Vascular Endothelial Cell Proteoglycans Are Susceptible to Cleavage by Neutrophils

Nigel S. Key, Jeffrey L. Platt, and Gregory M. Vercellotti

Heparan sulfate proteoglycan associated with vascular endothelial cells in vivo plays an important role in a number of endothelial functions, including the inhibition of intravascular coagulation and extravasation of plasma proteins and blood cells. In this report, we demonstrate that polymorphonuclear neutrophils, as well as cell-free neutrophil supernatants, lead to a rapid and dramatic loss of proteoglycan from endothelial cells in the absence of evidence of cell lysis. This cleavage appears to be relatively (although not absolutely) selective for heparan sulfate and is mediated by neutrophil-derived serine proteases. Inhibitors of neutrophil elastase appear to be the most effective inhibitors of proteoglycan release. Furthermore, purified human neutrophil elastase also leads to cleavage of cellular proteoglycans, although not to the same extent as neutrophils or neutrophil supernatants. Proteoglycans compared with all other protein-containing macromolecules appear to be especially vulnerable to neutrophil-mediated cleavage. The results of this study may be germane to the interaction of neutrophils with endothelium during the inflammatory process, during which the loss of endothelial heparan sulfate proteoglycan may play a critical role in the vascular injury that often accompanies inflammation. (Arteriosclerosis and Thrombosis 1992;12:836–842)

**KEY WORDS** • neutrophils • endothelial cells • heparan sulfate proteoglycans

**P**roteoglycans help to maintain the structural and functional integrity of the endothelium. These complex macromolecules consist of a protein core covalently linked to one or more glycosaminoglycan (GAG) chains. The most extensively studied proteoglycan associated with endothelial cells (ECs) is heparan sulfate proteoglycan (HSPG), which is found within the extracellular matrix and on the cell surface. Functionally, HSPGs contribute to the selective nature of vascular permeability by regulating the passage of proteins, ions, and blood cells. Within the extracellular matrix, HSPGs bind growth factors, such as basic fibroblast growth factor, thereby protecting them from degradation and providing a reservoir for overlying cells. HSPGs also participate in the maintenance of an anticoagulant surface by accelerating certain hemostatic enzyme-protease inhibitor interactions. In addition, cell surface–associated HSPG serves as a ligand for a number of extracellular enzymes, including lipoprotein lipase, which catalyzes the hydrolysis of triacylglycerol in circulating lipoprotein particles, and superoxide dismutase (SOD), which protects against oxygen radical–mediated injury. Thus, the loss of EC-associated proteoglycans might perturb a number of vessel wall functions, leading to extravasation of fluid and plasma components, intravascular thrombosis, and sensitivity to oxidant-mediated tissue injury.

Because of the diversity of functions of HSPGs in ECs, we tested the hypothesis that during inflammation, polymorphonuclear neutrophils (PMNs) may mediate the loss of EC HS. Our in vitro findings suggest that PMNs rapidly solubilize a large fraction of EC-associated proteoglycan (which is defined as the sum of proteoglycans within the cell and in the extracellular matrix) by proteolytic cleavage of core protein. Such loss might be important in diverse clinical syndromes in which inflammation is associated with vascular injury and intravascular thrombosis.

**Methods**

**Reagents**

Hanks' balanced salt solution (HBSS) and Dulbecco's modified Eagle's medium (DMEM) were obtained from Gibco Laboratories (Grand Island, N.Y.). RPMI-1640 was purchased from Hazleton Laboratories (Lenexa, Kan.). Fetal bovine serum (FBS) was from Hyclone Laboratories (Logan, Utah). The elastase-specific chloromethyl ketone inhibitor N-methoxyxuccinyl-Ala-Ala-Pro-Val chloromethyl ketone (AAPV-CK) was from Enzyme Systems, Inc. (Livermore, Calif.). Hydroxyethylstarch (Hespan) was purchased from American Hospital Supply Corp. (Irvine, Calif.), and Percoll was obtained from Pharmacia Fine Chemicals (Piscataway, N.J.). [35S]Sulfate and [35S]methionine were obtained from ICN Biochemicals (Costa Mesa, Calif.). Na2[35]CrO4 was from Amersham Corp., Arlington Heights, Ill. Bovine kidney HSPG, sodium salt, was obtained from Seikagaku America, Inc. (St. Petersburg, Fla.). Neutrophil elastase, purified as previously described, was...
Pro-Valp-nitroanilide (0.1 mM in 1% dimethylsulfoxide in PBS, pH 7.4) and incubated for 15 minutes at 37°C. The reaction was then terminated by placing the samples on ice, and the absorbance of duplicate samples was measured at 410 nm. The amount of elastase is expressed as a percentage of maximal releasable elastase, which is defined as the enzymatic activity in the supernatant derived from a similar number of granulocytes preincubated for 15 minutes at 37°C with cytochalasin B (5 µg/ml) and N-formyl-Met-Leu-Phe (fMLP, 10⁻⁷ M).

**Biosynthetic Labeling and Extraction of Proteoglycans**

EC monolayers were biosynthetically labeled with [³⁵S]sulfate (100 µCi/ml) in culture media for 18 hours. The cells were then washed three times with HBSS-HEPES (pH 7.4) and incubated at 37°C in a 5% CO₂ incubator with PMNs (usually 20 PMNs:1 EC), cell-free PMN-conditioned supernatants, or purified PMN elastase in HBSS-HEPES at a final volume of 250 µl for 60 minutes (unless otherwise indicated). The PMN suspension was removed, and each well was washed once with 500 µl buffer. PMNs and wash buffer were centrifuged at 300g for 10 minutes, and the cell-free supernatant fraction was saved for further analysis. The pelleted PMNs contained <1% of the total biosynthetically labeled EC proteoglycan pool. The EC fractions were extracted by adding 4 M guanidine hydrochloride, 0.05 M sodium acetate, 0.01 M EDTA, 0.1 M 6-aminohexanoic acid, 1 mg/ml benzamidine, and 1 mM iodoacetamide, pH 5.8, to the monolayer at 4°C for 24 hours. Cell extracts and supernatant fractions were thoroughly dialyzed in 3,500-d exclusion tubing against 0.5 M NaOH before counting for ³⁵S. An aliquot of each fraction was then counted and the percent proteoglycan release was determined as

\[
\frac{\text{cpm medium}}{\text{cpm medium} + \text{cpm cells}} \times 100\%
\]

For experiments that examined the release of [³⁵S]methionine-labeled macromolecules, ECs were labeled with [³⁵S]methionine (100 µCi/ml) in culture media for 18 hours. After washing and exposure to PMNs (as detailed above), [³⁵S]methionine-labeled macromolecules were obtained from the PMN-free supernatant fraction by precipitation with ice-cold trichloroacetic acid (15% [vol/vol]). The pellet was resuspended in 1 M NaOH before counting for ³⁵S. The EC fraction was removed by scraping the wells with a rubber policeman and repeated washing with HBSS-HEPES; trichloroacetic acid precipitation of [³⁵S]methionine-labeled macromolecules and resuspension in 1 M NaOH were carried out before scintillation counting.

For determination of EC cytotoxicity, monolayers were washed with HBSS-HEPES and then radiolabeled with ³¹Cr (2 µCi/well) for 3 hours at 37°C. Cells were then washed three times with buffer and incubated with PMNs (20 PMNs:1 EC) at 37°C for 60 minutes. The PMNs were removed, the wells were washed twice, and a supernatant fraction (A) was collected. Adherent ECs (fraction B) were extracted with 1N NaOH, and the radioactivity of each fraction was counted. The control ECs were incubated in buffer alone and the control supernatants (fraction C) counted. The specific release
of $^{51}$Cr is expressed as a percentage using the following formula:

\[
\frac{(A-C)}{(A+B-C)} \times 100\%
\]

Characterization of Endothelial Cell Proteoglycans

EC extracts and supernatant fractions that had been dialyzed as described above to remove free $^{35}$S were prepared for ion-exchange chromatography by dialysis into buffer containing 6 M urea, 0.1 M NaCl, 0.05 M tris(hydroxymethyl)aminomethane, and 0.2% CHAPS with protease inhibitors, pH 7.0. Each sample was then applied to a DEAE-Sephadex column and eluted with a linear 0.1-1.0 M NaCl gradient in the same buffer. Fractions representing chromatographic peaks were pooled and lyophilized in 10,000-cpm aliquots.

Characterization of the proteoglycans according to their hydrodynamic properties was performed by gel filtration chromatography on a 100×0.6-cm Sepharose CL-4B column equilibrated and eluted with 4 M guanidine hydrochloride in 0.05 M sodium acetate (void volume $V_v$, 32.0 ml; total volume $V_t$, 81.8 ml). Eluted fractions of 1 ml were collected, and the radioactivity was monitored in a scintillation counter.

Chromatography on a hydrophobic matrix was employed to resolve proteoglycans in control versus PMN-treated HUVECs according to the hydrophobicity of their core proteins. A 10,000-cpm aliquot was applied to a 0.7×4-cm octyl Sepharose CL-4B column that was equilibrated with 4 M guanidine hydrochloride in 0.05 M sodium acetate. The column was then eluted with a 0–0.8% gradient of Triton X-100 in 4 M guanidine hydrochloride. One-milliliter fractions were collected, and the radioactivity was quantified. The concentration of Triton X-100 was assayed by comparing the absorption at 283 nm of the sample to a standard curve composed of known concentrations of the detergent.

Low pH (pH 1.5) nitrous acid digestion was employed to assess the HSGAG content of the various fractions. The carbohydrate moieties were first released from the core protein by alkaline $\beta$-elimination with 0.05 M sodium hydrochloride. One-milliliter fractions were collected, and the radioactivity was quantified. The concentration of NaOH was then neutralized with glacial acetic acid to pH 5.0, and the sample was dialyzed into water and lyophilized. The GAGs were then applied to a 50×0.6-cm G-50 Sephadex column that was equilibrated and eluted with 0.1 M CH$_3$OH $\cdot$ H$_2$O, pH 6.0 (V$_v$, 12.0 ml; V$_t$, 32.0 ml). Intact GAGs eluted in the void volume of the column. Another GAG aliquot was digested with nitrous acid according to the method of Shively and Conrad and applied to the G-50 column. The HS content was calculated as the radioactivity present in the included fraction (after nitrous acid digestion) expressed as a percentage of the total counts applied to the column.

Statistics

Unless otherwise stated, all results are reported as mean±SEM and differences assessed using the Student's $t$ test.
labeled molecules 60 minutes after the addition of PMNs (20 PMNs:1 EC), after which time it reached a plateau. Based on this result, an incubation time of 60 minutes was chosen for further experiments.

The loss of proteoglycans increased in proportion to the number of added PMNs as shown in Figure 1B. When PMNs were activated by the addition of phorbol myristate acetate (PMA; final concentration, 100 ng/ml), the loss of EC proteoglycans was significantly increased; for example, with only one added PMN per EC, in the presence of PMA, there was a 63.0±0.2% release of proteoglycans compared with 19.9±1.4% in the absence of PMA (p<0.01; Figure 1B). The enhancement of PMN-mediated proteoglycan loss is not due to a direct effect of PMA on the endothelium, as the release of labeled macromolecules in control wells incubated with the same concentration of PMA in the absence of PMNs was <5%. Enhancement of PMN-mediated proteoglycan loss was also seen when 10⁻⁷ M fMLP was used to stimulate PMNs (data not shown). Furthermore, the profound release of [³⁵S]sulfate macromolecules (essentially consisting of proteoglycans only) was in marked contrast to the modest release of [³⁵S]methionine macromolecules (consisting of a variety of EC proteins, including the core proteins of proteoglycans) under the same conditions (Figure 1B). Specifically, at a PMN to EC ratio of 20:1 in the presence of PMA, the release of [³⁵S]methionine macromolecules amounted to only about 8% of the starting material, approximately 10-fold less than the release of labeled proteoglycans under the same conditions. Cell-free supernatants from an equivalent number of PMNs incubated at 37°C for 1 hour solubilized 63.7±9.0% of endothelial proteoglycans, whereas supernatants collected from PMNs at 4°C led to 21.9±6.1% solubilization. The propensity of PMN releasates to cause EC proteoglycan loss paralleled their elastase enzymatic activity; thus, the conditioned supernatants prepared from unstimulated PMNs at 37°C contained 17.1±2.83% of the maximal releasable elastase activity, whereas the 4°C supernatants contained 4.6±2.4% of the maximal releasable elastase activity. The release of proteoglycans was not due to EC lysis because PMA-stimulated PMNs at a ratio of 20 PMNs:1 EC caused only a 4% specific release of ³⁵Cr from ECs after 1 hour of incubation, whereas the release of [³⁵S]labeled macromolecules under these conditions was 81%. ECs from an alternative source (porcine aorta) did not appear to be as susceptible as HUVECs to PMN-mediated proteoglycan loss; in the presence of unstimulated PMNs (at a ratio of 20:1), total proteoglycan release reached a plateau of only 30.4±9.9% after 60 minutes.

Characterization of Proteoglycans

Further experiments were undertaken to characterize the media and cell-associated [³⁵S]macromolecules after treatment of HUVECs with PMNs. The [³⁵S]-labeled moieties were partially purified by anion-exchange chromatography on DEAE-Sepharose and then added to a Sepharose CL-4B column. The CL-4B profiles of cell-associated proteoglycans from control and PMN-treated ECs are compared in Figure 2A. Proteoglycans from control cells eluted at a \( K_v \) of 0.20 (peak I), whereas after PMN treatment, cell-associated proteoglycans became markedly altered, with the appearance of a new peak at a \( K_v \) of 0.45 (peak II). The elution position of this moiety is consistent with the possibility that these are glycopeptides rather than free GAG chains. Material eluting at a similar position (\( K_v \) of 0.44) was also present in the media fractions of PMN-treated ECs (Figure 2B; peak II). In addition, a second new peak (\( K_v \) of 0.82) was seen in the cellular fraction of treated ECs (Figure 2A; peak III); its low molecular weight and absence from the corresponding media fractions imply that it may represent material that is undergoing further degradation. Similar elution profiles were obtained for the media and cell fractions of HUVECs exposed to cell-free conditioned supernatants from PMNs (data not shown).

The technique of low pH nitrous acid digestion to quantify HSGAGs is based on the susceptibility of N-sulfated glucosamine residues, present only in HS and heparin, to deamination. We found that approximately 85% of the GAGs released into the media by PMNs were susceptible to deaminative cleavage with nitrous acid. In contrast, the media from untreated control cells contained only 50% HS by this method of analysis. Thus, although not absolutely specific, there appears to be a relatively selective cleavage of endothelial cell HSPG by PMNs.
Octyl Sepharose chromatography of proteoglycans. 35S-labeled proteoglycans were purified by anion-exchange chromatography (see “Methods”) before chromatography on octyl Sepharose CL-4B. Panel A: Profile of control human umbilical vein endothelial cells (HUVECs). Peak I (32% of the starting material) represents nonadherent proteoglycans. Peak II (58%) elutes at a Triton X-100 concentration of —0.03% and peak III (10%) at a concentration of —0.17%. Panel B: Profile of polymorphonuclear neutrophil-treated HUVECs.

The elution profiles of cell-associated 35S-labeled material from control and PMN-treated ECs analyzed by hydrophobic matrix chromatography with octyl Sepharose CL-4B is shown in Figure 3. For control cells (Figure 3A), approximately 32% of the starting material failed to adhere to the column and was eluted as a single peak (I). A major peak (II) accounting for approximately 58% of the radioactivity eluted at a Triton X-100 concentration of —0.03%; in addition, a minor peak (III) eluting at a Triton X-100 concentration of —0.17% contained approximately 10% of the starting material. In contrast, the elution profile of the PMN-treated ECs (Figure 3B) was markedly altered, with 95% of the added material failing to adhere to the column matrix (peak I); a small residual peak corresponding to peak II for control cells accounted for the remaining 5% of 35S-labeled material. Thus, the content of hydrophobic cell-associated proteoglycans has been grossly altered by exposure to PMNs.

Identification of the Active Secreted Product of Polymorphonuclear Neutrophils

Because serum contains a variety of protease inhibitors and scavengers of oxygen free radicals, the effect of adding human serum to PMNs during the period of incubation with endothelium was tested. Serum alone added to ECs did not increase the release of endothelial proteoglycans (Figure 4). However, at concentrations >1%, serum effectively inhibited the ability of unstimulated PMNs to cause the release of HSPG. Therefore, further experiments to identify the relevant PMN product were performed. Inhibitors that target serine proteases, including α-1 protease inhibitor and PMSF, were the most effective in preventing 35S-macromolecule release mediated by intact neutrophils or cell-free neutrophil supernatants (Table 1). The chloromethyl ketone AAPV-CK, which is a specific elastase inhibitor, reduced supernatant-mediated proteoglycan release by 77.9±1.0%. The oxygen-radical scavengers SOD and catalase either alone or in combination did not inhibit PMN-mediated proteoglycan loss (Table 1), suggesting that these otherwise important PMN products are not directly involved. Inhibitors of metalloproteases (1,10-phenanthroline, 0.3 mM), cysteine proteases (leupeptin, 0.5 mM), and plasmin (α-amino-n-caproic acid, 10 mM) reduced proteoglycan release by <10% (data not shown).

TABLE 1. Inhibition of Polymorphonuclear Neutrophil-Mediated Release of Proteoglycans From Human Umbilical Vein Endothelial Cells by Inhibitors

<table>
<thead>
<tr>
<th>Inhibitor added</th>
<th>Percent inhibition of 35S-labeled molecule release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibitor</td>
<td>Neutrophils</td>
</tr>
<tr>
<td>α-1 PI</td>
<td>64.20±5.88</td>
</tr>
<tr>
<td>1 mg/ml</td>
<td>70.58±4.88</td>
</tr>
<tr>
<td>10 mg/ml</td>
<td>45.36±7.23</td>
</tr>
<tr>
<td>PMSF (1 mM)</td>
<td>ND</td>
</tr>
<tr>
<td>AAPV-CK (10⁻³ M)</td>
<td>ND</td>
</tr>
<tr>
<td>SOD/catalase</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Heparin (10 µg/ml)</td>
<td>8.61±3.68</td>
</tr>
</tbody>
</table>

35S-labeled human umbilical vein endothelial cells were exposed to polymorphonuclear neutrophils (PMNs) or cell-free PMN supernatants prepared from PMNs at 37°C. In each case, the inhibitor(s) shown were added to the PMN suspension before subsequent addition to the endothelial monolayer. The numbers shown are percent inhibition of 35S-macromolecule release caused by PMN or PMN supernatants in the absence of inhibitors. α-1 PI, α-1 protease inhibitor; PMSF, phenylmethylsulfonyl fluoride; AAPV-CK, methoxysuccinyl-Ala-Ala-Pro-Val chloromethyl ketone; SOD, superoxide dismutase; ND, not done.
fully account for the loss induced by intact PMNs or that proteoglycan release by purified elastase did not micellar structures, are possible. We also observed of core proteins or even persistent association of pro-

Although this may reflect a global decrease in integral PMN-mediated alteration of the hydrophobic domains of PAD ECs, are the source of the released material is

whether this or other HS-containing proteoglycans on EC function may be

shown). Similarly, in the presence of de-N-sulfated N-acetylated heparin or bovine kidney HSPG (10–50 μg/ml), there was <10% inhibition of 35S-labeled human umbilical vein endo-

Finally, purified human neutrophil elastase in HBSS-HEPES was added to 35S-labeled HUVECs in culture at concentrations ranging from 0.1 to 5 μg/ml for 60 minutes at 37°C. Although elastase alone was effective in causing release of proteoglycans from ECs, the release peaked at 46.5±11.2% with an elastase concentra-

EC, endothelial cell. 35S-labeled human umbilical vein endothelial cells were exposed to varying concentrations of purified neutrophil elastase in Hank’s balanced salt solution-N-(2-hydroxy-

we did not find evidence for free GAGs or GAG fragments by gel filtration. In other studies, fMLP-stimulated PMNs degraded proteoglycan in the extra-

under conditions similar to ours, these cells have been reported to contain a pre-

This study demonstrates that proteoglycans associated with ECs are highly susceptible to neutrophil-mediated cleavage. The data obtained from gel filtration and nitrous acid digestion studies indicate that the released material probably consists of glycopeptides that are enriched in HSGAGs. Our model also suggests that a fraction of the released HSPGs are further degraded into low-molecular-weight fragments (Figure 2A, peak III), possibly by enzymes that include endoglycosidases. The characterization of proteoglycans associated with HUVECs has been reported in several previous studies. Under culture conditions similar to ours, these cell types have been reported to contain a pre-

whether this or other HS-containing proteoglycans, such as those recently described in rat fat pad ECs, are the source of the released material is unclear in the absence of detailed analysis of the core protein component. Based on octyl Sepharose CL-4B chromatography studies, our data, however, do suggest that there is a major loss of EC proteoglycans containing hydrophobic core proteins after exposure to PMNs. Although this may reflect a global decrease in integral membrane proteoglycans, other interpretations, such as PMN-mediated alteration of the hydrophobic domains of core proteins or even persistent association of pro-

We therefore considered the possibility that this enzyme might be at least partly responsible for the observed release of 35S-labeled EC macromolecules in our system. Accordingly, releasates of PMNs incubated at 4°C or 37°C in buffer for 60 minutes were collected and tested for their ability to cause proteoglycan release. The released enzyme did not appear to be an endoglucuronidase ("heparanase") because 1) its release was greater at 37°C than at 4°C; 2) heparin was unable to significantly inhibit PMN-mediated proteoglycan release (Table 1); 3) two other inhibitors of heparanase, de-N-sulfated N-acetylated heparin and bovine kidney HSPG, also did not inhibit PMN-mediated proteoglycan release; and 4) we did not find evidence for free GAGs or GAG fragments by gel filtration. In other studies, fMLP-stimulated PMNs degraded proteoglycan in the extra-

Degradation of matrix HS by this enzyme releases low-molecular-weight GAG fragments into the medium. However, this effect could be nullified by serine protease inhibitors, such as PMSF, which led the authors to conclude that proteolysis of the extracellular matrix is a necessary prerequisite for the subsequent degradation of HS side chains by neutrophil heparanase. We therefore considered the possibility that this enzyme might be at least partly responsible for the released material is insufficient to explain the total proteoglycan loss.

Discussion

This study demonstrates that proteoglycans associated with ECs are highly susceptible to neutrophil-mediated cleavage. The data obtained from gel filtration and nitrous acid digestion studies indicate that the released material probably consists of glycopeptides that are enriched in HSGAGs. Our model also suggests that a fraction of the released HSPGs are further degraded into low-molecular-weight fragments (Figure 2A, peak III), possibly by enzymes that include endoglycosidases. The characterization of proteoglycans associated with HUVECs has been reported in several previous studies. Under culture conditions similar to ours, these cell types have been reported to contain a pre-

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Several in vitro studies have addressed the interaction of inflammatory cells with ECs and the subendothelial matrix. Weiss and Regiani reported that PMN-derived elastase solubilized a HUVEC-derived matrix. Matzner and colleagues demonstrated that HS in the subendothelial matrix produced by bovine corneal and vascular ECs was degraded after prolonged (24-hour) incubation with human neutrophils. This was mediated by a neutrophil-derived heparanase, which is secreted by unstimulated neutrophils incubated at 4°C at pH 6.3. Degradation of matrix HS by this enzyme releases low-molecular-weight GAG fragments into the medium. However, this effect could be nullified by serine protease inhibitors, such as PMSF, which led the authors to conclude that proteolysis of the extracellular matrix is a necessary prerequisite for the subsequent degradation of HS side chains by neutrophil heparanase. We therefore considered the possibility that this enzyme might be at least partly responsible for the observed release of 35S-labeled EC macromolecules in our system. Accordingly, releasates of PMNs incubated at 4°C or 37°C in buffer for 60 minutes were collected and tested for their ability to cause proteoglycan release. The released enzyme did not appear to be an endoglucuronidase ("heparanase") because 1) its release was greater at 37°C than at 4°C; 2) heparin was unable to significantly inhibit PMN-mediated proteoglycan release (Table 1); 3) two other inhibitors of heparanase, de-N-sulfated N-acetylated heparin and bovine kidney HSPG, also did not inhibit PMN-mediated proteoglycan release; and 4) we did not find evidence for free GAGs or GAG fragments by gel filtration. In other studies, fMLP-stimulated PMNs degraded proteoglycan in the extracellular matrix derived from neonatal rat vascular smooth muscle cells or lung fibroblasts. Similar to the findings in our studies, elastase appeared to be responsible for cleavage of the proteoglycan core proteins, resulting in the generation of glycopeptides. Our studies extend these observations by demonstrating that granulocytes readily release proteoglycans from intact ECs. The exact site(s) of origin of the released proteoglycans still remain to be determined; although it is likely that the majority is derived from the EC surface and/or matrix (the predominant cellular locations for HS proteoglycans), we cannot exclude the possibility that some fraction is derived from intracellular pools.

The potential consequences of such a dramatic loss of HS-containing proteoglycans on EC function may be protean. Recent studies have shown that ECs in culture and on freshly prepared bovine aortic segments synthetize anticoagulantly active HSPG. A proportion of HS chains (1–10%) are characterized by a specific pentasaccharide sequence, distinguished by the presence of a 3-O-sulfated glucosamine residue, which allows high-affinity binding and activation of antithrombin III. This results in a dramatic acceleration of the ability of antithrombin III to inactivate the target
coagulation proteases, factors IIa (thrombin), and to a lesser extent factors IXa, Xa, and XIa. Although earlier studies with cloned bovine aortic ECs suggested a surface location for molecules with anticoagulant activity, recent evidence has shown that the majority (>90%) of antithrombin III binding sites in ECs are located within the extracellular matrix. We speculate that a gross diminution of endothelial proteoglycans (either on the cell surface, within the extracellular matrix, or even intracellularly) may therefore promote thrombosis through the loss of anticoagulantly active HSGAGs.

We have recently described another situation in which a dramatic loss of preformed endothelial HSPG occurs. During hyperacute rejection of transplanted xenogeneic organs, a process that is mediated by antienzymes and complement, there is prominent intravascular thrombosis and inflammatory cell infiltration. We demonstrated that antibody binding to ECs and subsequent complement activation caused a rapid cleavage and loss of HS from the endothelium. It thus appears that the loss of HS from ECs may be a common mechanism underlying a variety of physiologic changes in the endothelium associated with inflammation.

Acknowledgments

The authors would like to thank Dr. Beulah Gray for supplying purified neutrophil elastase. We are grateful to Dr. Harry S. Jacob for his continued support and advice, to Dong Tuong and Bonnie Lindman for expert technical assistance, and to Carol Taubert for secretarial assistance.

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doi: 10.1161/01.ATV.12.7.836

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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