Effects of Platelet-derived Growth Factor and Transforming Growth Factor-β1 on the Synthesis of a Large Versican-like Chondroitin Sulfate Proteoglycan by Arterial Smooth Muscle Cells*

Elke Schönherr‡, Hannu T. Järvelainen‡, Linda J. Sandell§, and Thomas N. Wight¶

From the §Department of Pathology, School of Medicine, University of Washington, Seattle, Washington 98195 and the ¶Departments of Orthopaedics and Biochemistry, University of Washington and Veterans Administration Medical Center, Seattle, Washington 98108

Platelet-derived growth factor (PDGF) and transforming growth factor-β1 (TGF-β1) increase [35S]sulfate incorporation into proteoglycan (PG) by monkey arterial smooth muscle cells but have opposite effects on cell proliferation. The combination of these two growth regulatory peptides has an additive effect on PG synthesis but no effects on cell proliferation. The time course of sulfate incorporation after stimulation indicates that both growth factors cause maximal incorporation of sulfate into glycosaminoglycan chains by 12–18 h. The PG that is most affected is a large CSPG (Mₐ 1.2 × 10⁶) which can be immunoprecipitated by an antibody against versican, a large CSPG synthesized by human skin fibroblasts. The hydrodynamic size of this molecule increases after PDGF and TGF-β1 stimulation, but the size of the core glycoprotein (Mₐ ~450,000) remains the same. Treatment with either growth factor leads to an increase in the amount of core glycoprotein for this PG. This increase correlates with an increase in the steady state level of mRNA identified by hybridization to a cDNA encoding versican. The two growth factors also increase the glycosaminoglycan chain length of this PG accounting for the greater hydrodynamic size of the molecule after stimulation. In contrast, PDGF and not TGF-β1 changes the composition of the glycosaminoglycan chains attached to this PG by reducing the ratio of chondroitin 6-sulfate to chondroitin 4-sulfate. These results indicate that although both of these growth factors increase the net synthesis of a large versican like CSPG, they differ in their effects on the structure of the glycosaminoglycan chains. These post-translational modifications may relate to the growth state of the cells.

Proteoglycans (PGs) are a heterogeneous group of macromolecules that contain glycosaminoglycan (GAG) side chains as a common feature (1, 2). They are present in all tissues in various amounts and compositions. In the arterial wall, PGs are only a minor component, but their accumulation in intimal lesions of blood vessels is typical in the early phases of atherosclerosis and in other vascular diseases (3). Proteoglycans have been shown to interact with a number of different macromolecules via their core proteins as well as via their GAG chains (4). These interactions influence such arterial properties as viscoelasticity, permeability, lipid metabolism, hemostasis, and thrombosis (5–7). Therefore, a quantitative and/or qualitative modification of arterial PGs may have profound effects on the function of this tissue (3).

Although PGs are known to accumulate in various phases of vascular diseases, little is known as to what factors are important in regulating the synthesis of these macromolecules. To address this question, we have compared the effect of PDGF and TGF-β1 on the synthesis of PGs by the major cell type present in the arterial wall, the smooth muscle cell. Both of these growth factors are known to be present in the arterial wall and are thought to contribute to a number of events leading to arterial disease (8).

PDGF is a peptide mitogen and a chemotactic agent for a number of different cell types including arterial smooth muscle cells (ASMCs) (for reviews see Refs. 9–11 and references therein). This growth factor has many different effects on its target cells, such as the stimulation of a variety of secondary messengers which may be involved in the initiation of DNA synthesis and cell proliferation, as well as in the expression of new proteins. Some of the proteins whose synthesis and secretion are stimulated by PDGF in different cell types are components of the extracellular matrix (ECM) such as different types of collagen (12–15), fibronectin (16), thrombospondin (17), and PGs (18). In addition, PDGF stimulates the synthesis of enzymes that degrade components of the ECM such as collagenase (19). The exact relationship between these ECM changes and the proliferative state of the cell is uncertain.

TGF-β1 on the other hand generally acts as an inhibitor of cell proliferation (20). In addition, TGF-β1 has a marked effect on ECM production by a variety of different cell types.

* This work was supported by National Institutes of Health Grant HL 18646, National Institute of Dental Research Grant DE-08229 to T. N. W., National Institutes of Health Grant AR36994, Department of Veterans Affairs to L. J. §, National Institutes of Health Grant F05TW04222 to E. S.), and the Department of Orthopaedics and Biochemistry, University of Washington and Veterans Administration Medical Center. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Pathology, School of Medicine, SM-30, University of Washington, Seattle, WA 98195.

¶ The abbreviations used are: PG(s), proteoglycan(s); CSPG, chondroitin sulfate proteoglycan; PDGF, platelet-derived growth factor; TGF-β1, transforming growth factor-β1; ASMC, arterial smooth muscle cell; ECM, extracellular matrix; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; GAG, glycosaminoglycan; C-6, chondroitin-6-sulfate; C-4, chondroitin-4-sulfate; CPC, cetylpolyethylene chloride; NBS, newborn calf serum; CS, chondroitin sulfates; DS, dermatan sulfates; HS, heparan sulfates.

Printed in U.S.A.
This peptide stimulates the synthesis and secretion of different types of collagen and fibronectin (21), thrombospondin (22), as well as PGs (23–25). TGF-β1 also influences the expression of integrins (26) and decreases the synthesis of proteases such as collagenase (27). TGF-β1 has been shown to be chemotactic for several different cell types (20, 21).

Although both of these growth factors have been shown to promote the synthesis and secretion of PGs by ASMCs, it is not clear whether these two growth factors affect the same population of PGs and whether they both regulate PG synthesis at the transcriptional/translational or post-translational level. We demonstrate that both of these growth factors increase the steady state level of mRNA transcripts that hybridize to cDNA for versican (28), a large CSPG. In addition, both of these growth factors influence the post-translational processing of the large CSPG by causing GAG chain elongation. However, PDGF and not TGF-β1 causes an enrichment in the chondroitin 6-sulfate (C-6-S) content in the GAG chains of this CSPG. This change in the qualitative nature of the CSPG is positively associated with the proliferation of ASMCs.

EXPERIMENTAL PROCEDURES

Materials
Guainidine HCl (GdnHCl, grade I), Tris base, cetylpyridinium chloride (CPC), urea, N-ethylmaleimide, phenylmethysulfonyl fluoride, DEAE-Sephacel, bovine serum albumin, dextran, dextran sulfate, Nonidet P-40, Ficol 400, and polyvinylpyrrolidone-40 were purchased from Sigma; 6-aminohexanoic acid, benzamidine HCl and XAR-2 film were from Eastman Kodak Co.; chondroitin ABC lyase, chondroitin AC II lyase were from Seikagaku Kogyo Co. Ltd., through ICN Biomedicals; Triton X-100 was from Boehringer Mannheim; Sepharose CL-2B, Sepharose CL-4B, Sepharose CL-6B were from Pharmacia LKB Biotechnology Inc.; grade 3MM filter paper was from Whatman Ltd.; electrophoresis chemicals were bought from Bio-Rad; Na[35S]SO4 (43 Ci/mg S) (carrier free) and Universal scintillation fluid were from ICN Biomedicals; L-[4,6-3H]leucine (74 Ci/ mmol) was from Amersham Corp.; recombinant TGF-β1 was obtained from R&D Systems; PDGF AB purified from human platelets was a gift of Elaine W. Raines, University of Washington. All other chemicals were reagent grade.

Cell Culture

General Procedure—Arterial smooth muscle cell cultures were established from strips of intimal-medial tissue from thoracic aortae of 3–4-year-old piglet monkeys (Macaca nemestrina). The cells were cultured as previously described (29–31). Cells between 4th–12th passage were plated into 35-mm diameter dishes (1.3 × 105 cells/dish), 100-mm diameter dishes (8 × 105 cells/dish), or 24-well trays (25 × 104 cells/well) and maintained in Dulbecco-Vogt modified Eagle’s minimal medium with high glucose, pyruvate and nonessential amino acids supplemented with 100 U/ml penicillin, 10 μg/liter streptomycin (GIBCO), and 5% newborn calf serum (NBS, Biolabs), which was changed every second day. The NBS was not heat-inactivated. Cultures reaching visual confluence were made quiescent by lowering the serum concentration to 0.1% NBS for 2 days.

Titration and Metabolic Labeling—The medium was replaced by fresh medium containing 0.1% NBS 24 h prior to harvest with or without added growth factors. The amounts of TGF-β1 (1 ng/ml medium) and PDGF (10 ng/ml medium) that gave maximal incorporation of [35S]sulfate under these conditions were determined by dose response curves (see “Results”). Over the same 24 h, the medium were labeled with 50 μCi/ml [35S]sulfate alone or with an additional 5 μCi/ml [3H]leucine in complete medium as indicated. To determine the time course of PG stimulation, quiescent cells were stimulated with 1 ng/ml TGF-β1 or 10 ng/ml PDGF and labeled with 200 μCi/ml [35S]sulfate at 6 h intervals from 0 to 48 h. The medium and the cell layer were harvested at the end of each labeling period as described below. For each experiment, the number of cells at the end of the experiment was determined in parallel dishes with a particle counter (Particle Data) after trypsinization.

PROTEOGLYCANS

Measurement of Sulfate Incorporation into Proteoglycans

Proteoglycans were extracted from medium and cell layer as described (30, 31). The medium was removed from the culture dishes and solid GdnHCl was added to the medium to a concentration of 4 M. Serum was added to the medium to a final concentration of 5% before PG isolation to minimize endogenous protease activity. The cell layer was washed once with phosphate-buffered saline and then extracted for 15 min with 4 M GdnHCl, pH 5.8, containing 2.5 mM EDTA, 0.1 M 6-aminohexanoic acid, 5 mM benzamidine HCl, 10 mM N-ethylmaleimide and harvested by scraping. The incorporation of [35S]sulfate into PG was measured by CPC precipitation (32, 33). Briefly, aliquots of the cell layer fraction and the medium were spotted on filter paper and washed five times for 1 h in 1% CPC with 0.05 M NaCl. The amount of precipitate on the dried filter paper was determined by liquid scintillation counting.

Proteoglycan Isolation

The medium (20 ml) and the extracts of the cell layer (10 ml) were pooled separately and concentrated to about 1.5 ml by contact desiccation at room temperature (Aquacide I, Calbiochem). The samples were dialyzed against 4 M GdnHCl, pH 7.0, containing the same proteinase inhibitors as the extraction buffer to remove free [35S]sulfate. They were then mixed with 100 μCi of 1,12-D3-carrier from rat chondrosarcoma (34) before application to a Sepharose CL-2B column (1 × 110 cm) equilibrated with 4 M GdnHCl. Proteoglycans were eluted with the same buffer under a pressure of 50 cm of H2O. Fractions of 1.2 ml were collected and an aliquot of each fraction (100 μl) was assayed for radioactivity by liquid scintillation counting. The two main peaks were pooled and concentrated by contact desiccation. Following dialysis against 8 M urea, 2 mM EDTA, 0.5% Triton X-100, 50 mM Tris-HCl, pH 7.5, the samples were further purified on DEAE-Sephacel minicolumns (1 ml of resin) equilibrated with the dialysis buffer. The columns were washed extensively with the same buffer containing 0.25 M NaCl to remove glycoproteins and the PGs were eluted with the same buffer containing 3 M NaCl. The isolated material was further concentrated by contact desiccation and dialyzed extensively against distilled water. Aliquots of the dialysates were counted and lyophilized for analyses.

Proteoglycan Characterization

Analytical Gel Filtration—The hydrodynamic sizes of the PGs were determined by gel filtration in 4 M GdnHCl, 0.1 M Na2SO4, 0.1 M Tris-HCl, pH 7.0, 0.025 M EDTA on a 1 × 120-cm Sepharose CL-2B column. Glycosaminoglycans, prepared by reductive β-elimination (see below) were chromatographed on a Sepharose CL-6B column (0.7 × 63 cm) in 0.2 M Tris-HCl, pH 7.0, 0.2 M NaCl (35). Chondroitin ABC and AC II lyase digests (see below) were run on a Sepharose CL-4B column (0.7 × 50 cm) in 50 mM Tris-HCl, pH 7.0, 0.2 M NaCl, 0.2% SDS. The void volume of the columns was determined by the elution of tritium labeled DNA from Escherichia coli (Sepharose CL-2B, CL-4B, CL-6B). The elution position of free [35S]sulfate was used as a marker for the total volume.

Chemical and Enzymatic Degradation—Glycosaminoglycans were chemically released from PGs by reductive β-elimination (36). The alkaline β-elimination was done with 1 M sodium borohydride in 50 mM NaOH for 24 h at 45 °C. The reaction was terminated by neutralizing the sample with glacial acetic acid. Heparan sulfate was chemically degraded with nitrous acid at pH 1.5 for 90 min (37).

Chondroitin sulfate and dermatan sulfate GAGs were degraded with 0.03 units/ml chondroitin ABC lyase in 0.3 M Tris-HCl, pH 8.0, 0.6 M bovine serum albumin, 18 mM sodium acetate for 3 h at 37 °C. Chondroitin sulfate GAGs were digested with chondroitin AC II lyase under similar conditions at pH 7.4. The enzymatic degradation products were separated on a Sepharose CL-4B column as described above or by SDS-PAGE (see below). The determination of the relative proportion of 6- and 4-sulfated disaccharides was carried out by descending paper chromatography (38) of chondroitin ABC lyase digests of isolated PG subclasses. Core proteins were prepared from [3H]leucine and [35S]sulfate double-labeled PG subclasses. The samples were digested with chondroitin ABC lyase for 1 h as described above except for the presence of proteinase inhibitors (5 mM benzamidine HCl, 1 mM phenylmethysulfonyl fluoride, 10 mM N-ethylmaleimide, and 0.1 M 6-aminohexanoic acid) (34). After digestion, samples were lyophilized and analyzed by SDS-PAGE.

Gel Electrophoresis—SDS-PAGE was performed according to the procedure of Laemmli (39) on 3–12% gradient slab gels with a 3%
staining. 14C-Labeled high molecular weight standards purchased from Bethesda Research Laboratories Life Technologies, Inc. were used for the estimation of the apparent molecular weights of the proteins. The labeled PGs and proteins were visualized by fluorography of dried gels previously treated with Enlightening enhancer (Du Pont-New England Nuclear) and exposed to Kodak XAR-2 film at -70°C.

**Immunoprecipitation**—Large versican like CSPG was immunoprecipitated with an affinity purified polyclonal antibody against a fusion protein for amino acid residue 387-692 of versican (28), a large human fibroblast CSPG. Briefly, secreted PGs from ASMC cultures treated as described before were labeled with [35S]sulfate and [3H]leucine and partially purified on DEAE-Sephadex. Samples were dialyzed into Ipp buffer (50 mM Tris-HCl, pH 8.0, 0.1 M NaCl, 0.1% bovine serum albumin, 1% Nonidet P-40, 0.5% Triton X-100) and preadsorbed with 50 μl of 20% Omnisorb cell (Calbiochem) previously saturated with normal rabbit serum for 2 h at room temperature. The preadsorbed antigen (3× 106 dpm [35S]sulfate, ~300,000 dpm [3H]leucine in 1 ml of volume) was incubated in bovine serum albumin coated microfuge tubes overnight at 4°C with 20 μl of the undiluted antiserum. The immunocomplexes were removed with 100 μl of 20% Omnisorb cells and washed twice with Ipp buffer, twice with Ipp buffer, 1 M NaCl, and again twice with Ipp buffer. Immunoprecipitates were incubated with chondroitin ABC lyase as described for core protein preparation and once again washed with Ipp buffer, before the antigen was released by boiling in SDS-PAGE sample buffer prior to electrophoresis.

**Northern Blot Hybridization**

RNA was isolated from cell cultures using the single step method as described by Chomczynski and Sacchi (40). RNA samples were electrophoresed overnight (15-16 h) in a denaturing 1% agarose gel (41) and transferred overnight to a nitrocellulose filter (Bethesda Research Laboratories, Life Technologies, Inc.) according to Thomas (42). After transfer, the filters were baked at 80°C for 2 h. The filters were prehybridized for at least 2 h at 42°C in a solution containing 50% formamide (Bethesda Research Laboratories, Life Technologies, Inc.), 5× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate), 25 mM Na2HPO4, 0.02% bovine serum albumin, 0.02% Ficoll-400, 0.02% polyvinylpyrrolidone-40, and 0.25 mg/ml Torula yeast RNA (Calbiochem). Filters were specifically hybridized to a 1.7-kilobase cDNA encoding the carboxyl-terminal region (base pairs between 6412 through 8125) of a large human cartilage PG from rat, called versican (28) or a 0.8-kb cDNA encoding the carboxyl terminal and the 3′-untranslated region (base pairs 922 through 1705) of the human elongation factor 1-α (44) as a reference gene. The nick-translated cDNA probes (Nick Translation System, Bethesda Research Laboratories) had a specific activity of about 1× 106 cpm/μg DNA. Hybridizations of the filters with 35P-labeled cDNA probes were carried out at 42°C using the same solutions as above, but also containing 10% dextran sulfate. The hybridization time was at least 16 h after which the filters were washed twice with 2× SSPE, 0.1% SDS (1× SSPE = 0.15 M NaCl, 0.2 M NaH2PO4, 20 mM Na2EDTA) and twice with 0.1× SSPE, 0.1% SDS for 20 min each at 43–52°C depending on the cDNA probe used. After washing the filters were air-dried and then autoradiographed. The autoradiograms were scanned with a gel scanner (Hoeffer Scientific Instruments).

**RESULTS**

Both PDGF and TGF-β1 increased [35S]sulfate incorporation into PGs in a dose-dependent fashion when added to ASMC culture media in the presence of low serum (0.1% NBS). Maximal incorporation was achieved with 10 ng/ml (330 pm) of PDGF and 1 ng/ml (40 pm) of TGF-β1 when added to quiescent cells for 24 h (Fig. 1, A and B). Both PDGF and TGF-β1 caused an approximate 2-fold increase in labeled PGs secreted into the medium and retained in the cell layer. To examine whether the effect on PG synthesis was confined to a particular time interval following growth factor addition, a series of short 6-h pulses of [35S]sulfate was administered over a 48-h period (Fig. 2, A and B). The results indicate that both PDGF and TGF-β1 gave maximal incorpora-
PDGF and TGF-β1 Affect the Synthesis of a Versican-like CSPG

Stimulation of growth and proteoglycan synthesis in ASMC cultures in the presence of PDGF and TGF-β1

| Conditions     | Cells/cm² | [35S]sulfate<sup>3</sup> | dpm/1000 cells<sup>4</sup> |
|---------------|-----------|--------------------------|-----------------------------|
| 0.1% NBS      | 39,541 ± 4,817 | 407 ± 5.45 | 475 ± 28.80 |
| TGF-β1        | 37,013 ± 2,367 | 484 ± 8.59 | 986 ± 36.40 |
| PDGF          | 55,847 ± 5,469<sup>5</sup> | 546 ± 13.88 | 1569 ± 37.40<sup>6</sup> |
| PDGF + TGF-β1 | 42,069 ± 5,099<sup>5</sup> | 604 ± 23.20 | 1569 ± 37.40<sup>6</sup> |

<sup>1</sup> Cells were counted 48 h after stimulation, n = 8.
<sup>2</sup> Sulfate incorporation was determined by CPC precipitation 24 h after stimulation, n = 4.
<sup>3</sup> Nonsignificant (Student’s t test).
<sup>4</sup> p < 0.001.

Values are means ± S.D.

To investigate whether PDGF and TGF-β1 affect the composition of this particular PG class, aliquots of C₁ and M₁ were isolated from Sepharose CL-2B columns and treated with chondroitin ABC and/or AC II lyase to identify chondroitin sulfates (CS) and dermatan sulfates (DS) or chondroitin ABC lyase and nitrous acid (pH 1.5) to identify heparan sulfates (HS) in addition to CS and DS. The digestion products were separated by size exclusion chromatography and the proportions of CS, DS, and HS in C₁ and M₁ were determined by a differential enzyme subtraction assay (see “Experimental Procedures”). Analysis of the C₁ peak from the quiescent cultures revealed a mixture of CS (63%) and HS (34%) with a small amount of DS (Table II). Treatment with PDGF or TGF-β1 significantly reduced the relative amounts of HS in this peak while not affecting the relative amounts of DS (Table II). However, the absolute amount of [35S]sulfate in HS in this
peak was decreased only by PDGF treatment but not by TGF-β1 (data not shown). Analysis of the M₁ peak revealed a composition that contained mostly PG sensitive to chondroitin AC I1 lyase (87–92%). The relative amounts of CS, DS, and HS in this peak were not altered by either PDGF or TGF-β1 (Table II). The incorporation of [35S]sulfate on a per cell basis into the large CSPG in peak C₁ and peak M₁ was determined after subtraction of the amounts of sulfate incorporated into HSPG in these peaks. This calculation showed that both treatments primarily increased the incorporation of [35S]sulfate into large CSPG synthesized by the ASMCs (Fig. 4).

Since both treatments seemed to quantitatively affect the same size class of PGs, we examined whether the qualitative nature of the large CSPG was also affected similarly by PDGF and TGF-β1. To determine whether PDGF and TGF-β1 altered the size and amount of the core protein(s) in C₁ and M₁, [3H]leucine- and [35S]sulfate-labeled peaks were isolated and digested with chondroitin ABC lyase. The PGs from the C₁ peak (results not shown) and from the M₁ peak had under all conditions a single core protein after chondroitin ABC lyase of M₉, ~450,000 (Fig. 5A). The same core protein could be immunoprecipitated with a polyclonal antibody raised against a fusion protein encoding for amino acid residues 387–692 of the large CSPG from human skin fibroblasts, called versican (28) (Fig. 5B).

To examine whether these two growth factors exerted their influence at the mRNA level, Northern blot analysis was performed. Although we have demonstrated that ASMCs synthesize a large CSPG that is capable of aggregating with hyaluronic acid (30) and which can be immunoprecipitated with an antibody against versican, the exact identity of this large CSPG is not known. Therefore, two different cDNA probes were used, one identifying an aggregating CSPG from cartilage (aggrecan) (43) and one identifying versican (28). Results indicate that these cells express mRNA transcripts that hybridize in detectable amounts only to the versican probe, but not to the aggrecan probe (Fig. 6A). It was further examined whether the expression of these mRNA transcripts that hybridize to the versican probe is modulated in ASMC cultures by PDGF or TGF-β1. Scans of Northern blots demonstrated that stimulation with PDGF and TGF-β1 increased the steady state level of mRNA that hybridized to versican cDNA 3-fold over unstimulated cells (Fig. 6B). This result was confirmed by slot-blot analysis (results not shown). Estimates of the [3H]leucine incorporation into the core protein of the large CSPG in peak M₁ revealed a similar increase in agreement with the results obtained by mRNA analysis (result not shown).

To analyze whether PDGF and TGF-β1 affected the GAG moiety of the large CSPG, the GAG chains in C₁ and M₁ were released by reductive β-elimination and separated on Sepha-
rose CL-6B columns. Treatment with PDGF and TGF-β1 caused a significant shift in the elution position of the isolated GAG chains. TGF-β1 caused a shift from $K_v = 0.35$ to $K_v = 0.29$ while PDGF caused a shift from $K_v = 0.35$ to $K_v = 0.25$ (Fig. 7, A and B). A shift in $K_v$ from 0.34 to 0.24 accounts for a change in the $M_r$ of approximately 45,000 to 70,000 (35). These results indicate that both growth factors cause elongation of the CS chains, and this increase partially accounts for the increased sulfate incorporation observed when these cells are stimulated by the two growth factors.

To investigate whether PDGF and TGF-β1 influence the composition of the CS chains in C1 and M1, the isolated chains were digested with chondroitin ABC lyase and the disaccharides were separated by paper chromatography. On the average the GAG chains in C1 and M1 from quiescent cells had a C-6-S to C-4-S ratio of approximately 2.0 (Fig. 8). TGF-β1 either did not influence this ratio or slightly lowered it, while PDGF increased the ratio to 3.6–3.7 (Fig. 8). This ratio is very similar to that obtained for the CS chains from the large CSPG when the cells were stimulated to proliferate with 5% serum (data not shown). It was also found that in the presence of both growth factors, when the cells were not proliferating (Table I), the GAG chains showed the same ratio as GAG chains from quiescent cells (result not shown). Thus, it appears that the higher ratio of C-6-S/C-4-S in the large CSPG is typical for ASMC cultures that have been stimulated to proliferate.

**DISCUSSION**

Two forms of large aggregating CSPGs have been identified in most connective tissues. One form called versican is synthesized by human fibroblasts (28) and the other form, aggregan, is the principle PG of cartilage (43). Previous studies have shown that cultured ASMCs synthesize a large CSPG ($M_r \sim 1.2 \times 10^6$) that aggregates in the presence of hyaluronic acid (30, 31). However, it is not known whether this PG belongs to the versican or aggregan family of large CSPGs or represents a different family. The sequence of the core protein of versican ($M_r \sim 260,000$) has been deduced from overlapping cDNA clones and shown to contain a hyaluronic acid binding region, no keratan sulfate attachment sites, 15 serine-glycine sequences, which are potential attachment sites for CS chains, 20 asparagine-X-serine (threonine) sequences for N-linked oligosaccharides, and more than 200 serines and threonines suitable for O-linked oligosaccharides (28). Biochemical (30, 31) and electron microscopic (31) studies of the ASMC-derived CSPG and a CSPG present in bovine aorta (45–47) indicate a core glycoprotein of $M_r \sim 450,000$ with 15–20 GAG chains of approximately $M_r \sim 45,000$ and a large number of O-linked oligosaccharides (~300). This molecule is clearly different from aggregan that is present in cartilage. Aggrecan is highly substituted with CS chains (113 presumed serine-glycine attachment sites) and several keratan sulfate attachment sites (43). Tryptic maps of the core proteins of aggregan and the large CSPG in aorta reveal distinctly different patterns for both of these core proteins (48). The structural similarities between versican and the vascular large CSPG together with our findings of abundant transcripts for versican and a large CSPG that can be immunoprecipitated with an antibody against versican in cultures of monkey ASMCs indicate that versican or a versican-like CSPG is a principle PG in vascular tissue. Recent results using Western blot analysis indicate that monkey and human aortic tissue contain versican or a versican-like PG that has the same $M_r$ as the CSPG from monkey ASMC cultures. The expression of versican-like transcripts has also been demonstrated for human and rat ASMC cultures (49, 50) as well as in rat aorta (60).

The synthesis of this PG by ASMCs is regulated in part by the growth regulatory peptides PDGF and TGF-β1. The site of regulation appears to be at both the transcriptional/translational and the post-translational levels. Both growth factors increase versican transcripts to about the same extent (~3-fold) in ASMCs made quiescent by culturing them in the presence of low serum. At this point, it is not clear whether this increase in mRNA is due to increases in transcription or decreases in degradation of this specific mRNA. These changes appear not to reflect a general stimulation in overall PG synthesis since the steady state level of mRNA for PG-II/decorin (51), a small interstitial DSPG synthesized by these cells was not affected under the same conditions by either of these growth factors. Furthermore, the increase in the expression of transcripts for versican when ASMCs are stimulated to proliferate appears to be accompanied by a decrease in type I and III collagen transcripts (52, 53) suggesting that these two different sets of ECM molecules are regulated differently when these cells are stimulated to divide. However, cell proliferation is not necessarily a prerequisite for the increase in the expression of versican since inhibition of ASMC prolif-

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$^3$E. Schonherr, H. T. Jarvelainen, L. J. Sandell, and T. N. Wight, manuscript in preparation.
eration by TGF-β1 also leads to increases in the steady state level of mRNA for this specific PG. These results indicate that the expression of versican by ASMCs may be regulated by a number of different factors.

PDGF and TGF-β1 also influence the post-translational processing of the CS chains by causing chain elongation. This increase in the length of CS chains may be due to increases in the amount and/or activity of enzymes responsible for CS synthesis. For example, actively proliferating bovine ASMCs synthesize GAG chains that are longer than those synthesized by non-proliferating cells (54). This change is accompanied by increases in the activity of several GAG synthesizing enzymes, such as xylosyl transferase, N-acetylgalactosaminy transferase I, and chondroitin sulfotransferases (54). Our finding that GAG chains could be lengthened by TGF-β1 in the absence of proliferation suggests that either proliferation is not essential for this post-translational modification and/or more than one controlling mechanism for chain elongation is responsible. Studies are currently in progress to distinguish between these two possibilities.

Although GAG chain elongation as well as increased core protein synthesis occurs in the absence of ASMC proliferation, the increase in the ratio of C-6-S/C-4-S in the CS chains of the ASMC-derived large CSPG to values greater than 2 appeared to be linked to the proliferative state. For example TGF-β1 effectively blocks the proliferative effect of PDGF when added to ASMCs in the concentrations used in this study. However, TGF-β1 and PDGF act synergistically to increase the level of CSPG above the level achieved with either growth factor alone (see Table I). Yet, this treatment does not change the ratio of C-6-S/C-4-S over than seen for the CSPG synthesized by quiescent cells (result not shown). Thus, it appears that TGF-β1 can block this post-translational modification of the CS isomers as well as ASMC proliferation induced by PDGF. Similar changes in the ratio of C-6-S/C-4-S of CS chains have been observed in other systems characterized by active cell proliferation, such as in developing chick bone marrow (55), proliferating mononuclear leukemic cells (56), and in cultures of ASMCs exhibiting elevated proliferative potential (57). The enzyme responsible for the formation of C-6-S isomer is 6-O-sulfotransferase. Conditions which promote cell proliferation increase the activity of this enzyme. For example, bioactive polycarboxylate which are elevated when cells start to proliferate (58) can activate 6-o-sulfotransferase (59). In addition, the PDGF stimulated uptake of putrescine (60), a precursor of bioactive polycarboxylates, peaks at about the same time as PG synthesis by ASMCs. It has also been shown for ASMCs that PDGF induces ornithine decarboxylase, a key enzyme in the synthesis of bioactive polycarboxylates (61). Whether these agents are operative in stimulating the specific synthesis of C-6-S when ASMCs are stimulated to divide is under investigation.

The functional significance of the diverse post-translational modifications of PGs associated with cell proliferation is not known. A preference for the C-6-S isomer in proliferating systems may be important in providing the proper microenvironment to sustain the proliferative response. For example, may be that this isomer is more effective in binding certain growth factors. A number of studies indicate that the C-6-S isomer interacts more avidly with basic residues in various proteins (61) and recent studies do indicate that the GAG moiety of some PGs interacts with growth factors such as basic fibroblast growth factor (63), granulocyte-macrophage colony-stimulating factor, interleukin-3, pleiotropin, and platelet factor 4 (for review, see Ref. 64). A possible adverse consequence of the accumulation of C-6-S in the microenvironment surrounding ASMCs is the binding and trapping of low density lipoprotein, setting the stage for probable lipid accumulation in blood vessels during atherogenesis (6). Previous studies have shown that PGs containing the C-6-S isomer exhibit a binding preference for low density lipoproteins (6, 65) and PG-low density lipoprotein complexes enriched in C-6-S have been isolated from human atherosclerotic lesions (66).

In summary, this study has shown that two important regulators of ASMC growth are also involved in the regulation of the synthesis of a specific class of PG. These effects may contribute to changes in the pattern of ECM composition that occur in blood vessel development and disease. Further studies are necessary to determine whether these specific modifications influence subsequent cellular events involved in these processes.

Acknowledgments—We are grateful to Drs. K. Doege (Shriners' Hospital for Crippled Children and Department of Biochemistry and Molecular Biology, Oregon Health Science University, Portland, OR) and E. Ruoslahti (La Jolla Cancer Research Center, La Jolla, CA) and M. Hickey (Veterans Administration Hospital, Seattle, WA) for providing us with the cDNA probes for aggrecan, versican, and elongation factor 1-α, respectively, and Dr. R. LeBaron and Dr. E. Ruoslahti (La Jolla Cancer Research Center, La Jolla, CA) for providing us with the polyclonal antibody against versican.

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