Endothelial Cell Membranes Contain Podocalyxin—the Major Sialoprotein of Visceral Glomerular Epithelial Cells

Reinhard Horvat, Anny Hovorka, Gerhard Dekan, Helga Poczewski, and Dentscho Kerjaschki
Institute of Pathological Anatomy, University of Vienna, School of Medicine, Vienna, Austria

Abstract. Podocalyxin is the major sialoprotein in the glycocalyx of glomerular podocytes. Here we report on its extraglomerular localization, using a monospecific antibody which was obtained by affinity purification of IgG on nitrocellulose transfers of glomerular podocalyxin. By indirect immunofluorescence, podocalyxin was found in the blood vessels of several organs (lung, heart, kidney, small intestine, brain, pancreas, aorta, the periportal blood vessels in liver, and the central arteries of follicles of the spleen, but not in the endothelia that line the sinusoids of the latter organs). By immunoelectron microscopy—using immunogold conjugates in diffusion (“pre-embedding”) and surface (“postembedding”) procedures—podocalyxin was localized on the luminal membrane domain of endothelial cells, in a patchy distribution. The presence of podocalyxin was confirmed in SDS extracts of lung tissue by immunoblotting.

We conclude that (a) podocalyxin is a widespread component of endothelial plasma membranes, (b) it is restricted to the luminal membrane domain, and (c) it is distributed unevenly on the endothelial cell surface.

Materials and Methods

Animals
Male Sprague-Dawley rats (150–200 g) were obtained from the Tierzuchtinstitut der Universität Wien.

Materials
SDS, acrylamide, bis-acrylamide, diethylenetriol, nitrocellulose membranes, and high molecular mass standards were from Bio-Rad Laboratories (Richmond, CA). Wheat germ agglutinin (WGA) and Aquacide II were from Calbiochem-Behring (San Diego, CA). ~125I-Protein A was from Amersham Corp. (Arlington Heights, IL). Fluorescein isothiocyanate (FITC)-conjugated goat-anti rabbit IgG was from Behring Corp. (Marburg, Germany). Goat anti-rabbit IgG-10-nm gold conjugate was from Jannsen Pharmaceutical (Beerse, Belgium). The acrylic resin LR White was purchased from the London Resin Company (Basingstoke, UK). Nonidet P-40, dimethyl sulfoxide, and Ponceau S were from Sigma Chemical Co. (St. Louis, MO). Protein A–Sepharose 4B was from Pharmacia Fine Chemicals (Uppsala, Sweden).

Preparation and Characterization of Anti-Podocalyxin IgG
Rabbit IgG against glomerular podocalyxin was prepared as described previously (9). A rabbit was immunized with the WGA-binding fraction of a Triton X-100 glomerular lysate. The resulting antibodies were found to be specific for podocalyxin by immunoprecipitation and by immunostaining (9).

Affinity Purification of Anti-Podocalyxin IgG
The IgG fraction of rabbit serum was isolated with Protein A–Sepharose 4B and was further purified to obtain monospecific anti-podocalyxin IgG. This was achieved by adsorption to podocalyxin which had been electrophoretically transferred onto nitrocellulose paper, according to the method of Talian et al. (20). Briefly, a nitrocellulose transfer of glomerular proteins which had been separated by 5–10% gradient SDS PAGE was stained with 0.5% Ponceau S in 5% trichloroacetic acid. The position of the podocalyxin band was determined by immunoblotting.
by overlaying of nitrocellulose strips with crude anti-podocalyxin IgG and 125I-protein A (9). Subsequently, the region of the transfer which contained podocalyxin was excised. This strip was then quenched in 10% fetal calf serum and 1% bovine serum albumin in phosphate-buffered saline (PBS) for 1 h at 20°C, followed by incubation in 10 ml PBS containing 5 μg rabbit IgG and 1% bovine serum albumin for 12 h at 4°C. After washing, the bound IgG was eluted in 5 ml of 20 mM glycine–HCl buffer, pH 2.3, for 2–3 min at 4°C. The eluate was neutralized with 1 M NaOH, and diluted to 15 ml with PBS, followed by dialysis against PBS (12 h, 4°C, 5 liters) and concentration with Aquacide II to 2 ml.

Transfer of Lung and Glomerular Extracts

Rat lungs were flushed blood-free by perfusion with PBS (25 ml at 4°C) via the right ventricle. They were minced with a razor blade, and homogenized with 3–5 ml 2x SDS sample buffer (containing 7.2% SDS, 36 mM dithiothreitol, 9 mM EDTA, 100 mM Tris-HCl, pH 6.8, 20% glycerol) with 10 strokes in a Potter-Elvehjem homogenizer, followed by boiling for 2 min. The homogenate was then centrifuged 5 min in a Microfuge (10,000 g).

The proteins of the lung extracts and of kidney glomeruli were separated on 5–10% gradient SDS gels and transferred electrophoretically onto nitrocellulose membranes, as described previously (8). The transfers were stained with 0.5% Ponceau S in 5% trichloroacetic acid, destained in 10% acetic acid, and photographed.

Overlays with 125I-WGA and Anti-Podocalyxin IgG

The nitrocellulose transfers were cut into strips, each containing the protein patterns of lung, glomerular extract, and of molecular mass standards. These strips were quenched in 1% hemoglobin in PBS at 20°C for 1 h, and incubated with 1–3 × 106 cpm of 125I-WGA (which was labeled by the chloramine T method) as described previously (9), washed three times in PBS with 0.04% NP-40, and exposed for autoradiography on Kodak Xomat-L film with a Cronex intensifying screen at ~70°C for 6–12 h.

The immunoreactivity with affinity-purified anti-podocalyxin IgG was performed as described previously for crude IgG (8). Briefly, the nitrocellulose strips were quenched in 1% hemoglobin in PBS as described above. Incubation in IgG (10 μg/ml) in PBS with 0.1% hemoglobin was performed for 12 h at 4°C, followed by three 10-min washes in PBS with 0.04% NP-40. Subsequently the strips were exposed for autoradiography as described above.

Immunofluorescence

Some major organs of rats were flushed blood-free by perfusion with 50 ml PBS via the left ventricle after opening of the right atrium. Small blocks of several organs (kidney, heart, small intestine, pancreas, liver, spleen) were excised. The lung was slightly inflated by a balloon connected to the trachea after thoracotomy, and it was perfused with PBS via the right ventricle after opening of the left auricle. The samples were frozen in 2% cooled isopentane, and 2-μm cryostat sections were prepared. These sections were fixed with 1% paraformaldehyde-lysine-periodate mixture (11) for 5 min. The sections were then washed three times for 10 min in PBS with 0.04% NP-40, and exposed for autoradiography on Kodak Xomat-L film with a Cronex intensifying screen at ~70°C for 6–12 h. The sections were then washed three times for 10 min in PBS with 0.04% NP-40, and incubated in goat anti-rabbit IgG-gold conjugate (10 nm, diluted 1:25), followed by washing and incubation in veronal acetate–buffered osmium and 0.2% aqueous uranyl acetate. Ultrathin sections were stained with aqueous uranyl acetate and lead citrate, and examined in a Zeiss EM9 electron microscope.

In control experiments the first antibody was omitted, or replaced by another IgG of defined specificity other than podocalyxin.

Immunogold Staining by the Surface (“Postembedding”) Method

We have developed an embedding procedure in LR White specifically for immunoelectron microscopy with gold conjugates. The embedding protocol was based on the previous procedures of Newman et al. (12) for immunoperoxidase, and Ellinger and Pavelka (4) for lectin binding. All steps were carried out on ice. Small fixed tissue samples were first incubated in 10 mM ammonium chloride in PBS for 20 min, washed three times for 10 min in PBS, and dehydrated for 30 min in 70%, 80%, and twice for 20 min in 90%, ethanol. Then the specimens were incubated for 1 h in 90% ethanol which was saturated with LR White, followed by two 6-h incubations in pure resin. For polymerization, one drop of accelerator was added to 10 ml of ice-cold LR White, and aliquots were distributed into dry, cold gelatine capsules to which the tissue blocks were added. Polymerization took place within 30–60 min on ice. Ultrathin sections were collected on Formvar-carbon coated nickel grids.

The sections were quenched in PBS containing 2% swine serum (PBS-SS) for 30 min, followed by incubation in anti-podocalyxin IgG (25 μg/ml) in PBS-SS for 1–12 h at 20°C. After five 10-min washes in PBS-SS, the grids were incubated for 60 min in goat anti-rabbit gold conjugate (10 nm, diluted 1:25), washed three times for 10 min in PBS-SS and once for 10 min in PBS, followed by incubation in 2.5% glutaraldehyde in 100 mM cacodylate buffer, pH 7.2. After three 10-min washes in H2O, the sections were placed on drops of aqueous 2% osmium tetroxide for 30 min, washed in water, and stained in aqueous 2% uranyl acetate (15 min), and alkaline lead citrate (5 min).

The same controls were used as listed above for the diffusion protocol.

Quantitation of the Distribution of Podocalyxin

For quantitative assessment of the distribution of gold particles in relation to endothelial vesicles and diaphragms, micrographs (×45,000) from nonfenes-
Figures 2-5. Localization of podocalyxin by indirect immunofluorescence in various organs, using affinity-purified anti-podocalyxin IgG as first antibody. Podocalyxin is present in the kidney (Fig. 2) not only in the glomeruli, but also in the interstitial capillaries. It is further found in the blood vessels of the pancreas (Fig. 3), in the heart (Fig. 4), and in the alveolar capillaries of the lung (Fig. 5). Bar, 10 μm.

Results

Characterization of Podocalyxin and Anti-Podocalyxin IgG

Podocalyxin was shown previously (9) to stain in SDS gels with silver salts and with “Stains All,” indicating that it is negatively charged. In addition, it was found to bind WGA (Fig. 1, lane C), and this affinity was abolished by digestion with neuraminidase.

The specificity of a rabbit IgG—which was raised against a WGA-binding fraction of glomerular molecules—for podocalyxin was demonstrated previously by immunoprecipitation and immuneoverlaying (9). The antibody is presumably directed against the protein core of the molecule and/or some proximal components of the N-linked carbohydrate chains, because digestion with Endoglycosidase F and neuraminidase did not interfere with its binding (10).

The IgG used in this study was further purified by adsorption to glomerular podocalyxin which was immobilized onto nitrocellulose (20). When this affinity-purified IgG and 125I-protein A were used for immuneoverlaying on nitrocellulose transfers of glomerular proteins, only the 140-kD band—which is characteristic for podocalyxin—was detected, even after long times of exposure for autoradiography (Fig. 1, lane D).
Figures 6 and 7. Localization of podocalyxin by indirect immunofluorescence in liver (Fig. 6) and in spleen (Fig. 7). The molecule is present only in the major blood vessels in the periportal area of the liver (bc indicates a bile duct), and in the central artery (ca) of a spleen follicle. It is not detectable in the lining cells of the sinusoids in either organ. Bar, 10 μm.

Biochemical Detection of Podocalyxin in Lung

When SDS extracts of lung tissue—which is particularly rich in blood vessels—were analyzed by SDS PAGE, numerous bands were observed, indicating a wide spectrum of proteins of different molecular masses (Fig. 1, lane E). In overlays with $^{125}$I-WGA, several bands bound the lectin, including one with a molecular mass of 140 kD i.e., the same electrophoretic mobility as glomerular podocalyxin (Fig. 1, lane F).

In immuneoverlays of lung extracts with affinity-purified anti-podocalyxin IgG, a single band with an apparent molecular mass of 140 kD was observed (Fig. 1, lane G), i.e., the same mobility in SDS PAGE as podocalyxin from glomerular lysates. When extracts of several other organs were used, the signal obtained by immuneoverlaying was very weak, probably due to the relatively low amount of endothelial membrane proteins present.

Immunofluorescence

In cryostat sections of kidney (Fig. 2), pancreas (Fig. 3), heart (Fig. 4), lung (Fig. 5), and a small intestine (not shown), the blood vessels of all calibers were strongly labeled by the affinity-purified anti-podocalyxin IgG. By contrast, in liver only the branches of the hepatic artery and the portal vein in the periportal area were stained (Fig. 6). In the spleen, the staining was restricted to the major segmental blood vessels, and to the central arteries of the follicles (Fig. 7). The cells lining the sinusoids of liver and spleen were consistently negative. When the first antibody was omitted or when another IgG of different specificity was used, no fluorescence was observed in the vasculature.

Immunogold Methods for Electron Microscopical Localization of Podocalyxin

Podocalyxin was localized by immunogold techniques with affinity-purified anti-podocalyxin IgG in lung (Figs. 8-11), pancreas (Figs. 12-14), kidney cortex (Figs. 15-18), and heart, small intestine, and aorta (not shown).

A diffusion (pre-embedding) procedure was used because of its relatively high sensitivity. For confirmation of the results obtained by this protocol, surface (postembedding) immunostaining was performed, because in this method antigenic sites are exposed which could be inaccessible for the immunocytochemical reagents in diffusion procedures.

Both methods revealed the localization of podocalyxin on the luminal domain of the endothelial plasmalemma in a discontinuous, patchy distribution in all vessels which were examined. The number of gold particles per cluster was, however, smaller in the surface technique, probably due to its relatively low sensitivity (Figs. 15-18). Similar results were obtained when protein A-gold was used instead of IgG-gold conjugates (not shown). All controls failed to show vascular labeling.

Nonfenestrated Capillary Endothelia

In the capillaries of the lung (Figs. 8-11) and the heart (not shown), podocalyxin was found in a patchy distribution on the luminal plasmalemma. In most cases the diaphragms of the plasmalemmal vesicles were not decorated (Figs. 9 and 11). Many coated pits on the luminal plasmalemma were associated with podocalyxin (Figs. 10 and 11), but smooth endothelial vesicles were usually devoid of gold particles (Figs.
Figures 8–11. Localization of podocalyxin in the capillaries of the lung by the immunogold technique in a diffusion (pre-embedding) protocol, using affinity-purified anti-podocalyxin IgG as the first antibody. At low magnification (Fig. 8), the gold particles are seen to be distributed unevenly in small patches. At higher magnification (Figs. 9–11), the patches of gold particles appear on the luminal plasmalemma in association with coated pits (cp) in Figs. 9 and 11. The label is absent from most endothelial vesicles and their diaphragms (arrows in Figs. 9 and 10). The basement membrane is marked bm, and the type I alveolar epithelial cells, el. Bars, 1 μm (Fig. 8) and 0.1 μm (Figs. 9–11).

Quantitative evaluation of 619 clusters of gold particles in relation to endothelial vesicles indicates that 86% were outside and 14% were inside a radius of 100 nm around the infundibula of vesicles. These data are accumulated from evaluation of lung and heart capillary endothelia which show similar distribution.

Fenestrated Capillary Endothelia

In the capillaries of the pancreas (Figs. 12–14), the peritubular capillaries of the kidney cortex (Figs. 15–17), and in the small intestine (not shown), podocalyxin was detected in patches on the luminal plasmalemma in both immunogold methods which were used in this study. Quantitative assessment of the distribution of gold clusters on capillary endothelia of kidney cortex and pancreas indicated that 91% of the clusters (out of a total of 320) were located outside, and 9% within 100 nm of the diaphragms.

Podocalyxin was localized by the surface method in kidney glomeruli to avoid problems which were previously encountered in diffusion procedures by the limited accessibility of antigenic molecules close to the glomerular basement membrane (9). On ultrathin sections of LR White embedded kidney glomeruli, podocalyxin was found restricted to the luminal surface of the fenestrated endothelial cells (Fig. 19).

Podocalyxin in the Endothelium of Arterial and Arteriolar Vessels

Podocalyxin was also found in blood vessels other than capillaries. For example, in the endothelium of all branches of the coronary artery (Fig. 4), in the arterioles of the kidney (Fig. 18), and in the abdominal aorta (not shown), podocalyxin was found in a patchy distribution similar to that in capillaries. A systematic study comparing the concentrations of podocalyxin in different segments of the vasculature is in progress.

Discussion

Endothelial cells are endowed with a polyanionic surface coat (2, 3, 18, 19). The electronegative charges are carried in part by proteoglycan molecules, of which a heparan sulfate-containing species was recently identified (1, 16). There is also evidence for the presence of sialoproteins (2, 3, 16), which were, however, not yet defined by biochemical methods. Here we report that podocalyxin—the major glomerular sialoprotein (9)—is a widespread component of endothelial cells in several organs of rats. Moreover, podocalyxin is concentrated at the luminal domain of the endothelial plasmalemma, and it is distributed there unevenly in a patchy pattern.

Podocalyxin was initially localized in the vasculature of several organs by immunocytochemistry. Therefore, it was necessary to confirm its presence separately by biochemical techniques. We have immuneoverlayed nitrocellulose transfers of SDS extracts of lung tissue, which is particularly rich in endothelial cells, with affinity-purified anti-podocalyxin IgG, and we have detected a molecule with the same electrophoretic mobility and with identical antibody- and WGA-binding characteristics as podocalyxin from kidney glomeruli.
Figures 12–14. Localization of podocalyxin in capillaries of the pancreas by the diffusion (pre-embedding) technique. In the survey view (Fig. 12), a patchy distribution of gold grains is seen, similar to that in the pulmonary endothelia. One fenestrum is devoid of label (f), whereas another is covered by gold particles (f'). Few gold particles (indicated by circles) are found in this preparation on the outside of the endothelial cells probably due to unspecific sticking of the gold conjugate to structures of the basement membrane. Figs. 13 and 14 are higher magnifications of a capillary wall in which several podocalyxin-containing areas of the plasmalemma are seen, but the fenestral diaphragms (f) are free of label. Note that the gold particles are separated by a 50-nm interspace from the cell membranes (Figs. 13 and 14), probably due to a layer of intercalated IgG molecules and probably also by a part of the podocalyxin molecules. Bars, 1 μm (Fig. 12) and 0.1 μm (Figs. 13 and 14).

Based on the similar properties of lung (vascular) and glomerular (predominantly podocytic) podocalyxin, it is likely that the molecules from both sources are identical, or at least very similar. It is reasonable to assume, therefore, that the antibody detects authentic podocalyxin also in the endothelia of other, less vascularized organs, in which the fraction of endothelial membrane proteins in SDS extracts is probably below the sensitivity of the immuneoverlay technique.

Glomerular podocalyxin is relatively rich in sialic acid. Direct chemical analysis has shown that it contains 15–20 sialic acid residues per molecule (10). Therefore, it could contribute negative charges to the polyanionic surface coat of endothelia.

Because podocalyxin has a high affinity to WGA in nitrocellulose transfers, it is likely to be a component of the WGA binding sites in endothelium, which were recently discovered by a combination of lectin-histochemistry and digestion with neuraminidase (13, 17). Currently it is not known if podocalyxin is the only endothelial sialoprotein, or a member of a larger family.

By indirect immunofluorescence with affinity-purified IgG, podocalyxin was localized exclusively to blood vessels of all calibers in lung, heart, small intestine, and pancreas. By contrast, it was absent from the endothelia of the sinusoidal capillaries of liver and spleen. The significance of this differential distribution is not known. By contrast, another protein which was recently discovered on glomerular podocytes and on endothelia ("podoendin" [7]), was also localized on the endothelia of liver sinusoids.

The precise localization of podocalyxin in the vascular wall
Figures 15–18. Localization of podocalyxin on the endothelium of several peritubular capillaries of kidney cortex (Figs. 15–17) and of an afferent arteriole (Fig. 18) by surface (postembedding) immunolabeling of acrylic resin LR White-embedded tissue. Gold particles are found in all figures in patches on the luminal plasma membranes of the endothelial cells (brackets), in a similar distribution as seen by the diffusion (pre-embedding) method. Very few gold particles (indicated by circles) are localized on the abluminal aspect of the endothelial cells, and on adjacent nonvascular cells. In Fig. 17 several fenestral diaphragms (f) of the endothelium of an interstitial capillary are visible, of which only one is associated with gold particles (f'). BM, basement membrane. Bars, 1 μm (Fig. 15), and 0.1 μm (Figs. 16–18).

was established by immunoelectron microscopy. Immunogold staining with affinity-purified IgG of nonfenestrated capillaries in lung, heart, and kidney, and fenestrated capillaries in pancreas and small intestine, indicates that podocalyxin is present selectively in the luminal domain of the endothelial plasmalemma and is absent from the abluminal aspect. This
of the endothelial plasmalemma remains to be determined.

The functions of endothelial sialoproteins are not known in detail. Several investigations indicate that they are involved in important biological processes, such as the sticking of platelets (5) and granulocytes (6). With the definition of podocalyxin as an endothelial sialoprotein and the availability of specific antibodies it will be possible to clarify its role in normal and in pathological conditions.

The authors are indebted to Drs. M. G. Farquhar and G. E. Palade for helpful discussion and critical reading of the manuscript.

This work was supported by Research Grant P5900 from the Fonds zur Förderung der Wissenschaftlichen Forschung.

Received for publication 16 July 1985, and in revised form 27 August 1985.

References

1. Buonassisi, V. 1973. Sulfated mucopolysaccharide synthesis and secretion in endothelial cultures. Exp. Cell Res. 76:563-570.

2. DeBruyne, P. P. H., and S. Michelson. 1979. Changes in the random distribution of sialic acid at the surface of the myocardial sinusoidal endothelium resulting from the presence of diaphragmated fenestrate. J. Cell Biol. 82:708-714.

3. DeBruyne, P. P. H., S. Michelson, and R. P. Becker. 1978. Nonrandom distribution of sialic acid over the cell surface of bristle coated endocytic vesicles of the sinusoidal endothelium cells. J. Cell Biol. 78:379-389.

4. Ellinger, A., and M. Pavelka. 1985. Postembedding localization of glycoconjugates by means of lectins on thin sections of "LR White" embedded tissue. Histochem. J. in press.

5. Görög, P., I. Schraufstatter, and G. V. R. Born. 1982. Effect of removing sialic acids from endothelium on the adherence of circulating platelets in arteries in vivo. Proc. R. Soc. Lond. B Biol. Sci. 214:471-480.

6. Hoover, R. L., R. T. Briggs, and M. J. Karmovsky. 1978. The adhesive interaction between polymorphonuclear leukocytes and endothelial cells in vivo. Cell. 14:423-428.

7. Huang, T. W., and J. C. Langlois. 1985. Podoenin: a new cell surface protein of the podocyte and the endothelium. J. Exp. Med. 162:245-267.

8. Kerjaschki, D., L. Noroinha-Blub, B. Sacktor, and M. G. Farquhar. 1984. Microdomains of distinctive glycoprotein composition in the kidney proximal tubule brush border. J. Cell Biol. 98:1505-1513.

9. Kerjaschki, D., D. J. Sharkey, and M. G. Farquhar. 1984. Identification and characterization of podocalyxin—the major sialoglycoprotein of the renal glomerular epithelial cell. J. Cell Biol. 98:1591-1596.

10. Kerjaschki, D., A. T. Vernillo, and M. G. Farquhar. 1985. Reduced sialylation of podocalyxin—the major sialoglycoprotein of the rat kidney glomerulus—in aminoaculade nephrosis. Am. J. Pathol. 118:343-349.

11. McClanahan, W., and P. F. Nakane. 1974. Periodate-lysine-paraformaldehyde fixative. A new fixative for immuno-elecron microscopy, J. Histochem. Cytochem. 22:1079-1083.

12. Newman, G. R., B. Jasani, and E. D. Williams. 1983. A simple postembedding system for the rapid demonstration of tissue antigens under the electron microscope. Histochem. J. 15:543-555.

13. Simionescu, M., N. Simionescu, and G. E. Palade. 1982. Differentiated microdomains on the luminal surface of capillary endothelium: distribution of lectin receptors. J. Cell Biol. 94:406-413.

14. Simionescu, M., N. Simionescu, and G. E. Palade. 1982. Preferential distribution of anionic sites on the basement membrane and the abluminal aspect of the endothelium in fenestrated capillaries. J. Cell Biol. 95:425-434.

15. Simionescu, M., N. Simionescu, F. Santoro, and G. E. Palade. 1985. Differentiated microdomains of the luminal plasmalemma of muscle capillaries: segmental variations in young and old animals. J. Cell Biol. 100:1396-1470.

16. Simionescu, M., N. Simionescu, E. D. Silbert, and G. E. Palade. 1981. Differentiated microdomains on the luminal surface of the capillary endothelium. II. Partial characterization of their anionic sites. J. Cell Biol. 90:614-621.

17. Simionescu, N. 1979. The microvascular endothelium: segmental differ-entiation, transcytosis, selective distribution of anionic sites. In Advances in Inflammation Research. G. Weissmann, B. Samuelson, and R. Paoletti, editors. Raven Press, New York. 64-70.

18. Simionescu, M., N. Simionescu, and G. E. Palade. 1981. Differentiated microdomains of the luminal surface of the capillary endothelium. I. Preferential distribution of anionic sites. J. Cell Biol. 90:605-613.

19. Skutelsky, E., Z. Rudich, and D. D. Anon. 1975. Surface charge prop-erties of the luminal front of blood vessel walls: an electron microscopic analysis. Thromb. Res. 7:633-632.

20. Talian, J. C., B. Olmstead, and R. D. Goldman. 1983. A rapid procedure for preparing fluorescein-labeled specific antibodies from whole antiscum: its use in analyzing cytoskeletal architecture. J. Cell Biol. 97:1277-1282.