The eluted band was then precipitated by the addition of 3.5 volumes of 95% ethanol containing 1.5% KAc w/v and pelleted by centrifugation (10,000 rpm for 10 min). The pelleted material was then subjected to chondroitinase ABC lyase digestion as previously described [Schönherr et al., 1997]. The digested material was electrophoresed on 4%–12% SDS–PAGE gels with 3% stacking gels as described [Laemmli, 1970]. Following SDS–PAGE, the separated proteins were transferred to PVDF membrane (Bio-Rad) and stained with coomassie R250 (0.1 w/v R250 in 40% MeOH, 1% HAc) and destained in 50% MeOH. An autoradiograph was then prepared from the PVDF blot by placing it against film for 48 h. Using the autoradiograph as a guide, a swatch of the PVDF membrane containing the 23 kDa band was cut and submitted for N-terminal sequencing (Midwest Analytical, St. Louis, MO).

RESULTS

Northern blot analysis of mRNA isolated from ASMC treated with 10^{-10} M IL-1β for 6 h following stimulation up to 24 h confirmed our previous findings that IL-1β significantly reduced versican mRNA levels (Fig. 1A). IL-1β had the opposite effect on decorin mRNA in that IL-1β increased mRNA transcript for decorin at the 12–18 h time point. The level of biglycan mRNA was not affected by IL-1β treatment at any of the time points analyzed. Because our previous studies had suggested that the nature

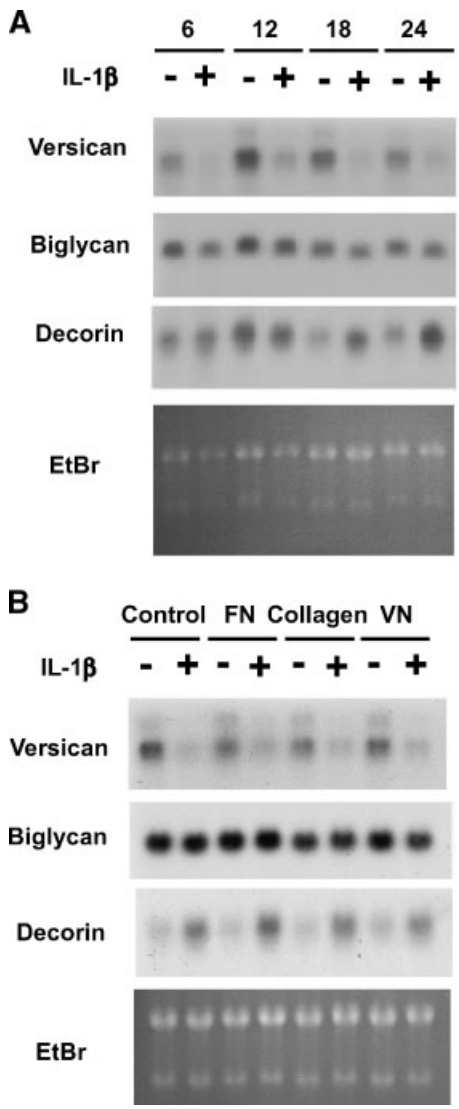


Fig. 1. Changes in ASMC proteoglycan mRNA levels in response to IL-1. **A**: Time course of the response to Il-1β. Cells were stimulated with IL-1β (10^{-10} M) 24 h after plating and harvested at the indicated times. Top panels: Northern blot of RNA isolated from cultures was probed for the expression of versican, biglycan, and decorin RNAs. Bottom panel: RNA in gel stained with ethidium bromide (EtBr) prior to transfer. **B**: Northern blot of RNA isolated 24 h after IL-1β treatment, from cultures grown on different substrates. Top and bottom panels as in **A.** fn, fibxonectin; collagen, type I collagen, vn; vitronectin.

of the substrate may in part regulate the IL-1β response by these cells, Northern blot analysis was performed on ASMC grown on either type I collagen, fibronectin, or vitronectin in the presence or absence of IL-1β for 24 h (Fig. 1B). Results show that none of the substrates influenced the selective effects of IL-1β on versican and decorin mRNA.

To determine if changes in versican mRNA following IL-1β treatment were due to transcriptional or post-transcriptional events, nuclear run-on experiments were performed. Hybridization analyses of radiolabeled transcripts in the nuclear extracts revealed no differences in versican RNA between IL-1β-treated and control cultures when normalized to 28S RNA (Fig. 2A,B). These results suggest that the decrease in mRNA for versican by IL-1β in these cells is not due to a direct effect on transcription. To determine if versican mRNA stability was affected by IL-1β, cultures were treated with actinomycin D at various time points from 1 to 10 h following IL-1β addition and Northern blot analyses performed at these time points. IL-1β treatment increased the versican mRNA decay rate by threefold over the controls (Fig. 2C). These results indicate that IL-1β decreases versican mRNA levels primarily by post-transcriptional mechanisms.

To determine if IL-1β negatively regulated the synthesis and accumulation of versican in these cultures, ASMC were treated with IL-1β for up to 24 h, pulse-labeled for 6 h with ^{35}S-sulfate before harvest, and proteoglycans were isolated from the medium. SDS–PAGE analyses of radiolabeled proteoglycans show that IL-1β reduced the amount of radiolabel in the versican, previously identified as the proteoglycan band that remains in the stacking gel [Schönherr et al., 1991, 1997] (Fig. 3A) at all time points. IL-1β (10^{-10} M) was chosen as a dose since a dose-response experiment with IL-1β revealed that 10^{-10} M gave a maximal effect in decreasing the amount of radioactivity in the versican band over the time points in the study (data not shown). In addition, others have shown that similar concentrations of IL-1β regulate the synthesis of matrix molecules and metalloproteinases by ASMC [Amento et al., 1991; Keen et al., 1994; Ye et al., 1997]. IL-1β increased the intensity of the unidentified (but see below) radiolabeled band that separated at the interface of the stacking and separating gels but had no effect on the intensity of the band previously identified as biglycan [Schönherr et al., 1993]. However, IL-1β did increase the radiolabel intensity in the band previously shown to contain decorin [Järveläinen et al., 1991; Schönherr et al., 1993].

To determine if the nature of the substrate had an effect on this IL-1β response, ASMC were cultured on fibronectin, Type I collagen, or vitronectin and stimulated with IL-1β. None of the substrates affected the selective effects of IL-1β on the synthesis of proteoglycans by these cells (Fig. 3B).

To determine if IL-1β affected the synthesis of proteoglycan core proteins, cultures were labeled with [^{35}S]-labeled amino acids following the same protocol as for cells labeled with [^{35}S]-sulfate. Following purification of the labeled proteoglycans by DEAE chromatography, the same four bands could be identified by 4%–12% gradient SDS–PAGE (Fig. 4). As shown for the [^{35}S]-sulfate labeled samples, IL-1β reduced the intensity of the versican band but increased the intensities of the band at the gel interface as well as the band that contains decorin without affecting the band that contains biglycan (Fig. 4). The changes detected in the interface band after IL-1β treatment in the [^{35}S]-amino acid-labeled samples were not as dramatic as those observed in the [^{35}S]-sulfate-labeled samples (compare Fig. 3 and Fig. 4), suggesting that IL-1β had a greater effect on the glycosaminoglycan component of this band. Digestion of the samples with chondroitin ABC lyase eliminated all four radiolabeled bands and generated a series of new bands that migrated to different positions on the gel (Fig. 5A,B). Comparison of the banding patterns between IL-1β-treated and control cultures reveals that the same bands are present but differ in their intensities. Distinct bands were seen after chondroitin ABC lyase digestion at 450, 350, 215, 127, 48, 40, and 23 kDa. IL-1β treatment caused decreases in the 450 and 350 kDa bands by 6 h following treatment and this decrease lasted through 24 h of treatment (Fig. 5A). No change in intensity was seen in the 215 kDa band but IL-1β decreased the intensity of a band found at 127 kDa while increasing intensities of the 48 and 40 kDa bands. A faint band appeared at approximately 23 kDa (Fig. 5A) which also was increased by IL-1β by 12 h (Fig. 5B). While differences in the intensities of these bands could be seen as early as 6 h following IL-1β

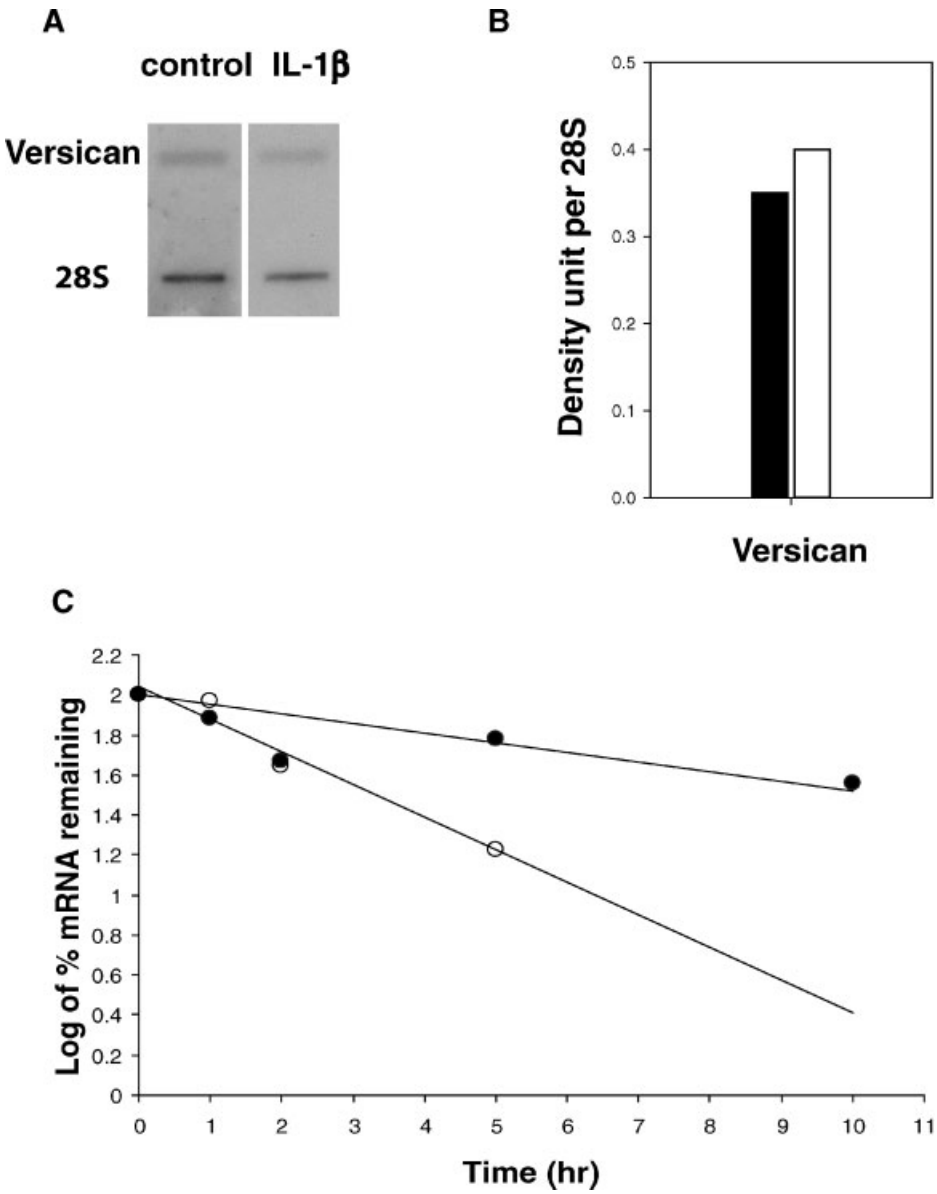
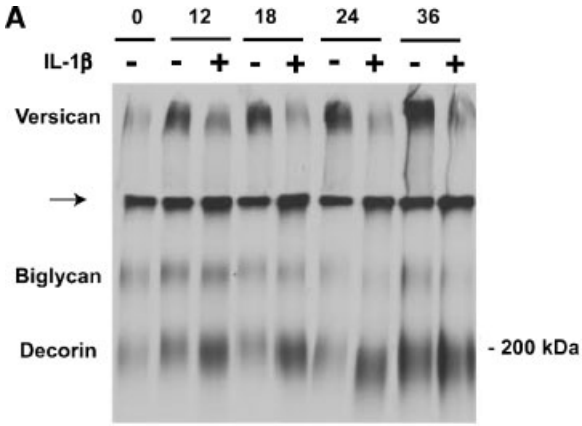


Fig. 2. Nuclear run-on transcription. **A**: Nuclear run-on transcriptional analysis of untreated cells and cells exposed to 10^{-10} M IL-1β for 6 h was performed as described under Materials and Methods. The labeled transcripts of control and IL-1β-treated cells were hybridized to human versican cDNA and 28S plasmid that had been previously slot blotted and bound to the filter. **B**: Relative levels of the transcripts for versican were normalized per 28S and expressed in the bar graph. Solid bar represents control cells and open bar represents IL-1β-treated cells. **C**: Effect of IL-1β on versican mRNA decay rate. Confluent cells were treated in the presence or absence of IL-1β (10^{-10} M) for 6 h and subsequently up to 10 h in the presence of 10 μg/ml Actinomycin D. Total RNA was isolated at 0, 1, 2, 5, 10 h and Northern analysis was performed. Closed circles represent control cells and open circles represent IL-1β-treated cells. The decay of versican mRNA was found to have a half-life of 6 h in control and 2 h in IL-1β-treated cells. All scanning data from Northern blots were subjected to linear analysis of semilog plots of the percentage of mRNA remaining versus time [Song et al., 2000].

treatment, maximum differences were evident at 24 h (Fig. 5A,B).

Proteoglycans isolated from the media of control cultures and cultures treated with IL-1β were subjected to chondroitin ABC lyase digestion and probed with an antibody to human versican. This antibody was raised against total recombinant versican V1 isoform expressed by CHO cells [LeBaron et al., 1992; du Cros et al., 1995] and thus is expected to react with all the versican splice variants. Versican antibody reacted only with protein cores at 450 and 350 kDa (Fig. 6, lanes 2, 3) and not with the radioactive bands at 215, 127, 48, 40, and



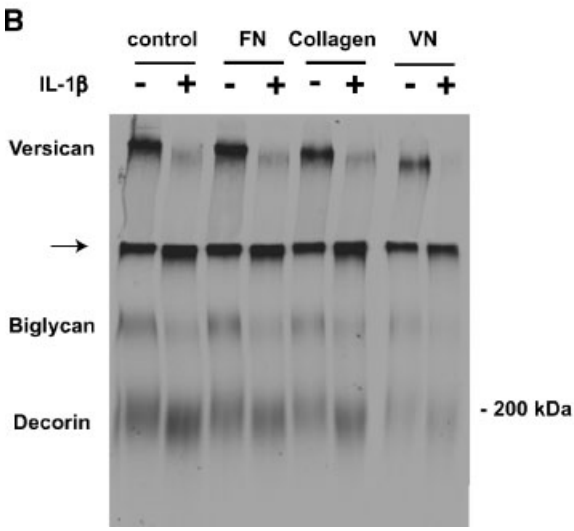


Fig. 3. Time course of the incorporation of [^{35}S]-sulfate into proteoglycans in response to IL-1β. **A**: Cells were stimulated with IL-1β (10^{-10} M) 24 h after plating. [^{35}S]-sulfate was added to the culture medium 6 h before harvest at the indicated times. Proteoglycans were isolated form the culture medium and equal volumes were separated on SDS–PAGE gels and autoradiographed. The number of hours after IL-1β addition is indicated. The position of the 200 kDa marker is indicated. **B**: Cells growing on different substrates, as specified, were treated with IL-1 and labeled as described above. Medium was collected 24 h after IL-1 treatment and equal volumes of radiolabeled proteoglycans were separated by SDS–PAGE. Arrow points to radiolabeled proteoglycans that separate at the stacking/resolving gel interface.

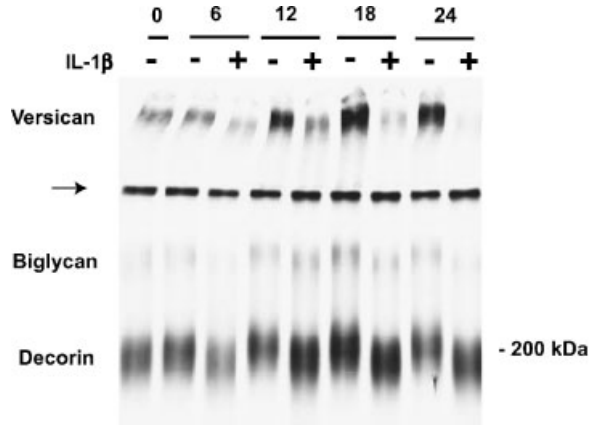


Fig. 4. Time course of incorporation of [^{35}S]-amino acids into proteoglycans in response to IL-1β. Cells were stimulated with IL-1β (10^{-10} M) 24 h after plating. Cultures were labeled with [^{35}S]-amino acids 6 h before harvest at the indicated times. Proteoglycans were isolated from the culture medium. Amounts proportional to the cell number were separated on SDS–PAGE gels and autoradiographed. Arrow points to radiolabeled proteoglycans that separate at the stacking/resolving gel interface. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

23 kDa, which can be detected by autoradiography after the decay of the chemiluminescent signal (lanes 5, 6). The bands of immunoreactivity below 350 kDa may represent degradation products, which appear as faint bands after SDS–PAGE of [^{35}S]-amino acid-labeled core proteins (Fig. 5A). By comparing the size of these bands with the versican splice variants expressed by glioma cells [Dours-Zimmermann and Zimmerman, 1994], it is likely that the 450 kDa band corresponds to the V0 isoform and the 350 kDa band corresponds to the V1 isoform, which are both expressed by ASMC [Yao et al., 1994; Lemire et al., 1999]. IL-1β decreased the intensity of the 450 and 350 kDa immunoreactive bands showing IL-1β selective effect on versican core protein synthesis.

Western analysis of chondroitin ABC lyase-digested proteoglycans from the media of control cultures, and of cultures treated with IL-1β for 24 h, was used to identify the core proteins of decorin and biglycan, which are dermatan sulfate proteoglycans (Fig. 6B). Biglycan immunoreactive core proteins, at approximately 50 and 43 kDa, were detected in both IL-1β and control samples, but the intensity of the signal did not change in response to IL-1β (Fig. 6B, lane 3, IL-1; lane 2, control). On the other hand, decorin immunoreactivity increased in response to IL-1β and migrated at approximately 48 and 40 kDa (Fig. 6B, lane 6, IL-1; lane 5, control). After the short-lived chemiluminescent Western signal had decayed, the blots were subjected to autoradiography to detect the [^{35}S]-labeled core proteins, which are present in greater amount in the IL-1β-treated sample (Fig. 6B, lane 9, IL-1; lane 8, control). Biglycan immunoreactivity corresponded predominately with the top of the 48 kDa [^{35}S]-labeled core protein (compare to Fig. 6B, lanes 2, 3). Decorin immunoreactivity corresponded with both the

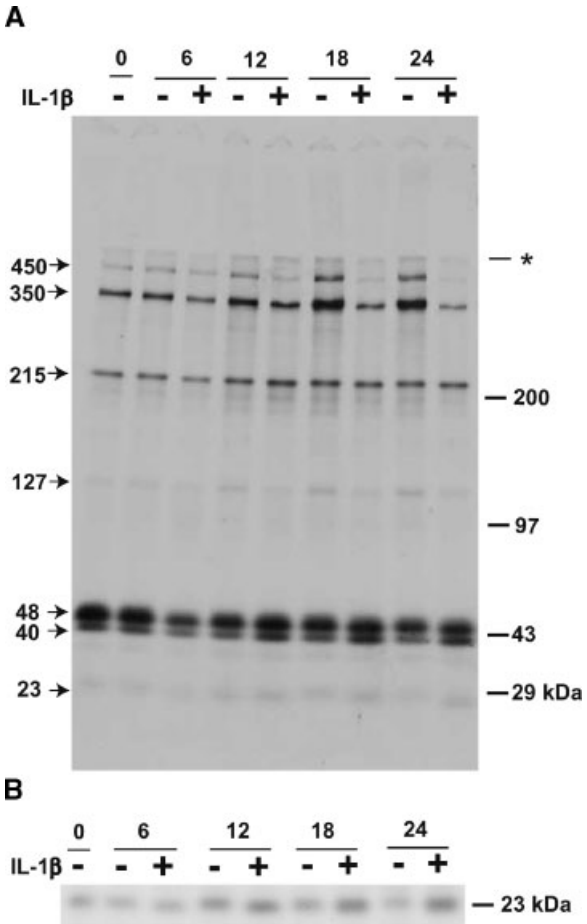


Fig. 5. Time course of incorporation of [^{35}S]-amino acids into proteoglycan core proteins in response to IL-1β. **A**: Cells were stimulated with IL-1β (10^{-10} M) 24 h after plating. Cultures were labeled with [^{35}S]-amino acids 6 h before harvest at the indicated times. Proteoglycans were isolated from the culture medium and were subjected to chondroitin ABC lyase digestion prior to loading on gels. Amounts proportional to the cell number were separated on SDS–PAGE gels and autoradiographed. The asterisk indicates the top of the separating gel. The positions of the size markers are indicated, at right. Arrowheads, at left, are labeled with the calculated size of the core protein bands. **B**: Portion of an SDS–PAGE gel containing the 23 kDa protein at various times after IL-1β treatment. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

48 and 40 kDa core proteins (compare to Fig. 6B, lanes 5, 6).

To identify the specific protein core(s) in the smallest proteoglycan that increases in response to IL-1β, the bands from control and IL-1-treated samples were excised from a gel similar to Figure 4. The electroeluted bands were digested with chondroitin ABC lyase and probed with antibodies to decorin and biglycan (Fig. 6C). The small band from IL-1 and control treated cultures contained both decorin (Fig. 6C, lanes 5, 6) and biglycan cores (lanes 2, 3). The similarity between the Western signal for decorin (Fig. 6C, lanes 5, 6) and the autoradiographic pattern (Fig. 6C, lanes 8, 9) suggests, however, that the lower molecular weight band contains predominately decorin. These results support the interpretation that decorin but not biglycan was influenced by IL-1β treatment.

To identify the presence of specific proteoglycan cores in the radiolabeled proteoglycan bands, [^{35}S]-amino acid-labeled bands were cut from the gel, digested with chondroitin ABC lyase, electroeluted from the gel, and rerun on SDS–PAGE (Fig. 7). Recovery from the gels is not quantitative so comparisons of abundance among cores recovered from a single proteoglycan band, as well as between IL-1β-treated and control cultures, is not possible. Digestion of the radiolabeled band in the stacking gel from control and IL-1β-treated cultures gave protein bands at 450, 350, and 305 kDa (Fig. 7, lane 1). The 305 kDa band is likely to be a degradation product, as it is not routinely seen. The 450 and 350 kDa bands correspond to the versican cores, as identified by Western analysis in Fig. 6A.

Digestion of the radiolabeled band that separates at the interface between the stacking and resolving gel with chondroitin ABC lyase resulted in protein bands at 350, 215, and 23 kDa (Fig. 7, lane 2). These results show that versican is also present in the second radiolabeled band because the 350 kDa protein is recognized by the versican antibody (Fig. 6A). Two additional components (215 and 23 kDa) are radiolabeled in this band as well. No versican immunoreactivity was seen at the positions of the 215 and 23 kDa cores (see Fig. 6A), indicating that they are not derived from versican. Thus, of the seven protein cores identified following chondroitin ABC lyase treatment of ASMC-derived proteoglycans, only four corresponded to known proteoglycans synthesized by these cells. Furthermore, these three components of the interface band are only resolved from each other after chondroitin ABC lyase digestion of the sample, and not by boiling in gel loading buffer containing mercaptoethanol prior to cutting the interface band from the gel. These results suggest that the macromolecules that separate at the interface on the SDS–PAGE gel may be part of a complex formed by covalent linkage involving chondroitin sulfate chains or merely represent more than one

chondroitin sulfate proteoglycan with separate and distinct core proteins. Furthermore, it is of interest that chondroitin ABC lyase digestion of the biglycan band (Fig. 7, lane 3) generated a band at 127 kDa and at ~48 kDa, while chondroitin ABC lyase digestion of the smaller band, containing predominantly decorin (Fig. 6C) generated only bands at ~45 kDa and several lower molecular weight bands. The identity of the 127 kDa band is not known at this time.

Since the 23 kDa protein showed positive regulation by IL-1β, we sought to identify the source of this protein. Amino terminal sequencing of the 23 kDa band resulted in the elucidation of a stretch of 15 contiguous amino acids. A BLAST search [Altschul et al., 1997] of the NCBI non-redundant data base showed an exact match of this *Macaca nemstrina* sequence with amino acids 28–42 of the predicted sequence of proteoglycan 1, secretory granule isoform 1, from *Macaca mulatta*, the rhesus monkey (Fig. 8). This proteoglycan is also known as serglycin and is encoded by the *PRG1* gene. *Homo sapiens* and the Yerkes chimp, *Pan troglodytes* had a three amino acid insertion in comparison to the serglycin sequence found in both *Macaca* species; a different three amino acid insertion is found in rat and mouse. Thus, the 23 kDa band liberated by chondroitinase ABC lyase digestion of band at the interface is the monkey homologue of human serglycin.

DISCUSSION

Versican has been shown to be positively regulated by PDGF and TGF-β1 in ASMC [Chen et al., 1991; Schönherr et al., 1991, 1997; Evanko et al., 2001] and these molecules are likely to be at least partly responsible for the increase of versican seen in developing vascular lesions [Geary et al., 1996, 2002; Halpert et al., 1996; Matsuura et al., 1996; Lin et al., 1996b;

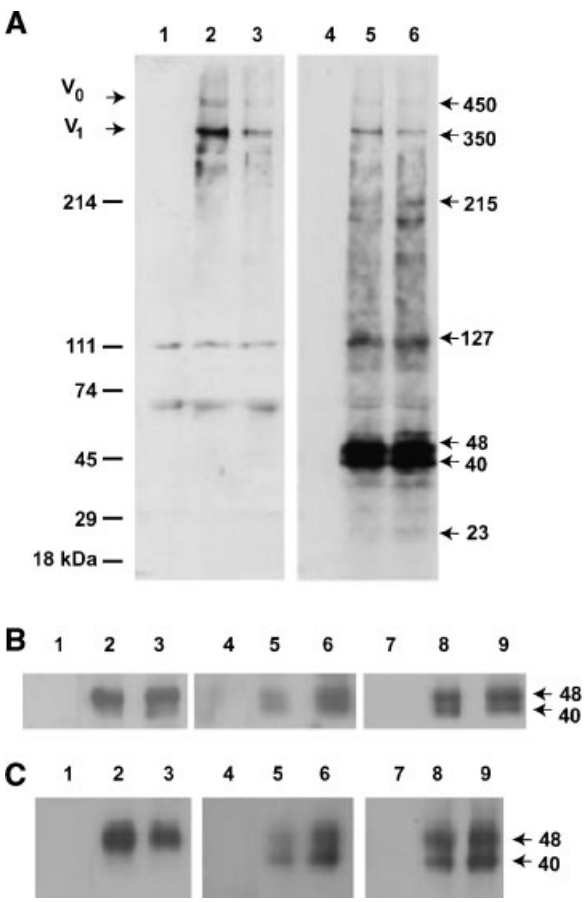


Fig. 6. Identification of the proteoglycan cores responding to IL-1β stimulation by Western blot analysis. **A**: Versican core proteins decrease in response to IL-1β. Proteoglycans were isolated from the medium of cells cultured for 24 h in absence (lane 2) or presence (lane 3) of IL-1β, subjected to chondroitin ABC lyase digestion, and electrophoresed on 4%–12% SDS–PAGE gels and transferred to nitrocellulose. Left panel: the blot was probed with an antibody to human versican. Lane 1 is a control sample of the chondroitin ABC lyase reaction mixture, lacking any proteoglycan sample. Right panel: autoradiography to detect [^{35}S]-label, after decay of chemiluminescent signal from the Western analysis. The positions of the radiolabeled cores are indicated with arrows. The position of the molecular size markers is indicated at left. **B**: Identification of the 48 and 40 kDa cores: the decorin core increases in response to IL-1β. Proteoglycans were isolated from the medium of cells cultured for 24 h in absence (lanes 2, 5, 8) or presence (lanes 3, 6, 9) of IL-1β, subjected to chondroitin ABC lyase digestion, and electrophoresed on 10% PAGE gels and transferred to nitrocellulose. Lanes 1, 4, 7; a control sample of the chondroitin ABC lyase reaction mixture, lacking any proteoglycan sample. Lanes 1–3, Western blot probed with antibody to biglycan; lanes 4–6, identical blot probed with antibody to decorin; lanes 7–9, same blot as lanes 4–6, autoradiographed to detect [^{35}S]-label, after decay of chemiluminescent signal from Western. **C**: Identification of the 48 and 40 kDa core proteins derived from the smallest proteoglycan band. Proteoglycans bands were cut from a gel, electroeluted, subjected to chondroitin ABC lyase digestion, reapplied to 8% SDS–PAGE gels, and subjected to Western analysis. Lanes 1, 4, 7; a control sample of the chondroitin ABC lyase reaction mixture, lacking any proteoglycan sample. Lanes 2, 5, 8, core proteins derived from the smallest proteoglycan band, from the medium of an untreated culture. Lanes 3, 6, 9, core proteins derived from the smallest proteoglycan band, from the medium of a culture treated with IL-1β for 24 h. Lanes 1–3, Western blot probed with antibody to biglycan; lanes 4–6, Western blot probed with antibody to decorin; lanes 7–9, same blot as lanes 4–6, autoradiographed to detect [^{35}S] label, after decay of chemiluminescent signal from Western blot.

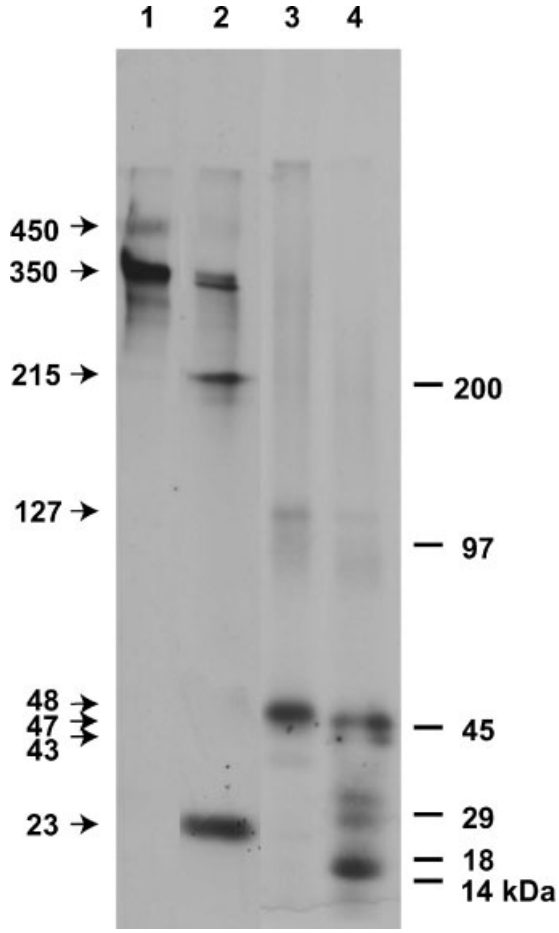


Fig. 7. Correlation of proteoglycans with their respective protein cores. Individual [^{35}S]-amino acid-labeled proteoglycan bands derived from the medium of cells cultured in the presence of IL-1β were cut from gels, digested with chondroitin ABC lyase, and their core proteins electrophoresed and autoradiographed. Lane numbers correspond to the position of the undigested proteoglycan bands on a PAGE gel, from top to bottom. Bands 1 (versican) and 2 (unknown) were subjected to chondroitin ABC lyase digestion before isolation from the gel slices and recovered from the reaction solution. Bands 3 (biglycan) and 4 (predominately decorin) were isolated from the gel slices by electroelution, ethanol precipitated, and subjected to chondroitin ABC lyase digestion. Arrowheads, at left, are labeled with the calculated size of the core protein bands.

Gutierrez et al., 1997; Wight et al., 1997; Evanko et al., 1998; O'Brien et al., 1998]. Although versican is widely distributed in loose connective tissue [Bode-Lesniewska et al., 1996; Westergren-Thorsson et al., 1998], restenotic lesions [Matsuura et al., 1996; Wight et al., 1997; Chung et al., 2002], condensing mesenchyme prior to chondrogenesis [Kimata et al., 1986], and the cycling hair follicle [du Cros et al., 1995], high-level expression of this proteoglycan is transient. Little is known about the removal of versican *in vivo* and whether it is regulated by increased degradation or decreased synthesis. Thus far, the only molecule known to decrease versican RNA abundance in ASMC is IL-1β [Ostberg et al., 1995]. While IL-1β appears to be a negative regulator of versican synthesis in ASMC, opposite effects are seen with cultured human embryonic lung fibroblasts [Tufvesson and Westergren-Thorsson, 2000]. Such results suggest that IL-1β effects on proteoglycan synthesis may be cell-type specific. In addition, antisense IL-1β expression reduces endogenous IL-1β levels in vascular smooth muscle cells but has no effect on versican mRNA levels when these cells are maintained in the presence of endothelial growth supplement [Hsu et al., 1999]. On the other hand, another member of the versican family, aggrecan, is decreased in response to IL-1β in cultured cartilage and in rabbit articular chondrocytes in monolayer culture [Tyler, 1985; Benton and Tyler, 1988; Demoor-Fossard et al., 1998].

While the present data indicate that IL-1β has definite effects on the synthesis of versican by ASMCs, IL-1β is known to induce the production of proteolytic enzymes capable of degrading proteoglycans and thus may additionally regulate versican content by influencing versican turnover. For example, IL-1β induces the degradation of aggrecan via the

Species	Accession #	Serglycin sequence
Macaca nemstrina	(this work)	1 YP ARYQWVRCNPDSN 15
Macaca mulatta	XP_001110742	28 YP ARYQWVRCNPDSN 42
Pan troglodytes	XP_001168827	27 YP{TQR}ARYQWVRCNPDSN 44
Homo sapiens	NP_002718	28 YP{TQR}ARYQWVRCNPDSN 45
Rattus novegicus	NP_064459	27 YP{ARR}ARYQWVRCKPD 42
Mus musculus	NP_035287	26 YP{ARR}ARYQWVRCKP 40

Fig. 8. Amino-terminal sequencing of the 23 kDa band revealed 100% homology to human serglycin from the Rhesus monkey. *Macaca* serglycin lacks three amino acids (TQR) in comparison to the human and Yerkes chimp sequence.

induction of matrix metalloproteinase and "aggrecanase," a molecule that is a member of the "ADAMTS" family [Hughes et al., 1995; Xu et al., 1996; Hsu et al., 1999; Tortorella et al., 1999]. Furthermore, ASMC-derived versican is a substrate for ADAMTS-1 and ADAMTS-4 [Sandy et al., 2001]. It is not known whether the metalloproteinases that are induced in ASMC in response to IL-1β [Galis et al., 1994; Fabunmi et al., 1996] degrade versican, or whether the ADAMTS members are regulated by IL-1β in ASMC.

Our finding that IL-1β positively regulates the synthesis and accumulation of decorin agrees with earlier studies that demonstrated that IL-1β derived from macrophages increased decorin synthesis by ASMCs [Edwards et al., 1990, 1994]. The fact that IL-1β positively regulates decorin levels via the transcription factor AP-1/c-jun in fibroblasts [Mauviel et al., 1996; Lin et al., 1997] suggests that the synthesis of this proteoglycan is controlled through transcriptional regulation by IL-1β. The lack of AP1 binding sites in the versican promoter [Naso et al., 1994] indicates that the synthesis of these two proteoglycans must be controlled differently.

The finding that serglycin was produced by these cells and remained as part of an aggregate between the stacking and resolving gel under reducing conditions was a surprise. Serglycin was once thought to be restricted to the haemopoietic lineage [Kolset and Gallagher, 1990; Schick and Senkowski-Richardson, 1992; Schick and Jacoby, 1995] but a number of studies have shown that serglycin is expressed outside the haemopoietic system including vascular endothelial and smooth muscle cells [Kulseth et al., 1999; Schick et al., 2001]. Our finding that IL-1β increased the amount of serglycin produced by ASMCs is the first demonstration that this cytokine positively regulates serglycin, although TNF alpha and IL-1β also stimulate serglycin production in endothelial cells [Kulseth et al., 1999]. In addition to serglycin being present in the radiolabeled band present at the gel interface, chondroitinase digestion of this band also released two proteins at 350 and 215 kDa. Western blot analyses identified the 350 kDa band as versican but the versican antibody did not react with the 215 kDa band. Interestingly, macrophages secrete serglycin covalently linked to MMP 9 and this complex was seen to separate at the interface of a 4.5%–7.5% gradient SDS–PAGE gel [Winberg et al., 2000] similar to what we have seen in our study. Whether this same complex exists for ASMCs awaits further study but this would seem unlikely since reducing conditions were used in the isolation of this aggregate in our study. Identification of the nature of the interaction of sergylcin with these and other components also awaits further study.

In summary, IL-1β specifically downregulates versican synthesis by cultured non-human primate ASMC by post-transcriptional events but positively regulates the synthesis of other proteoglycans synthesized by these cells, such as decorin and serglycin. These changes may be part of a specific response by ASMCs in response to this proinflammatory cytokine. Since versican accumulation is a hallmark of early vascular lesions, IL-1β may be useful as a treatment in negatively regulating versican synthesis by ASMC involved in vascular lesion development.

ACKNOWLEDGMENTS

This work was supported by NIH grant HL 18645 (to T.N.W). We thank Dr. Larry Fisher for the antibodies to biglycan and decorin. We thank Dr. Marian Young for the cDNA probe to biglycan and for the decorin cDNA. Both Dr. Fisher and Dr. Young are from the National Institutes of Dental Research, Craniofacial and Skeletal Branch, NIH, Bethesda, MD. The authors also thank Dr. Michael Kinsella and Dr. Susan Potter-Perigo for their critical reading of the manuscript, Dr. Virginia Green for editing, and Ms. Ellen Briggs for assistance in its preparation.

REFERENCES

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res 25:3389–3402.

Amento EP, Ehsani N, Palmer H, Libby P. 1991. Cytokines and growth factors positively and negatively regulate interstitial collagen gene expression in human vascular smooth muscle cells. Arterioscler Thromb 11:1223–1230.

Benton HP, Tyler JA. 1988. Inhibition of cartilage proteoglycan synthesis by interleukin I. Biochem Biophys Res Commun 154:421–428.

Bode-Lesniewska B, Dours-Zimmermann MT, Odermatt BF, Briner J, Heitz PU, Zimmermann DR. 1996. Distribution of the large aggregating proteoglycan versican in adult human tissues. J Histochem Cytochem 44:303–312.

Camejo G, Hurt-Camejo E, Wiklund O, Bondjers G. 1998. Association of apo B lipoproteins with arterial proteoglycans: Pathological significance and molecular basis. Atherosclerosis 139:205–222.

Cattaruzza S, Schiappacassi M, Ljungberg-Rose A, Spessotto P, Perissinotto D, Morgelin M, Mucignat MT, Colombatti A, Perris R. 2002. Distribution of PG-M/versican variants in human tissues and de novo expression of isoform V3 upon endothelial cell activation, migration, and neoangiogenesis in vitro. J Biol Chem 277:47626–47635.

Chait A, Wight TN. 2000. Interaction of native and modified low-density lipoproteins with extracellular matrix. Curr Opin Lipidol 11:457–463.

Chang Y, Yanagishita M, Hascall VC, Wight TN. 1983. Proteoglycans synthesized by smooth muscle cells derived from monkey (*Macaca nemestrina*) aorta. J Biol Chem 258:5679–5688.

Chen JK, Hoshi H, McKeehan WL. 1991. Stimulation of human arterial smooth muscle cell chondroitin sulfate proteoglycan synthesis by transforming growth factor-β. In Vitro Cell Dev Biol 27:6–12.

Chung IM, Gold HK, Schwartz SM, Ikari Y, Reidy MA, Wight TN. 2002. Enhanced extracellular matrix accumulation in restenosis of coronary arteries after stent deployment. J Am Coll Cardiol 40:2072–2081.

Day AA, McQuillan CI, Termine JD, Young MR. 1987. Molecular cloning and sequence analysis of the cDNA for small proteoglycan II of bovine bone. Biochem J 248:801–805.

Demoor-Fossard M, Redini F, Boittin M, Pujol JP. 1998. Expression of decorin and biglycan by rabbit articular chondrocytes. Effects of cytokines and phenotypic modulation. Biochim Biophys Acta 1398:179–191.

Dours-Zimmermann MT, Zimmerman DR. 1994. A novel glycosaminoglycan attachment domain identified in two alternative splice variants of human versican. J Biol Chem 269:32992–32998.

du Cros DL, LeBaron RG, Couchman JR. 1995. Association of versican with dermal matrices and its potential role in hair follicle development and cycling. J Invest Dermatol 105:426–431.

Edwards IJ, Wagner WD, Owens RT. 1990. Macrophage secretory products selectively stimulate dermatan sulfate proteoglycan production in cultured arterial smooth muscle cells. Am J Pathol 136:609–621.

Edwards IJ, Xu H, Wright MJ, Wagner WD. 1994. Interleukin-1 upregulates decorin production by arterial smooth muscle cells. Arterioscler Thromb 14:1032–1039.

Evanko S, Raines EW, Ross R, Gold LI, Wight TN. 1998. Proteoglycan distribution in lesions of atherosclerosis depends on lesion severity, structural characteristics and the proximity of PDGF and TGF-β1. Am J Pathol 152: 533–546.

Evanko SP, Angello JC, Wight TN. 1999. Formation of hyaluronan and versican-rich pericellular matrix is required for proliferation and migration of vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 19: 1004–1013.

Evanko SP, Johnson PY, Braun KR, Underhill CB, Dudhia J, Wight TN. 2001. Platelet-derived growth factor stimulates the formation of versican-hyaluronan aggregates and pericellular matrix expansion in arterial smooth muscle cells. Arch Biochem Biophys 394:29–38.

Fabunmi RP, Baker AH, Murray EJ, Booth RF, Newby AC. 1996. Divergent regulation by growth factors and cytokines of 95 kDa and 72 kDa gelatinases and tissue inhibitors or metalloproteinases-1, -2, and -3 in rabbit aortic smooth muscle cells. Biochem J 315(Pt 1):335–342.

Fisher LW, Stubbs JT 3rd, Young MF. 1995. Antisera and cDNA probes to human and certain animal model bone matrix noncollagenous proteins. Acta Orthop Scand Suppl 266:61–65.

Fisher LW, Termine J.D, Young MF. 1989. Deduced protein sequence of bone small proteoglycan I (biglycan) shows homology with proteoglycan II (decorin) and several nonconnective tissue proteins in a variety of species. J Biol Chem 264:4571–4576.

Galis ZS, Muszynski M, Sukhova GK, Simon-Morrissey E, Unemori EN, Lark MW, Amento E, Libby P. 1994. Cytokine-stimulated human vascular smooth muscle cells synthesize a complement of enzymes required for extracellular matrix digestion. Circ Res 75:181–189.

Geary RL, Williams JK, Golden D, Brown DG, Benjamin ME, Adams MR. 1996. Time course of cellular proliferation, intimal hyperplasia, and remodeling following angioplasty in monkeys with established atherosclerosis. A nonhuman primate model of restenosis. Arterioscler Thromb Vasc Biol 16:34–43.

Geary RL, Wong JM, Rossini A, Schwartz SM, Adams LD. 2002. Expression profiling identifies 147 genes contributing to a unique primate neointimal smooth muscle cell phenotype. Arterioscler Thromb Vasc Biol 22:2010–2016.

Gutierrez P, O'Brien KD, Ferguson M, Nikkari ST, Alpers CE, Wight TN. 1997. Differences in the distribution of versican, decorin, and biglycan in atherosclerotic human coronary arteries. Cardiovasc Pathol 6:271–278.

Halpert I, Sires U, Potter-Perigo S, Wight TN, Shapiro DS, Welgus HG, Wickline SA, Parks WC. 1996. Matrilysin is expressed by lipid-laden macrophages at sites of potential rupture in atherosclerotic lesions and localized to areas of versican deposits. Proc Natl Acad Sci USA 93: 9748–9753.

Hsu JY, Hsu MY, Sorger T, Herlyn M, Levine EM. 1999. Heparin/endothelial cell growth supplement regulates matrix gene expression and prolongs life span of vascular smooth muscle cells through modulation of interleukin-1. In Vitro Cell Dev Biol Anim 35:647–654.

Huang R, Merrilees MJ, Braun K, Beaumont B, Lemire J, Clowes AW, Hinek A, Wight TN. 2006. Inhibition of versican synthesis by antisense alters smooth muscle cell phenotype and induces elastic fiber formation in vitro and in neointima after vessel injury. Circ Res 98:370–377.

Hughes CE, Caterson B, Fosang AJ, Roughley PJ, Mort JS. 1995. Monoclonal antibodies that specifically recognize neoepitope sequences generated by 'aggrecanase' and matrix metalloproteinase cleavage of aggrecan: Application to catabolism in situ and in vitro. Biochem J 305 (Pt 3):799–804.

Iruela-Arispe ML, Hasselaar P, Sage H. 1991. Differential expression of extracellular proteins is correlated with angiogenesis in vitro. Lab Invest 64:174–186.

Järveläinen HT, Kinsella MG, Wight TN, Sandell LJ. 1991. Differential expression of small chondroitin/dermatan sulfate proteoglycans, PG-I/biglycan and PG-II/decorin,

by vascular smooth muscle and endothelial cells in culture. J Biol Chem 266:23274–23281.
Keen RR, Nolan KD, Cipollone M, Scott E, Shively VP, Yao JS, Pearce WH. 1994. Interleukin-1 beta induces differential gene expression in aortic smooth muscle cells. J Vasc Surg 20:774–784; discussion 784–786.
Kimata K, Oike Y, Tani K, Shinomura T, Yamagata M, Uritani M, Suzuki S. 1986. A large chondroitin sulfate proteoglycan (PG-M) synthesized before chondrogenesis in the limb bud of chick embryo. J Biol Chem 261:13517–13525.
Kolodgie FD, Burke AP, Farb A, Weber DK, Kutys R, Wight TN, Virmani R. 2002. Differential accumulation of proteoglycans and hyaluronan in culprit lesions: Insights into plaque erosion. Arterioscler Thromb Vasc Biol 22: 1642–1648.
Kolset SO, Gallagher JT. 1990. Proteoglycans in haemopoietic cells. Biochim Biophys Acta 1032:191–211.
Kulseth MA, Kolset SO, Ranheim T. 1999. Stimulation of serglycin and CD44 mRNA expression in endothelial cells exposed to TNF-alpha and IL-1alpha. Biochim Biophys Acta 1428:225–232.
Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.
LeBaron RG, Zimmermann DR, Ruoslahti E. 1992. Hyaluronate binding properties of versican. J Biol Chem 267: 10003–10010.
Lemire JM, Braun KR, Maurel P, Kaplan ED, Schwartz SM, Wight TN. 1999. Versican/PG-M isoforms in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 19:1630–1639.
Lemire JM, Merrilees MJ, Braun KR, Wight TN. 2002. Overexpression of the V3 variant of versican alters arterial smooth muscle cell adhesion, migration, and proliferation in vitro. J Cell Physiol 190:38–45.
Lin H, Ignatescu M, Wilson JE, Roberts CR, Horley KJ, Winters GL, Costanzo MR, McManus BM. 1996a. Prominence of apolipoproteins B, (a), and E in the intimae of coronary arteries in transplanted human hearts: Geographic relationship to vessel wall proteoglycans. J Heart Lung Transplant 15:1223–1232.
Lin H, Wilson JE, Roberts CR, Horley KJ, Winters GL, Costanzo MR, McManus BM. 1996b. Biglycan, decorin and versican protein expression patterns in coronary arteriopathy of human cardiac allograft: Distinctness as comared to native atherosclerosis. J Heart Lung Transplant 15:1233–1247.
Lin JM, Yamauchi M, Sato S. 1997. Effects of recombinant interleukin-1 beta on decorin gene expression in human periodontal ligament fibroblast and its possible transcriptional regulation. J Periodontal Res 32:225–232.
Matsuura R, Isaka N, Imanaka-Yoshida K, Yoshida T, Sakakura T, Nakano T. 1996. Deposition of PG-M/versican is a major cause of human coronary restenosis after percutaneous transluminal coronary angioplasty. J Pathol 180:311–316.
Mauviel A, Korang K, Santra M, Tewari D, Uitto J, Iozzo RV. 1996. Identification of a bimodal regulatory element encompassing a canonical AP-1 binding site in the proximal promoter region of the human decorin gene. J Biol Chem 271:24824–24829.
Mazzucato M, Cozzi MR, Pradella P, Perissinotto D, Malmstrom A, Morgelin M, Spessotto P, Colombatti A, De Marco L, Perris R. 2002. Vascular PG-M/versican variants promote platelet adhesion at low shear rates and cooperate with collagens to induce aggregation. FASEB J 16:1903–1916.
McGee M, Wagner WD. 2003. Chondroitin sulfate anticoagulant activity is linked to water transfer: Relevance to proteoglycan structure in atherosclerosis. Arterioscler Thromb Vasc Biol 23:1921–1927.
Merrilees MJ, Lemire JM, Fischer JW, Kinsella MG, Braun KR, Clowes AW, Wight TN. 2002. Retrovirally mediated overexpression of versican V3 by arterial smooth muscle cells induces tropoelastin synthesis and elastic fiber formation in vitro and in neointima after vascular injury. Circ Res 90:481–487.
Naso MF, Zimmermann DR, Iozzo RV. 1994. Characterization of the complete genomic structure of the human versican gene and functional analysis of its promoter. J Biol Chem 269:32999–33008.
O'Brien KD, Olin KL, Alpers CE, Chiu W, Ferguson M, Hudkins K, Wight TN, Chait A. 1998. A comparison of apolipoprotein and proteoglycan deposits in human coronary atherosclerotic plaques: Co-localization of biglycan with apolipoproteins. Circulation 98:519–527.
Ostberg CO, Zhu P, Wight TN, Qwarnstrom EE. 1995. Fibronectin attachment is permissive for IL-1 mediated gene regulation. FEBS Lett 367:93–97.
Sambrook J, Fritsch EF, Maniatis T. 1998. Molecular cloning: A laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
Sandy JD, Westling J, Kenagy RD, Iruela-Arispe ML, Verscharen C, Rodriguez-Mazaneque JC, Zimmermann DR, Lemire JM, Fischer JW, Wight TN, Clowes AW. 2001. Versican V1 proteolysis in human aorta in vivo occurs at the Glu441-Ala442 bond, a site that is cleaved by recombinant ADAMTS-1 and ADAMTS-4. J Biol Chem 276:13372–13378.
Schick BP, Gradowski JF, San Antonio JD. 2001. Synthesis, secretion, and subcellular localization of serglycin proteoglycan in human endothelial cells. Blood 97:449–458.
Schick BP, Jacoby JA. 1995. Serglycin and betaglycan proteoglycans are expressed in the megakaryocytic cell line CHRF 288-11 and normal human megakaryocytes. J Cell Physiol 165:96–106.
Schick BP, Senkowski-Richardson S. 1992. Proteoglycan synthesis in human erythroleukaemia (HEL) cells. Biochem J 282(Pt 3):651–658.
Schönherr E, Järveläinen HT, Sandell LJ, Wight TN. 1991. Effects of platelet-derived growth factor and transforming growth factor-β 1 on the synthesis of a large versican-like chondroitin sulfate proteoglycan by arterial smooth muscle cells. J Biol Chem 266:17640–17647.
Schönherr E, Järveläinen HT, Kinsella MG, Sandell LJ, Wight TN. 1993. Platelet-derived growth factor and transforming growth factor-β1 differentially affect the synthesis of biglycan and decorin by monkey arterial smooth muscle cells. Arterioscler Thromb 13:1026–1036.
Schönherr E, Kinsella MG, Wight TN. 1997. Genistein selectively inhibits platelet-derived growth factor stimulated versican biosynthesis in monkey arterial smooth muscle cells. Arch Biochem Biophys 339:353–361.

Song YL, Ford JW, Gordon D, Shanley CJ. 2000. Regulation of lysyl oxidase by interferon-gamma in rat aortic smooth muscle cells. Arterioscler Thromb Vasc Biol 20:982–988.

Tortorella MD, Burn TC, Pratta MA, Abbaszade I, Hollis JM, Liu R, Rosenfeld SA, Copeland RA, Decicco CP, Wynn R, Rockwell A, Yang F, Duke JL, Solomon K, George H, Bruckner R, Nagase H, Itoh Y, Ellis DM, Ross H, Wiswall BH, Murphy K, Hillman MC, Jr., Hollis GF, Arner EC, et al. 1999. Purification and cloning of aggrecanase-1: A member of the ADAMTS family of proteins. Science 284:1664–1666.

Tufvesson E, Westergren-Thorsson G. 2000. Alteration of proteoglycan synthesis in human lung fibroblasts induced by interleukin-1beta and tumor necrosis factor-alpha. J Cell Biochem 77:298–309.

Tyler JA. 1985. Articular cartilage cultured with catabolin (pig interleukin 1) synthesizes a decreased number of normal proteoglycan molecules. Biochem J 227:869–878.

Westergren-Thorsson G, Norman M, Bjornsson S, Endresen U, Stjernholm Y, Ekman G, Malmstrom A. 1998. Differential expressions of mRNA for proteoglycans, collagens and transforming growth factor-β in the human cervix during pregnancy and involution. Biochim Biophys Acta 1406:203–213.

Wight TN, Lara S, Reissen R, LeBaron R, Isner J. 1997. Selective deposits of versican in the extracellular matrix of restenotic lesions from human peripheral arteries. Am J Pathol 151:963–973.

Williams KJ, Tabas I. 1998. The response-to-retention hypothesis of atherogenesis reinforced. Curr Opin Lipidol 9:471–474.

Winberg JO, Kolset SO, Berg E, Uhlin-Hansen L. 2000. Macrophages secrete matrix metalloproteinase 9 covalently linked to the core protein of chondroitin sulphate proteoglycans. J Mol Biol 304:669–680.

Xu C, Oyajobi BO, Frazer A, Kozaci LD, Russell RG, Hollander AP. 1996. Effects of growth factors and interleukin-1 alpha on proteoglycan and type II collagen turnover in bovine nasal and articular chondrocyte pellet cultures. Endocrinology 137:3557–3565.

Yao LY, Moody C, Schönherr E, Wight TN, Sandell LJ. 1994. Identification of the proteoglycan versican in aorta and smooth muscle cells by DNA sequence analysis, in situ hybridization and immunohistochemistry. Matrix Biol 14:213–225.

Ye L, Mora R, Akhayani N, Haudenschild CC, Liau G. 1997. Growth factor and cytokine-regulated hyaluronan-binding protein TSG-6 is localized to the injury-induced rat neointima and confers enhanced growth in vascular smooth muscle cells. Circ Res 81:289–296.

Zimmermann DR, Ruoslahti E. 1989. Multiple domains of the large fibroblast proteoglycan, versican. EMBO J 8: 2975–2981.