Regulation of Vascular Smooth Muscle Cell Growth by Endothelial-Synthesized Extracellular Matrices

Ira M. Herman and John J. Castellot, Jr

Previous work has demonstrated that aortic endothelial cells (EC) produce a heparin-like inhibitor of smooth muscle cell (SMC) growth when both cell types were cultured on plastic. We have now tested the influence of the extracellular matrix on this EC-SMC interaction. Specifically, we examined: 1) the role of different substrates (plastic, fibronectin, monomeric, and fibrillar collagens I and III, and EC-derived matrices) on the growth rate and population density of SMC; 2) the heparin-sensitivity of SMC on these diverse substrates; and 3) the effect of these same substrates on EC ability to secrete heparin-like and polypeptide inhibitors of SMC growth. SMC demonstrated a sixfold difference in sensitivity to heparin when grown on different substrates, with the following rank order: EGTA matrix > collagens = plastic = fibronectin > deoxycholic acid (DOC) matrix. Maximally, we found a 10-fold difference in the potency of the inhibitory activity secreted by EC grown on different substrates, with the following order: plastic = EGTA matrix > fibronectin > collagens = DOC matrix. Treatment of the conditioned mediums with heparinase and trypsin indicated that 58% to 76% of the inhibitory activity was due to heparin-like species, and 24% to 42% was due to protein(s). When EC cultured on EGTA matrix are compared to those plated on DOC matrix, the potency of the heparin-like and peptide inhibitory activities increased 8- and 17-fold, respectively. Hypothetically, one would predict a 60-fold change in the potency of the antiproliferative effect if the contributions of substrate to EC production of inhibitors and SMC sensitivity were additive. In practice, we obtained 30- to 40-fold changes in potency between the different substrate combinations. These results indicate that EC-derived matrices strongly influence both the EC production of heparin-like and polypeptide inhibitory activity and the SMC response to the growth regulators. (Arteriosclerosis 7:463–469, September/October 1987)

Extracellular matrix and vascular cell interactions have been intensively studied during the past decade. While the precise biochemical mechanisms involved remain obscure, it is generally agreed that the integrity of the blood vessel wall, the selective nature of the endothelial cell permeability barrier, and the ability of the intima to withstand blood shearing forces are among the functions dependent on the ability of vascular cells to synthesize, organize, and adhere to molecules of the extracellular matrix. The form and composition of the extracellular matrix that surrounds the intimal endothelial and medial smooth muscle cells may influence distinctive events in both the artery wall and in monolayer cultures of endothelial cells (EC) and smooth muscle cells (SMC) established for in situ and in vitro analyses. In vitro, the fundamental cell func-

From the Department of Anatomy and Cellular Biology, Tufts University School of Medicine and the Department of Pathology, Harvard Medical School, Boston, Massachusetts.

This work was supported by NIH Awards HL 30901, 35570, and 17747 and American Heart Association Established Investigatorship Awards to both authors.

Address for reprints: Ira M. Herman, Ph.D., Department of Anatomy and Cellular Biology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, Massachusetts 02111.

Received September 2, 1986; revision accepted March 30, 1987.
the SMC proliferation caused by this injury. This class of sulfated glycosaminoglycan (GAG) has also proven effective in arresting SMC growth in vitro. A physiologic role for heparin is suggested by the finding that a heparin-like inhibitor of SMC growth can be obtained from culture media conditioned by aortic EC. Our current work addresses whether the extracellular matrix produced by the vascular endothelium influences EC production of the heparin-like inhibitor for SMC growth. We also tested whether the same matrices alter the SMC sensitivity to heparin and the endothelial-derived heparin-like growth regulator. The data indicate that vascular cell-extracellular matrix interactions strongly influence SMC growth and suggest that extracellular signaling of vascular cell behavior may help determine whether the outcome of intimal injury will be atherogenesis or restitution of a normal-functioning intima.

Methods

Cell Culture

All cells were cultured at 37°C in a humidified, 5% CO2/95% air atmosphere. All growth media contained 4 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin and all cells were used between passages 2 through 6.

Smooth Muscle Cells

Rat aortic SMC from Sprague-Dawley rats (Charles River, Wellesley, Massachusetts, CD strain) were isolated, cultured, and characterized as previously described. Briefly, the abdominal segment of the aorta was removed and the fascia was cleaned away under a dissecting microscope. The aorta was cut longitudinally and small pieces of media were carefully stripped from the vessel wall. Two or three such strips were placed in 60-mm tissue culture dishes. Within 1 to 2 weeks, SMC migrated from the explants; they were capable of being subcultured about a week after the first appearance of cells. SMC were grown in RPMI-1640 medium containing 20% fetal calf serum (FCS). SMC were identified and characterized by the presence of nonmuscle actin, and by the characteristic "hillocks and valleys" appearance of confluent cultures.

Vascular Endothelium

EC were isolated from bovine aortas, were cultured and were characterized as previously described. Cells were released by one of two methods including: 1) collagenase irrigation of aortas (0.1% CL II, Worthington Biochemicals, Malvern, Pennsylvania) for 30 minutes at room temperature, or 2) scraping of the intima with a sterile scalpel blade. Cells were dispensed as clusters into the enzyme solution before centrifugation and plating at differential population densities in Dulbecco's Modified Eagle's Medium containing 5% calf serum (CS). EC verification was carried out by demonstration of Factor VIII immunofluorescent staining, by the uptake of di-acyl-LDL, by the presence of nonmuscle actin, and by the characteristic pattern of nonoverlapping contact-inhibited growth seen at confluence. No differences were noted in the biological activities, population densities, or morphology of EC or in the EC-derived matrices by these isolation procedures.

Preparation of Extracellular Matrices

Endothelial-Synthesized Matrices

Deoxycholic Acid Matrix. To prepare endothelial-synthesized matrices, we modified the procedure for the preparation of fibroblast pericellular coats. Aortic EC were plated into 24-well plates at seeding densities of 1 x 10^5 cells/well. At this seeding density, the cultures were confluent within 6 hours after plating. Cells were fed every third day. At 7 days after seeding, the population was washed three times in phosphate-buffered saline (PBS, 0.015 M sodium phosphate, pH 7.5, 0.15 M NaCl) before treatment with 0.5% sodium deoxycholate (DOC) (7-deoxycholic acid, Sigma Chemical Company, St. Louis, Missouri, Lot 57C0456) dissolved in 0.02 M Tris-Cl (pH 7.8) 0.015 M NaCl, 0.001 M EGTA, 0.001 M PMSF. Two room temperature detergent treatments, each lasting 15 minutes, were followed by five 1-minute washes with PBS before buffer aspiration. DOC-prepared, EC-synthesized matrices that contain fibronectin, laminin, and collagen were then inoculated with SMC or EC for further studies.

Ethylene Glycol-bis(β-aminomethyl Ether)-N,N,N',N'-tetraacetic Acid (EGTA) Matrix. To prepare these EC-synthesized matrices, we grew and washed EC as described above for the DOC-release procedure. Washed EC were then incubated for two 15-minute periods with 0.02 M EGTA dissolved in PBS without Ca^2+ or Mg^2+. After the first 15-minute incubation, the endothelial monolayer detached as a sheet into the aqueous phase. Careful aspiration of the monolayer left the tissue-like sheet intact; it could then be reused or discarded. As in the DOC experiment, these PBS-washed matrices were used for SMC proliferation studies.

Purified Extracellular Matrix Molecules

Multiwell plates were treated with purified extracellular matrix molecules as previously described. Briefly, purified bovine fibronectin (Biomedical Technologies, Stoughton, Massachusetts) or rat-tail tendon collagens I and III (Collaborative Research, Cambridge, Massachusetts) were diluted and dispensed in phosphate-buffered saline before spreading over the well bottoms. After a 60- to 120-minute incubation at room temperature, the wells were washed with sterile PBS before inoculation with either SMC or EC. Protein determination revealed that between 1 and 5 μg were bound for each well.

Collection of BAEC-Conditioned Mediums

Conditioned medium (CM) was collected from postconfluent cultures of EC as previously described. Briefly, cultures were grown as described above and were allowed to remain confluent for 5 to 7 days. The cells were then washed once with serum-free medium and incubated for 48 hours in RPMI + 0.5% to 1.0% CS or FCS (6 ml per 100 mm dish). At the end of this time, the medium was collected and centrifuged at 2500 g for 15 minutes to remove any
floating cells and cell debris. Cultures with noticeable debris or floating cells were discarded to minimize the possible contribution of lysed cell contents to the inhibitory activity. Aliquots of this conditioned medium could be stored at 4°C or subjected to further enzymatic treatments.

**Enzyme Treatments of Conditioned Medium**

Trypsin treatment of CM was accomplished by adding 30 μg/ml trypsin (Sigma Chemical Co., St. Louis, Missouri) for 2 hours at 37°C. A 10-fold molar excess of soybean trypsin inhibitor in a small volume (50 μl/ml CM) was added 30 minutes before mixing the CM with RPMI + 20% FCS. Chondroitinase treatment was carried out by incubating CM with 1 U/ml chondroitin ABC lyase for 90 minutes at 37°C. Heparinase digestion was carried out by exposing CM to 10 U/ml Flavobacterium heparinase for 90 minutes at 37°C. This preparation has no detectable protease activity and does not degrade chondroitin sulfates, dermatan sulfate, or hyaluronic acid (Miles Laboratories, Elkhart, Indiana).27 Samples treated with glycosaminoglycan hydrolases were then boiled for 5 minutes, a treatment that kills all the above enzyme activities but does not affect the heparin-like inhibitory activity.

**Growth Assays**

To assay the growth effects of EC-secreted products and heparin, SMC were sparsely plated into 24-well microplate dishes (6 to 8 × 10³ cells per cm² well). After 14 to 16 hours, control cultures were fed with RPMI + 20% FCS. Other cultures were exposed to RPMI + 20% FCS containing varying concentrations of EC-CM or heparin (Elkins-Sinn, Cherry Hill, New Jersey). Conditioned medium was mixed 1:1 with RPMI containing 40% FCS, thus making the final FCS concentration 20%. Cell numbers were measured either every other day or after 5 days (when growth had essentially ceased in both control and experimental cultures) in duplicate samples using a Coulter counter. Trypsinized cultures were routinely checked by direct microscopic examination to ascertain that the trypsinization procedure had not lysed the cells and to ensure that all cells were removed from the multwell plate. SMC were not fed during the experiment. The net growth of the cells in the control culture and the EC-CM or heparin-treated cultures were obtained by subtracting the starting cell number from the cell number at the end of the experiment. The range of the plating efficiencies of SMC varied between 65% and 85% in different experiments as determined by cell counts 14 to 16 hours after plating (i.e., before significant cell proliferation could occur). Within a given experiment, no significant differences were noted between any of the different substrates (i.e., <10%).

**Results**

Previous experiments that have examined EC secretion of heparin-like inhibitors and their ability to inhibit SMC growth were carried out using cells cultured on plastic substrates.27, 28 We were interested in whether the extracellular matrix could influence the production of the endothelial-derived, heparin-like inhibitor of smooth muscle growth and if such an endothelial-synthesized matrix could also affect the sensitivity of SMC to this growth regulator.

**Effect of Extracellular Matrix on Vascular Smooth Muscle Cell Growth**

To test the effects of different substrates on SMC growth, defined matrices were prepared from postconfluent aortic EC cultures using two different procedures. The DOC (detergent) method actually lysed EC in the presence of Tris-buffered saline containing millimolar amounts of proteolysis inhibitors; in contrast to this, the EGTA (chelator) method of matrix preparation detoxifies EC in a tissue-like sheet of intact cells. In addition to preparing these biosynthesized matrices, we also examined whether films of extracellular matrix molecules (fibronectin, collagen I monomer, and fibrils (rat-tail tendon/Collaborative Research) influenced the proliferative capacity of smooth muscle in vitro (Figure 1). While plating efficiency was not significantly altered by any of the matrices, both the proliferative rates, as well as the final population densities, were affected by matrix molecules. SMC responsiveness to purified fibronectin, monomeric and fibrillar collagen, and tissue culture plastic was similar with respect to the population doubling time (24 hours) and the final population density (5.2 × 10⁴ cells per cm²). In contrast to these matrices, the two-EC-derived matrix preparations modulated growth in distinctive manners (Figure 1). SMC grown on DOC-EC matrices had doubling times of 19 hours and achieved a final density of 1.1 × 10⁵ cells per cm². SMC cultured on EGTA-EC matrix had doubling times of 32 hours and attained a final density of 3 to 5 × 10⁴ cells per cm².

**Effect of Substrate on Smooth Muscle Cell Response to Heparin**

Because extracellular matrix components influenced the growth properties of SMC in vitro, we tested whether these same matrices could alter the responsiveness of SMC to heparin (Figure 2). Earlier studies on the SMC sensitivity to heparin employed cells that were growth arrested in G₁ (Gᵢ), before exposure to heparin.27, 28 This was done because the growth-arrested cells are 50 to 100 times more sensitive to heparin when compared to the exponentially-growing cells. In the experiments reported here, we were concerned that the SMC would significantly modify the matrix during the 72-hour period in low-serum conditions. We therefore chose to treat SMC with EC-conditioned media or heparin 14 to 16 hours after plating. This allowed cell attachment and spreading while leaving the population largely synchronized in G₁ and still quite sensitive to heparin (Castellot JJ unpublished observation).

EGTA-EC matrix, which was least supportive of SMC growth in the absence of heparin, was the most effective in potentiating the growth-inhibitory response to SMC cultured in the presence of heparin (1 to 200 μg/ml). Interestingly, DOC-EC matrices, which allowed the fastest rate of SMC growth (Figure 1) rendered SMC less sensitive to heparin since both the efficacy (degree of growth inhibition) as well as the potency (the dose required for 50% growth inhibition) of heparin were altered (Figure 2).
Matrix Influences Endothelial Cell Production of Inhibitory Activity

We were able to demonstrate that the matrices produced by EC in vitro modulated SMC growth sensitivity in the presence or absence of exogenously-added heparin; this did not address whether the extracellular compartment also altered EC production of the heparin-like SMC growth inhibitor. To test this possibility, aortic EC populations were plated onto tissue culture plastic or defined extracellular matrices at high densities (see Methods for details). This was done to minimize the time required for a monolayer of EC to form (6 hours), thus reducing the possibility of significant matrix modifications before the EC became confluent; but, it should be pointed out that the EC monolayer is almost certainly modifying the matrix during the 7 days after plating. On the seventh day after inoculation, the culture media was removed, replaced, and then collected 24 hours later. This was done because earlier studies had demonstrated that EC did not produce the heparin-like growth inhibitor until at least 5 days after confluence. These EC-conditioned mediums were tested for SMC growth-inhibitory activity (Figure 3). The EGTA-EC matrices were the most effective substrates influencing the EC-synthesized inhibitory activity; EC-CM from this matrix contained the equivalent of 20 μg/ml heparin and inhibited SMC growth by 59%. In contrast, EC-CM from DOC-EC matrix contained the equivalent of only 2 μg/ml heparin and inhibited SMC proliferation by 18%. Noteworthy is the fact that the DOC-EC matrices behaved similarly to the collagens I and III if the percent inhibition and equivalent heparin doses were compared (Figure 3). Thus, despite the fact that EC may be altering the matrix before CM is collected (or perhaps even because of it), there is still a substantial difference in the amount of growth-inhibitory activity secreted by EC on EGTA compared to DOC matrices.

ED_{50} for heparin when SMC are grown on EGTA-EC matrices was 10 μg/ml; the ED_{50} for heparin on DOC-EC matrices was 65 μg/ml. It should be noted that the percent growth was calculated for each substrate using growth in the absence of heparin on that substrate as the 100% control. Thus, there were inherent differences in sensitivity to heparin observed on EGTA-EC and DOC-EC matrices rather than changes due simply to the different growth rates of SMC supported by these matrices.

We considered two trivial explanations for the above results. First, it was possible that the soluble heparin we added to SMC bound differentially to the substrates and thus altered the degree of growth inhibition. To examine this question, we soaked the substrates overnight in a 1 mg/ml of heparin in RPMI medium solution before plating SMC. Growth assays in the presence and absence of heparin were performed. There were no differences in the growth rates or final densities of SMC plated onto heparin-saturated substrates compared to untreated substrates, nor were there any changes in the sensitivity of SMC to exogenously supplied heparin (data not shown). The second possibility was that the effect of the different substrates on SMC growth and heparin sensitivity was a function of cell density. Experiments were carried out using starting cell densities ranging from 2 × 10^3 to 1.6 × 10^4 cells per well. The differences between the substrates remained essentially unchanged throughout this range (data not shown).

**Figure 1.** Effect of extracellular matrix on vascular smooth muscle cell growth. 6 to 8 × 10^3 smooth muscle cells were plated onto defined substrates in normal growth medium (RPMI + 20% fetal calf serum) and allowed to proliferate as described in Methods. Cells were not fed during the experiment. Doubling times and final population densities were significantly affected over the time course of the experiment. The data are the mean values of duplicate samples from at least six separate experiments. SEMs are less than ±5%.

**Figure 2.** Substrate affects the vascular smooth muscle cell response to heparin. The relative sensitivity of smooth muscle cells to heparin was tested as a function of the extracellular matrix. Cells were counted 6 days after exposure to heparin. As seen in Figure 1, the EGTA-EC matrix was the most effective matrix at inhibiting growth and in lowering the ED_{50} for heparin. Values are the means of duplicate samples in six separate experiments. The SEM for each point is less than 5%. Student's t test indicated the difference between the DOC and EGTA matrices was statistically significant (p < 0.01), as was the difference between the EGTA matrix and the FN, collagens, plastic group (p < 0.02) or between the DOC matrix and the FN, collagens, plastic group (p > 0.05). The differences within the FN, collagens, and plastic group are not significant (p > 0.05). The number of cells per well in the absence of heparin (100% level) was the same as for Day 8 in Figure 1.
SUBSTRATE

**Figure 3.** Production of the endothelial-derived growth inhibitor for smooth muscle cells is influenced by the extracellular matrix. Smooth muscle cells were exposed to the indicated EC-CMs as described in Methods. The cell number was determined in duplicate samples after 6 days. Error bars represent the standard error of the mean from at least six experiments. The numbers at the top of bars represent the equivalent heparin doses in Table 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Inhibition</th>
<th>% Inhibitor Activity Destroyed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic</td>
<td>52</td>
<td>0</td>
</tr>
<tr>
<td>Trypsin</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Heparinase</td>
<td>13</td>
<td>75</td>
</tr>
<tr>
<td>Heparinase, then trypsin</td>
<td>3</td>
<td>94</td>
</tr>
<tr>
<td>EGTA</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td>Trypsin</td>
<td>30</td>
<td>43</td>
</tr>
<tr>
<td>Heparinase</td>
<td>23</td>
<td>58</td>
</tr>
<tr>
<td>Heparinase, then trypsin</td>
<td>8</td>
<td>85</td>
</tr>
<tr>
<td>DOC</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Trypsin</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>Heparinase</td>
<td>6</td>
<td>70</td>
</tr>
<tr>
<td>Heparinase, then trypsin</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1. Enzymatic Reversal of the Endothelial-Derived Inhibitor of Smooth Muscle Cell Growth

**Characterization of the Endothelial Cell-Synthesized Inhibitors of Smooth Muscle Cell Growth**

Proteases and glycosaminoglycan-degrading enzymes (GAGases) were used to examine whether we could ablate or abolish the EC-synthesized activity shown to modulate SMC growth (Table 1). Mediums derived from EC cultured on the various test substrates were incubated in saturating concentrations of either trypsin, heparinase, or both enzymes sequentially (heparinase followed by trypsin) before testing for SMC growth inhibition. In control experiments, heat-inactivated enzymes or GAG hydrolases specific for other species (hyaluronidase, chondroitinase) proved ineffective in reversing the inhibition of SMC proliferation. In contrast, trypsin or heparinase treatment significantly reduced the inhibitory effect. The effects of protease and heparinase treatment on the various substrates tested were nearly identical. Treatment with both heparinase and trypsin destroyed 94%, 85%, and 100% of the activity found in EC-conditioned mediums obtained from cells grown on plastic, EGTA-EC- and DOC-EC-derived matrices, respectively.

When the data on enzyme sensitivity (Table 1) and inhibitory activity (shown in Figure 3 as the equivalent heparin dose required to produce the growth inhibition found with various substrates) are combined, it is clear that secretion of both the heparinase-sensitive and protease-labile inhibitory factors are strongly matrix-dependent. To quantitate this, we calculated the fraction of total inhibitory activity that was heparinase- or trypsin-sensitive. We found that EC cultured on the EGTA-derived matrix secreted eightfold more heparin-like and 17-fold more peptide inhibitory activities than EC grown on the DOC-derived matrix. This data also suggest that the peptide inhibitor may be somewhat more matrix-dependent than the heparin-like factor, but the experimental errors and inherent assumptions made in calculating this result make a precise quantitative relationship between the peptide and heparin-like inhibitors difficult to determine.

**Combined Matrix Modulation of Smooth Muscle Cell Growth**

If the elevated EC production of inhibitory activity and the potentiated SMC sensitivity to heparin were additive effects, we would have expected to find that EC-synthesized matrices exerted a 60-fold difference in the potency of the antiproliferative activity. To examine this directly, we tested SMC growth sensitivity in combination with EC-inhibitor production as a function of matrix (Table 2). When the most potent growth-inhibitory mediums (EGTA-EC-derived) were added to SMC inoculated on the most inhibitory substrate (EGTA-EC derived), we found that SMC growth inhibition was approximately fourfold greater than SMC cultured on DOC-EC matrix and exposed to CM from EC grown on DOC-EC-derived matrix. This corresponds to a 30- to 40-fold difference in the potency of the growth inhibitory effect if expressed as the equivalent doses of heparin required to produce similar growth inhibition (the equivalent of 100 μg/ml heparin on EGTA-EC vs. 3 μg/ml on DOC-EC-derived substrates).
Table 2. Combined Effects of Substrates on Smooth Muscle Cell Growth

<table>
<thead>
<tr>
<th>Smooth muscle cell substrate</th>
<th>Endothelial cell substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGTA</td>
<td>72% (100)</td>
</tr>
<tr>
<td>Plastic</td>
<td>67% (70)</td>
</tr>
<tr>
<td>DOC</td>
<td>48% (20)</td>
</tr>
<tr>
<td>Plastic</td>
<td>65% (60)</td>
</tr>
<tr>
<td>DOC</td>
<td>59% (30)</td>
</tr>
<tr>
<td>Plastic</td>
<td>39% (10)</td>
</tr>
<tr>
<td>DOC</td>
<td>18% (3)</td>
</tr>
</tbody>
</table>

The data are given as the mean percent inhibitions from duplicate determinations of two separate experiments; the equivalent heparin dose present (μg/ml) is given in parentheses. All SEM are less than or equal to ± 5%.

EGTA = ethylene glycol tetraacetic acid; DOC = deoxycholic acid.

Discussion

Our studies have focused on the interrelationships between the extracellular matrix and the vascular cells of the elastic artery wall. We have demonstrated that: 1) EC-synthesized matrices influence the production of heparin-like and peptide inhibitors of SMC growth, and 2) these same matrices alter the sensitivity of SMC to these growth regulators. The results add support to the importance of extracellular signaling in regulating an interrelated array of cell functions including adhesion, motility, growth, and differentiation. 32-37

It is noteworthy that the substrates of purified extracellular matrix molecules (FN, collagen monomers, and fibrils) all yield similar growth responses of SMC in the presence or absence of heparin (Figures 1 and 2). However, extracellular matrices synthesized and organized by living EC affected SMC growth in distinctive manners, depending upon the method used to prepare the matrix. Because the DOC-prepared EC matrix lyzes the monolayer of cells, whereas the EGTA-derived matrix is prepared without cell disruption, the form and composition of the two EC-synthesized matrices may explain both the SMC growth properties and the altered EC synthetic activities. To address this point, we have begun biochemical characterization of these two different preparations of endothelial-synthesized matrices. Parallel experiments are also being conducted on the protease and GAG-hydrolase sensitivity of these same biomatrices. Results of these preliminary experiments indicate that biosynthetically labelled matrices prepared from the aforementioned methods possess different assortments of 35S-methionine-labelled protein pools. Gel electrophoresis and autoradiography demonstrate that the EGTA matrix is complex, rich in biosynthetically labelled proteins of varied molecular weights (20 to 300 kd). In contrast, the DOC matrix is relatively deficient in methionine-labelled proteins. Major protein components of the DOC matrix appear to be fibronectin and collagen (Yost J, Herman I unpublished observation). Furthermore, treatment of the matrices with heparinase and protease partially reverses the SMC growth inhibitory activity. Further biochemical characterization of the EC-derived matrices is currently underway.

Other work in vitro indicates that specific components of different matrices alter growth in vitro. 5, 6, 17, 34-37 For example, it has recently been shown that GAG or proteoglycan components of the extracellular matrix synthesized by EC may be capable of binding and concentrating polypeptide growth factors, thus providing a mechanism for autocrine stimulation of growth. 32, 40 Moreover, it has been suggested that inhibition of microvascular EC growth by heparin and cortisone may proceed via basement membrane dissolution. 41 The exact molecular makeup of this matrix or the in vitro synthesized EC matrices is still poorly understood and awaits further characterization.

In vitro studies cannot reveal the precise roles that extracellular matrix-vascular cell interactions play in regulating the structure and function of the artery wall. However, it is interesting to speculate that EC-synthesized matrix components influence the growth of cells of the intima and the media since EC production of growth inhibitors, as well as the SMC sensitivity to these same moieties, are strongly matrix-dependent events. For example, endothelial migration after injury correlates with a shift in sulfated proteoglycan synthesis for EC migrating at the wound zone on plastic substrates. 9 This supports earlier in situ studies on fluctuating proteoglycans accumulating in medias of injured and repairing blood vessels. In both these sets of studies, controls were enriched with heparan sulfate; but, with injury and repair the major sulfated proteoglycans present were the dermatans and chondroitins. 4, 6, 42

Our observations suggest that the use of a cell-derived matrix or "biomatrix" is important in the study of extracellular signaling. These results corroborate and extend earlier studies indicating that EC-synthesized extracellular molecules influence the proliferative capacity of vascular SMC. 27, 29 Work from several laboratories supports this idea. 3, 17, 33-37 Ultimately, this association of SMC, EC, and the extracellular matrix may direct whether the vascular cell responses to hemostatic or injurious stimuli will maintain SMC in a quiescent growth state, 43 promote intimal proliferation, 25 or induce the SMC regression seen following restitution of the intima after injury in situ.

Acknowledgments

We are grateful to Morris J. Karnovsky for valuable discussions during the course of these studies; to Tamara Pikulik, Martin Keisch, and Benjamin Caleb for expert technical assistance and to Tom Wright and Pat D'Amore for critical reading of the manuscript.

References


Index Terms: vascular endothelium • vascular smooth muscle • blood vessels • extracellular matrix • heparin • cytoskeleton • growth • cell culture • glycosaminoglycans
Regulation of vascular smooth muscle cell growth by endothelial-synthesized extracellular matrices.

I M Herman and J J Castellot, Jr

_Arterioscler Thromb Vasc Biol_. 1987;7:463-469
doi: 10.1161/01.ATV.7.5.463

_Arteriosclerosis, Thrombosis, and Vascular Biology_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/7/5/463

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Arteriosclerosis, Thrombosis, and Vascular Biology_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Arteriosclerosis, Thrombosis, and Vascular Biology_ is online at:
http://atvb.ahajournals.org/subscriptions/