Lung endothelial heparan sulfates mediate cationic peptide-induced barrier dysfunction: a new role for the glycocalyx

Randal O. Dull,1 Ramani Dinavahi,1 Lawrence Schwartz,1 Donald E. Humphries,2 David Berry,3 Ram Sasisekharan,3 and Joe G. N. Garcia4

1Anesthesiology and Critical Care Medicine and 4Division of Pulmonary and Critical Care, Department of Medicine, Johns Hopkins School of Medicine, Baltimore, Maryland 21287;
2Department of Veterans Affairs Medical Center, Boston 01230; and 3Harvard-Massachusetts Institute of Technology, Divisions of Health Science and Technology and Biological Engineering, Cambridge, Massachusetts 02139

Submitted 22 January 2003; accepted in final form 11 May 2003

Dull, Randal O., Ramani Dinavahi, Lawrence Schwartz, Donald E. Humphries, David Berry, Ram Sasisekharan, and Joe G. N. Garcia. Lung endothelial heparan sulfates mediate cationic peptide-induced barrier dysfunction: a new role for the glycocalyx. Am J Physiol Lung Cell Mol Physiol 285: L986–L995, 2003. First published May 16, 2003; 10.1152/ajplung.00022.2003.—The endothelial glycocalyx is believed to play a major role in microvascular permeability. We tested the hypothesis that specific components of the glycocalyx, via cytoskeletal-mediated signaling, actively participate in barrier regulation. With the use of polymers of arginine and lysine as a model of neutrophil-derived inflammatory cationic proteins, we determined size- and dose-dependent responses of cultured bovine lung microvascular endothelial cell permeability as assessed by transendothelial electrical resistance (TER). Polymers of arginine and lysine >11 kDa produced maximal barrier dysfunction as demonstrated by a 70% decrease in TER. Monomers of l-arginine and l-lysine did not alter barrier function, suggesting a cross-linking requirement of cell surface “receptors.” To test the hypothesis that glycosaminoglycans (GAGs) are candidate receptors for this response, we used highly selective enzymes to remove specific GAGs before polyarginine (PA) treatment and examined the effect on TER. Heparinase III attenuated PA-induced barrier dysfunction by 50%, whereas heparinase I had no effect. To link changes in barrier function with structural alterations, we examined actin organization and syndecan localization after PA. PA induced actin stress fiber formation and clustering of syndecan-1 and syndecan-4, which were significantly attenuated by heparinase III. PA-induced cytoskeletal rearrangement and barrier dysfunction did not involve myosin light chain kinase (MLCK) or p38 MAPK, as ML-7, a specific MLCK inhibitor, or SB-20358, a p38 MAPK inhibitor, did not alter PA-induced barrier dysfunction. In summary, lung endothelial cell heparan sulfate proteoglycans are key participants in inflammatory cationic peptide-induced signaling that links cytoskeletal reorganization with subsequent barrier dysfunction.

endothelium; inflammation; permeability

THE AGGREGATION of polymorphonuclear leukocytes (PMNs) is a hallmark of acute inflammation and represents a complex coordination of signals between microvascular endothelial cells and both circulating and adherent PMNs. Cytokines elaborated during infection and tissue injury activate both endothelial cells and PMNs, which promotes the sequence of PMN rolling, tethering, and ultimately, firm adhesion through the interaction of E- and P-selectins and vascular cell adhesion molecule-1 (23). Subsequently, ligation of the β2-integrin receptor (CD11/18) complex on PMNs promotes the release of inflammatory peptides from azurophilic granules that further propagates the inflammatory response (3).

An important consequence of this inflammatory process is an increase in vascular endothelial permeability that results in tissue edema with attendant changes in microvascular hemodynamics, tissue oxygenation, and subsequent organ failure. In fact, microvascular dysfunction underlies the etiology of multiorgan failure after sepsis, trauma, burn injuries, solid organ transplant failure, and a host of autoimmune diseases (22, 25, 26). Elucidating the fundamental mechanisms by which neutrophil-endothelial interaction produces subsequent changes in vascular permeability has important implications for attenuating the undesirable effects of inflammation.

Ligation of CD11/18 on PMNs initiates adhesion to microvascular endothelial cells and subsequent adhesion-dependent activation. Activated PMNs secrete cationic peptides such as heparin-binding protein/CAP37/azurocidin (HBP), elastase, and cathepsin G (3) that have been implicated in PMN-mediated vascular permeability changes, and inhibition of CD11/18-mediated adhesion of PMNs during acute lung injury (1, 19) prevents associated pulmonary edema. HBP/CAP37/azurocidin belongs to a family known as the serpocidins whose members include three active proteases (elastase, cathepsin G, and proteinase-3) and the inactive protease homolog HBP/CAP37. HBP has antimicrobial activities (33), has antiapoptotic effects in endothelium (31), and is a potent chemoattractant for

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajplung.org
monocytes and T cells (34). Importantly, HBP/CAP37 has been reported to account for all of the vascular permeability-enhancing activity of activated neutrophils (12). Whether receptors for HBP/CAP37 and other inflammatory cationic peptides are present on the luminal surface of vascular endothelium has not been established. However, the strong cationic nature of HBP in conjunction with intrinsic heparin-binding characteristics suggests that a search for the HBP receptors could potentially include cell surface glycosaminoglycans (GAGs), including heparin/heparan sulfate-like GAGs (HSGAGs), implicating a component of the endothelial glycocalyx, a thick surface layer of proteoglycans whose anionic GAG side chains form an entangled meshwork on the cell surface (36). The adsorption of serum proteins onto the GAG chains results in the formation of a gel-like layer that creates a direct physical barrier to both water and protein transport into the intercellular junction (6, 15). In this study, we hypothesized that specific components of the glycocalyx may play an active role in barrier regulation through GAG-mediated interactions with soluble cationic ligands (like HBP), thereby activating signaling pathways associated with the cytoplasmic domain of the proteoglycan core protein. We now report size- and polymer-specific effects of diverse cationic peptides on endothelial cell cytoskeletal rearrangement and transmonolayer permeability. Our results indicate that cell surface HSGAGs are actively involved in barrier regulation and directly mediate, in part, cationic peptide-induced signaling that leads to increases in endothelial permeability and cytoskeletal reorganization. These data further suggest that the syndecan family of heparan sulfate proteoglycans participate in polycation-induced signaling and highlights a novel role for the glycocalyx.

METHODS

Cell culture and reagents. Bovine lung microvascular endothelial cells (BLMVEC) were obtained from American Type Culture Collection (Rockville, MD) and Vec Technologies (Rensselaer, NY) and cultured in DMEM and MCDB-131, respectively, and supplemented with 10% fetal calf serum. Culture Collection (Rockville, MD) and Vec Technologies (Santa Cruz, CA). Monoclonal antibody (BB4) to syndecan-1 and anti-syndecan-4 antibodies followed by anti-IgG-labeled secondary antibody. Cell monolayers were then examined by fluorescence microscopy (Nikon Eclipse TE-200 inverted microscope).

Statistics. For all TER measurements, each monolayer served as its own control, and percentage change from baseline was determined. Data from each group were pooled and analyzed by one-way ANOVA, and groups were compared with a Student-Neuman-Keuls test using Sigma Stat (SPSS, Chicago, IL). Data are presented as percent change in TER ± SE.

RESULTS

Polycationic peptides increase endothelial permeability. We examined the role of the polycationic peptides PA and polylysine (PL) on endothelial permeability as measured by TER. Both PA (90 kDa) and PL (84 kDa) produced rapid decreases in TER as demonstrated in Fig. 1, A and B, with maximal permeability occurring at a concentration of 50 μg/ml. By comparison, dextran 70, a neutral polymer of similar size (70 kDa), did not
affect TER values at concentrations of 10, 50, and 100 μg/ml (data not shown).

We next tested the effect of polycation size on endothelial barrier function and demonstrated the multivalent requirement of cationic peptides for this response. Monomers of L-arginine failed to alter TER (Fig. 2A), whereas PA at each size tested (11.8, 38, and 90 kDa) produced maximal reductions in TER (Fig. 2A). Consistent with these results, L-lysine monomers did not significantly alter TER (Fig. 2B), whereas larger polymers of lysine (32 and 84 kDa) both reduced TER maximally (Fig. 2B). The observation that monomers of L-arginine and L-lysine failed to increase endothelial permeability supports the notion that signal activation may require cross-linking of cell surface domains. Although the polymers of arginine and lysine used in these studies were both polydispersed, the response to the smallest PA (11.8 kDa) differed dramatically from the response to the smallest PL (7.3 kDa). PA (11.8 kDa) induced maximal barrier dysfunction, whereas the 7.3-kDa PL polymer did not affect lung TER (Fig. 2B), suggesting specificity in the response evoked by interaction between the cationic peptide structure and the unknown binding site(s) on the endothelial surface.

**HSGAGs mediate PA-induced barrier dysfunction.**

We hypothesized that cationic peptides mediate cellular effects via interaction with anionic GAGs present on the endothelial surface composed primarily of HSGAGs and chondroitin sulfate-like GAGs. To examine the dependence of cationic peptide-induced decrease in TER on endothelial GAGs, we selectively removed either HSGAGs or chondroitin sulfate GAGs via enzymatic cleavage using heparinase III and chondroitinase ABC, respectively. Removal of either heparan sulfates or chondroitin sulfates had no effect on baseline TER; however, the removal of cell surface heparan sulfate with heparinase III significantly attenuated PA-induced barrier dysfunction (Fig. 3), whereas chondroitin sulfate removal did not alter PA-induced barrier dysfunction (Fig. 3). We next quantified the amount of heparan sulfate removed by heparinase III pretreatment to link enzyme activity with the alterations in PA-induced barrier dysfunction. Heparinase III (15 mU/ml) pretreatment removed ~67% of the releasable heparan sulfate pool (Table 1). Given this close linkage between the amount of heparan sulfate removed and the magnitude of attenuation of PA-induced barrier dysfunction, these data suggest that heparan sulfate proteoglycans are key participants in cationic peptide-induced endothelial barrier dysfunction.

To exclude persistent HSGAGs not removed with heparinase III as a possible explanation for the remaining 50% of the PA-induced barrier dysfunction
that was refractory to heparinase III, we pretreated monolayers with heparinase I alone and in combination with heparinase III, before PA stimulation. Heparinase III cleaves primarily between unsulfated or monosulfated saccharides within the GAG chain, whereas heparinase I generally cleaves at more highly sulfated saccharides (28). Heparinase I alone had no effect on PA-induced barrier dysfunction, and the mix of heparinase I and III provided no additional attenuation of the PA response compared with heparinase III alone. Enzyme activity assays demonstrated that heparinase I released 40% of labeled heparan sulfate (Table 1). The combination of heparinases I and III had no effect on PA-induced TER beyond that of heparinase III alone (Fig. 3), and enzyme activity assays demonstrated no additional release when the enzymes were used in combination. We next examined the role of chondroitin sulfate-like GAGs by predigesting the cell surface with chondroitinase ABC lyase (2.5 mU/ml) before PA treatment. This dose of chondroitinase I and III had no effect on PA-induced TER beyond that of heparinase III alone (Fig. 3), and enzyme activity assays demonstrated no additional release when the enzymes were used in combination. We next examined the role of chondroitin sulfate-like GAGs by predigesting the cell surface with chondroitinase ABC lyase (2.5 mU/ml) before PA treatment. This dose of chondroitinase II and III had no effect on PA-induced TER beyond that of heparinase III alone (Fig. 3), and enzyme activity assays demonstrated no additional release when the enzymes were used in combination.

To correlate these enzyme results with HSGAG structure, we characterized the disaccharide composition of the HSGAGs from lung microvascular endothelial cells by capillary electrophoresis (20). These studies demonstrated (Table 2) that 41% of the disaccharides were monosulfated and 59% were unsulfated disaccharides. The predominance of monosulfated and unsulfated disaccharides directly correlates with the observed activity of heparinase III (28) and the observed attenuation of the PA-induced permeability response by heparinase III pretreatment.

HSGAG sulfation level does not affect PA-induced permeability. To investigate the structural requirements of HSGAGs in mediating PA-induced signaling, we pretreated monolayers for 24 h with sodium chlorate in sulfate-free RPMI 1640, which reduces endothelial HSGAGs by 65% (Table 2) and chondroitin sulfate by 90% (16). Chlorate treatment failed to alter either baseline TER or PA-induced barrier dysfunction (Fig. 3), suggesting that simple charge interaction alone is not solely responsible for mediating PA binding and associated signal activation but requires an additional specific structural characteristic of the repeating disaccharide units.

Anti-syndecan and anti-heparan sulfate antibody cross-linking and endothelial barrier function. The size-dependent effect of polycations on barrier dysfunc-

---

**Fig. 2.** A: PA size response at 50 μg/ml on endothelial cell monolayer permeability. Monomers of L-arginine (L-Arg) had no effect on TER. All sizes of PA tested provoked maximal loss in TER. B: PL size response at 50 μg/ml on endothelial cell monolayer permeability. Monomers of L-lysine (L-Lys) and the 7.3-kDa polymer did not alter TER. PL at both 32 and 84 kDa caused maximal increase in permeability. Data are means ± SD (n = 6–8). #P < 0.05 vs. control; *P < 0.05 vs. monomers.
Table 1. Quantification of enzyme activity

| Enzyme          | Concentration, mU/ml | %35SO4 Released |
|-----------------|----------------------|-----------------
| Heparinase III  | 15                   | 67              |
| Heparinase I    | 15                   | 40              |
| Heparinase I + III | 15 + 15             | 62              |
| Chondroitinase  | 2.5                  | 16              |

Heparinase II and III had no effect on baseline TER (not shown). Chondroitinase ABC lyase (Cha'ese+) failed to alter PA-induced permeability. Cha'ese had no effect on baseline TER (not shown). Chlorate treatment (Chl+PA) failed to alter PA-induced permeability. Both the myosin light chain kinase inhibitor 1-(5-iodonaphthalene-1-sulfonyl)-1H-hexahydro-1,4-diazepine HCl (ML-7; 20 μM) and the p38 MAPK inhibitor SB-20358 (20 μM) failed to alter the PA-induced barrier dysfunction. Data are means ± SD (n = 6–8).

Table 2. Heparan sulfate disaccharide composition

<table>
<thead>
<tr>
<th>Disaccharide</th>
<th>BLMVEC in MCDB-131</th>
<th>BLMVEC in SF-RPMI 1640</th>
<th>BLMVEC in SF-RPMI 1640 + Chlorate, 50 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔU2SHNS,6S</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ΔU2SHNS</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ΔUH2S,6S</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ΔUH2S,6S,6S</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ΔUHNS,6S</td>
<td>0.83</td>
<td>0.63</td>
<td>0.25</td>
</tr>
<tr>
<td>ΔUH2S,6S,6S</td>
<td>12.91</td>
<td>14.46</td>
<td>5.56</td>
</tr>
<tr>
<td>ΔUH2S,6S,6S</td>
<td>27.77</td>
<td>26.23</td>
<td>11.27</td>
</tr>
<tr>
<td>ΔUHNS,6S</td>
<td>57.87</td>
<td>58.31</td>
<td>82.73</td>
</tr>
</tbody>
</table>

Heparan sulfate from bovine lung microvascular endothelial cells (BLMVEC) was digested completely, and disaccharides were identified by capillary electrophoresis. Disaccharide composition was measured in cells cultured in standard culture medium (MCDB-131), in sulfate-free RPMI 1640 (SF-RPMI 1640), and sulfate-free RPMI 1640 plus 50 mM sodium chlorate for 24 h. U, uronic acid, H, glucosamine, 2S, 2-O-sulfate, 6S, 6-O-sulfate, NAc, N-acetyl, NS, N-sulfate.
syndecan-1 and syndecan-4 localization and actin organization in response to PA challenge. In control endothelial cells, syndecan-1 distribution was limited to the cell periphery (Fig. 4A), and cells demonstrated the typical peripheral actin band with a few centrally located stress fibers (Fig. 4B). Treatment of endothelial monolayers with PA (90 kDa, 50 µg/ml) caused a significant change in syndecan-1 distribution, from a continuous peripheral pattern to a punctuate pattern consistent with clustering (Fig. 4C), and altered the polymerized actin cytoskeleton from a peripheral distribution to predominately cytoplasmic stress fibers (Fig. 4D). These changes in syndecan-1 and actin were associated with gap formation between adjacent endothelial cells (Fig. 4D, arrows), consistent with the associated increase in monolayer permeability reflected by TER.

Under basal conditions, syndecan-4 staining localized to the cell periphery, as did actin, specifically at areas of cell-cell contact; there was also significant perinuclear staining (Fig. 5, A and C). After PA treatment, syndecan-4 was notably absent from cell-cell contacts and demonstrated reduced staining in the perinuclear region but demonstrated enhanced localization in a linear pattern throughout the cytoplasm (Fig. 5B), suggestive of colocalization with actin (Fig. 5D) and/or microfilaments. Thus both syndecan-1 and syndecan-4 demonstrated significant changes in localization after PA stimulation, and these changes occurred in association with dramatic actin stress fiber formation.

Heparinase III pretreatment abolishes PA-induced actin cytoskeletal reorganization and syndecan local-
To confirm that HSGAGs were directly involved in mediating syndecan clustering and actin reorganization, cell surface heparan sulfates were removed with heparinase III before treatment with PA. Immunofluorescent studies revealed that removal of cell surface heparan sulfates completely abolished PA-induced actin reorganization, syndecan-1 clustering, and interendothelial gap formation (Fig. 4, E and F), whereas heparinase III treatment alone had no effect on actin or syndecan-1 localization (data not shown). Heparinase III pretreatment also attenuated PA-induced syndecan-4 clustering and actin reorganization (Fig. 5, E and F).

**Signaling pathways involved in cationic peptide-mediated barrier dysfunction.** Increases in endothelial cell stress fibers implicates an activation of the endothelial cell contractile apparatus. We examined the role of two major signaling pathways known to contribute to endothelial cell stress fiber formation, myosin light chain kinase (MLCK), and p38 MAPK (8, 11) on cationic peptide-mediated endothelial permeability. To examine the role of MLCK on myosin light chain phosphorylation, we first assessed the levels of myosin light chain (data not shown). Consistent with these results, 1-(5-iodonaphthalene-1-sulfonyl)-1H-hexahydro-1,4-diazepine HCl (ML-7), a specific MLCK inhibitor, was used at a concentration (20 μM) that has been shown to completely inhibit MLCK phosphorylation in response to numerous agonists (41) and failed to alter PA-induced barrier dysfunction as assessed by
TER (Fig. 3), suggesting that PA acts through MLCK-independent pathways to modulate cytoskeletal organization and barrier function. We have also previously reported a p38 MAPK pathway (11, 32) leading to marked stress fiber not associated with myosin light chain phosphorylation. To assess this signaling pathway, we tested SB-20358, a p38 MAPK inhibitor, which failed to affect the PA response on TER (Fig. 3). The concentration of SB-20358 used (20 μM) has been shown to significantly inhibit p38 MAPK (29). These results suggest a novel mechanism of stress fiber formation that does not require an increase in either MLCK or p38 MAPK activity.

DISCUSSION

In this study, we demonstrate that endothelial HSGAGs mediate cationic peptide-induced signaling that leads to endothelial cytoskeletal rearrangement and barrier dysfunction. This cationic peptide-mediated loss in barrier function was associated with syndecan-1 and syndecan-4 clustering and actin stress fiber formation; removal of cell surface HSGAGs attenuated these responses. Together, these observations suggest that syndecan heparan sulfates serve as an endothelial cell surface binding domain for cationic peptides and initiate stimulus/coupling responses that signal via the actin cytoskeleton to increase endothelial permeability. However, it is likely that there are additional participants involved in this complex signaling pathway that lead to changes in endothelial barrier function. These data provide the first account in identifying an endothelial-surface binding domain for inflammatory cationic peptides and provide mechanistic insight for the recent observations of Gautum et al. (12), who demonstrated that the neutrophil-derived cationic peptide, HBP, is responsible for the increase in vascular permeability produced by endothelial cell interaction with activated neutrophils.

Like HBP/CAP37, PA and PL are highly cationic molecules that have been utilized as models of neutrophil- and eosinophil-derived cationic peptides (30, 39). PL promotes edema formation when injected intradermally (30) and increases lung epithelial permeability when instilled into the intra-alveolar space (39). The mechanism(s) responsible for these effects was unknown, and the direct effects of cationic peptides on endothelial and epithelial cells could not be separated from secondary paracrine mediators, such as mast cell degranulation or macrophage activation. Polycation-induced permeability was postulated to occur through charge-mediated binding to anionic sites on the cell surface, presumably sulfated proteoglycans (30). The cellular effects of polycations are size dependent (30), suggesting that cationic peptides cross-link cell surface domains, and the greater the number of cross-linked “receptors” the greater the intensity of signal amplification. Our data support this concept, since monomers of L-arginine and L-lysine failed to alter endothelial barrier properties, whereas larger polymers induced maximal signal activation and greater changes in permeability.

Our results with the small polycation polymers require specific comment as the 11.8-kDa polymer of arginine induced maximal barrier dysfunction, whereas the smallest lysine polymer (7.3 kDa) did not alter endothelial permeability. Because the cationic peptides used in this study were all polydispersed, we expected similar results for these two peptides. The difference between the size response of PA (11.8 kDa) and PL (7.3 kDa) suggests there is structural specificity of the putative binding domain for these basic amino acid residues. The lack of effect of sodium chloride on PA-induced barrier dysfunction further suggests that the interaction of PA with its receptor involves more complex stereoselectivity as opposed to simple charge interaction.

The specificity for arginine-mediated binding to heparan sulfate has been reported by Liu and colleagues (24), who demonstrated that the binding of tissue factor pathway inhibitor-2/matrix-associated serine protease inhibitor to heparan sulfate and dermatan sulfate occurred selectively through arginine-mediated interaction. Likewise, Michel and colleagues (27) demonstrated that serum albumin reduces capillary permeability via arginine-mediated interaction with the endothelial cell surface, whereas modification of lysine residues had no effect on the ability of albumin to modulate permeability; similar results were obtained when modified albumins were tested on the permeability of cultured endothelial cells (35). Last, Vepa et al. (40) demonstrated that PA was more potent in activating endothelial cell phospholipase D compared with PL. Thus our data are consistent with a number of reports demonstrating that whereas arginine and lysine residues carry the same electrical charge, other structural characteristics allow endothelial proteins and HSGAGs to discriminate between these residues.

A specific focus of this work was to characterize the endothelial receptor for inflammatory cationic peptides with endothelial HSGAGs as likely candidates. Endothelial cells express three HSGAGs, including perlecain, syndecan, and glypican. Of the three, only the syndecans are known to influence cytoskeletal organization, cell-cell adhesion, and motility (42). Given that these processes are associated with changes in vascular permeability (7), syndecans were the most likely candidate molecule in our search for a cationic peptide receptor. To establish that syndecans participate in cationic peptide-induced signaling, we examined syndecan localization after stimulation with PA and found PA induced 1) actin stress fiber formation, 2) syndecan-1 to cluster around the cell periphery, and 3) syndecan-4 to distribute in a centralized linear pattern, suggestive of localization with actin or microfilaments and/or microtubules. Because antibody-mediated cross-linking of syndecan has been associated with syndecan clustering and subsequent stress fiber formation (38), we hypothesize that cationic peptides cross-link heparan sulfates to promote similar changes in
syndecan localization and actin organization. Syndecan-1 clustering precedes and likely mediates actin reorganization in response to PA as removal of heparan sulfates by heparinase III completely abolished both syndecan clustering and actin stress fiber formation while reducing the barrier disruption of PA by 50%. This suggests that other cell surface domains may be involved and/or other signaling pathways are activated that affect permeability independent of actin reorganization. Removal of chondroitin sulfate-like GAGs with chondroitinase did not affect PA-mediated permeability changes.

We offer three possible explanations regarding the complex relationship between the enzyme activity data (Table 1), the compositional analysis (Table 2), and the effects of enzyme pretreatment (Fig. 3) on PA-mediated barrier disruption. Briefly, heparinase III released 67% of labeled heparan sulfates and reduced PA-induced barrier dysfunction by 50%. Thus there is a close correlation between the amount of heparan sulfate released and the attenuation of the effects of PA on TER. Heparinase I, however, released 40% of labeled heparan sulfates but had no effect on attenuating PA-induced barrier dysfunction. The simplest explanation for this observation is that the heparan sulfates released by heparinase I were not carried on syndecans but, perhaps, carried by glypicans or perlecans and, therefore, not involved in barrier regulation. An alternative explanation is that the additional 27% of the heparan sulfates released by heparinase III (compared with heparinase I) surpassed a crucial cell surface GAG content that is required to participate in cross-linking or activation of intracellular signals that are associated with barrier regulation. Last, heparan sulfates can have similar global composition but possess markedly different focal sequences, referred to as “fine structure”. These differences in heparan sulfate focal sequence have been shown to differentially alter endothelial responses to fibroblast growth factor-mediated signaling (2). Thus the heparan sulfates removed by heparinase I may have had focal sequences that did not participate in barrier regulation.

HSGAG-mediated signaling has been well documented as numerous growth factors, including the fibroblast growth factor family and vascular endothelial growth factor-165 (4, 14) as well as matrix elements (5, 18), require HSGAGs as coligands for maximal receptor activation. Langford and colleagues (21) also demonstrated that both the number and position of heparan sulfates on the syndecan core protein can influence heparan-mediated signaling. Last, the fine structure of the heparan sulfates also influences heparan-mediated signaling and cell behavior (37). Thus through undetermined mechanisms, the heparan sulfates can modulate syndecan-induced signal activation and subsequent cell function.

Our data suggest that PA-induced cross-linking of syndecan heparan sulfates activate signaling pathways associated with syndecan clustering and actin cytoskeletal reorganization. The mechanism(s) responsible for clustering remains largely unknown. Polyca-
4. Capilia I and Linhardt RJ. Heparin-protein interactions. Ag-
5. Couchman JR and Woods A. Syndecan-4 and integrins: com-
binatorial signaling in cell adhesion. J Cell Sci 112: 3415–3420,
2000.
7. Dukek SM and Garcia JGN. Cytoskeletal regulation of pul-
mmonary vascular permeability. J Appl Physiol 91: 1457–1500,
8. Garcia JGN, Davis HW, and Patterson CE. Regulation of endothelial gap formation and barrier dysfunction. Role of myo-
1995.
9. Garcia JGN, Lazar V, Gilbert-McClain LI, Gallagher PJ, and Verin AD. Myosin light chain kinase in endothelium: mo-
lecular cloning and regulation. Am J Respir Cell Mol Biol 16:
T, Liu F, Birukova A, Jacobs K, Bogatcheva N, and Verin AD. Critical involvement of p38 MAP kinase in pertussis toxin-
12. Gautum N, Olofsson AM, Herwald H, Iverson LF, Lund-
gern-Akerlund E, Hedqvist P, Arfors KE, Flodgaard H, and Lindbom L. Heparin-binding protein (HPBP/CAP37): a missing link in neutral-phil-evoked alteration of endothelial per-
13. Giaever I and Keese CR. A morphological biosensor for mam-
16. Humphries DE, Lee SL, Fanburg BL, and Silbert JE. Ef-
fects of hypoxia and hyperoxia on proteoglycan production by bovine pulmonary artery endothelial cells. J Cell Physiol 126:
erson RD, Brakebusch C, Fassler R, and Weer UM. The cysteine-rich domain of human ADAM-12 supports cell adhesion through syndecans and triggers signaling events that lead to β1-integrin-dependent cell spreading. J Cell Biol 1495:
19. Kaslovsky RA, Horgan MJ, Lum H, McCandless BK, Gil-
boa N, Wright D, and Malik AB. Pulmonary edema induced by phagocytosing neutrophils. Protective effect of monoclonal anti-
21. Langford KJ, Stanely MJ, Con D, and Sanderson RD. Multiple heparan sulfate chains are required for optimal syn-
22. Leh HA, Bittinger F, and Kirkpatrick CJ. Microradial dis-
fusion in sepsis: a pathogenetic basis for therapy. J Pathol 190:
23. Ley K. Pathways and bottlenecks in the web of inflammatory adhesion, molecules and chemotactants. Immunol Rev 24:
24. Liu Y, Stack SM, Lakka SS, Khan AD, Woodley DT, Rao JS, and Rao CN. Matrix localization of tissue factor pathway inhib-
itor-2/matricellular serine protease inhibitor (TFPI-2/ 
MSPi) involves arginine-mediated ionic interactions with hepa-
118, 1999.
30. Needham L, Hellewell PG, Williams TJ, and Gordon JL. Endothelial functional responses and increased vascular perme-
W, and Lundgren-Akerlund L. Heparin-binding protein tar-
33. Pereira HA, Erdem I, Pohl J, and Spitznagel JK. Synthetic bactericidal peptide based on CAP37: a 37-kDa human neutro-
phil granule-associated cationic antimicrobial protein chemotac-
34. Pereira HA, Shafer WM, Pohl J, Martin LE, and Spitz-
38. Saoum S, Echtermeyer F, Denaz F, Nowken JL, Mosher DF, Robinson SD, Hynes R, and Goetinck PF. Syndecan-4 signals cooperatively with integrins in a Rho-depen-
40. Vepa S, Scribner WM, and Natarajan V. Activation of endoth-
41. Verin AD, Birukov A, Wang P, Lui F, Becker P, Birukov K, and Garcia JGN. Microtubule disassembly increases endo-
42. Zimmermann P and David G. The syndecans, tuners of trans-