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SPARC (secreted protein acidic and rich in cysteine) regulates endothelial cell shape and barrier function

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ABSTRACT **SPARC (secreted protein acidic and rich in cysteine) can be selectively expressed by the endothelium in response to certain types of injury and induces rounding in adherent endothelial cells *in vitro*. To determine whether SPARC might influence endothelial permeability, we studied the effect of exogenous SPARC on the movement of ^{14}C-labeled bovine serum albumin across postconfluent bovine pulmonary artery endothelial cells. SPARC increased ($P < 0.02$) transendothelial albumin flux in a dose-dependent manner at concentrations ≥ 0.5 μg/ml. At a fixed dose (15 μg/ml), exposure times ≥ 1 h augmented ($P < 0.005$) albumin flux by 1.3- to 3.6-fold; this increase was blocked by anti-SPARC antibodies but not by inhibition of protein synthesis. Barrier dysfunction was not associated with loss of cell viability. Monolayers exposed to SPARC exhibited a rounded morphology and intercellular gaps. Prior stabilization of F-actin with phallicidin protected against the changes in barrier function ($P = 0.0001$) that were otherwise induced by SPARC. Bovine aortic and retinal microvascular endothelia also responded to SPARC. We propose that SPARC regulates endothelial barrier function through F-actin-dependent changes in cell shape, coincident with the appearance of intercellular gaps, that provide a paracellular pathway for extravasation of macromolecules.**

The vascular endothelium presents a selective barrier that actively regulates movement of circulating macromolecules and cells into extravascular tissues and compartments (1, 2). Although mechanisms regulating this endothelial barrier are not well understood, a structure–function relationship appears to exist between endothelial cell (EC) shape and barrier function. Specific agonists can induce changes in EC shape coincident with the formation of intercellular gaps, which in turn provide a paracellular pathway for the flux of macromolecules (3–5). Actin organization is postulated to regulate EC shape as well as barrier function (5–12). Actin filament-disrupting agents increase endothelial permeability (6), prior stabilization of actin protects against increases in permeability (7–9), and mediators of permeability have been shown to induce cytoskeletal rearrangement (8–12).

SPARC (a secreted protein acidic and rich in cysteine), known also as osteonectin and BM-40 (13), is secreted constitutively by EC (14, 15) and binds transiently to specific components of the extracellular matrix (ECM) (15). Increased expression of SPARC can be induced by sparse plating density and by exposure to endotoxin (15), itself an established mediator of EC contraction, intercellular gap formation, and increased permeability (3). Exogenous SPARC induces a dose-dependent rounding of confluent EC, with partial detachment in the absence of cell injury (16, 17). In the present report, we offer evidence for an unusual mechanism for the regulation of endothelial barrier function. We have demonstrated that SPARC induces changes in EC shape, intercellular gaps, and dose- and time-dependent increments in the transendothelial flux of ^{14}C-labeled bovine serum albumin (^{14}C-BSA).

METHODS

Preparation of SPARC. Murine SPARC was purified from the conditioned medium of mouse PYS-2 cells (18). The preparations of SPARC pooled for these studies contained 0.005–0.02 ng of endotoxin/μg of protein as determined by the *Limulus* amoebocyte lysate assay (19).

EC Culture. Bovine pulmonary artery EC obtained from the American Tissue Culture Collection were grown in Dulbecco's modified Eagle's medium (Sigma) containing 20% (vol/vol) fetal bovine serum (FBS) (HyClone), 4 mM L-glutamine, nonessential amino acids, vitamins, penicillin (50 units/ml), and streptomycin (50 μg/ml) (4, 9). The cells were gently detached by brief exposure to trypsin at 0.5 mg/ml, counted, and seeded into assay chambers (2×10^5 cells per cm^2) or 24-well and 6-well tissue culture plates (5×10^4 cells per cm^2). Bovine aortic and retinal EC (passages 3–12), provided by J. Laterra (Kennedy Krieger Institute, The Johns Hopkins Medical Institutions, Baltimore), were cultured as described (20).

Assay of Albumin Flux. EC were grown to confluence in 0.5 ml of medium on gelatin-impregnated polycarbonate filters (13-mm diameter, 0.4-μm pore size; Nucleopore) mounted in chemotactic chambers (ADAPS, Dedham, MA) as described (4). These chambers, which served as the upper compartment for the assay chambers, were inserted into wells of 24-well plates, each well containing 1.5 ml of medium and serving as the lower compartment of the assay chamber. ^{14}C-BSA (Sigma; 30.1 μCi/mg of protein; 1 μCi = 37 kBq), the tracer molecule, was prepared in serum-free medium supplemented with BSA (34 mg/ml) to produce a final protein concentration equivalent to medium enriched with 10% FBS. The baseline barrier function of each monolayer was established by application of an equivalent amount of ^{14}C-BSA (1.1 pmol/0.5 ml) to each upper compartment for 1 h at 37°C, after which 0.5 ml from the lower compartment was added to 4.5 ml of Optifluor scintillation fluid (Packard) and assayed for radioactivity. Only monolayers retaining ≥95% of the tracer were studied. Trypan blue exclusion and lactate dehydrogenase (LDH) release were also determined (9).

Microscopy. EC cultured on polycarbonate filters were washed, fixed with 4% formaldehyde/1% glutaraldehyde in 0.1 M sodium cacodylate buffer brought to pH 7.25 with HCl, postfixed with 2% OsO_4 in *S*-collidine buffer, and dehydrated

Abbreviations: SPARC, secreted protein acidic and rich in cysteine; EC, endothelial cell(s); ECM, extracellular matrix; BSA, bovine serum albumin; FBS, fetal bovine serum; LDH, lactate dehydrogenase; PMSF, phenylmethylsulfonyl fluoride; NBD, 7-nitrobenz-2-oxa-1,3-diazole; LPS, lipopolysaccharide.

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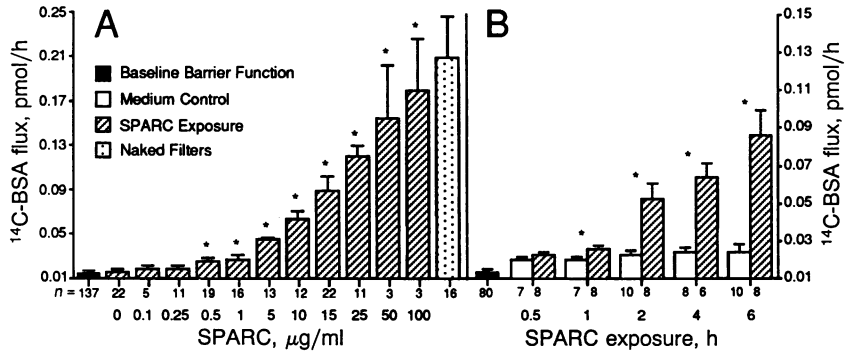

FIG. 1. Effects of SPARC on transendothelial flux of ^{14}C-BSA. Vertical bars represent mean (± SEM) transendothelial flux of ^{14}C-BSA in pmol/h. Baseline ^{14}C-BSA flux is shown by a closed bar in *A* and *B*. *n* indicates the number of monolayers studied, and each star indicates a significant increase compared with the simultaneous medium control ($P < 0.02$). (*A*) ^{14}C-BSA flux immediately after 6-h exposures to increasing concentrations of SPARC in the absence of FBS (crosshatched bars) and across naked filters (stippled bar). (*B*) ^{14}C-BSA flux immediately after increasing exposure times to SPARC at 15 μg/ml (crosshatched bars) and simultaneous controls (open bars) in the absence of FBS.

through graded ethanol solutions. Specimens were dried at the critical point, coated with gold, and viewed under an AMR 1000 scanning electron microscope. Alternatively, monolayers cultured on filters were fixed, rendered permeable with 0.5% Triton X-100 for 5 min, stained with fluorescein-phalloidin (Molecular Probes), and photographed through a Zeiss Axioskop 20 Microscope equipped for epifluorescence (9).

Quantitation of Total Protein and F- and G-actin. Postconfluent EC were detached with trypsin and counted in triplicate. Cells were centrifuged, washed, and lysed in 3% sodium dodecyl sulfate/1 mM dithiothreitol/10 μM phenylmethylsulfonyl fluoride (PMSF)/1 mM EDTA/50 mM Tris·HCl, pH 8.0. Lysates were assayed for protein concentration with the Bio-Rad DC Protein Assay. Total EC protein was used to standardize measurements of F- and G-actin. The F-actin pool was measured fluorometrically as described (9) with minor modifications. Monolayers cultured in six-well plates were washed in 75 mM KCl/3 mM $MgSO_4$/1 mM EGTA/10 mM imidazole, 0.2 mM dithiothreitol/10 μg of aprotinin per ml/0.1 mM PMSF, fixed in 3.7% formaldehyde for 15 min, rendered permeable in 0.2% Triton X-100 for 5 min, stained with 0.165 μM 7-nitrobenz-2-oxa-1,3-diazole (NBD)-phallicidin (Molecular Probes) for 20 min, and extracted with methanol overnight at −20°C. Intraendothelial fluorescence was measured in a Perkin–Elmer LS30 Luminescence spectrometer (465-nm excitation/535-nm emission) and expressed in arbitrary fluorescence units/mg of total EC protein. G-actin was measured by the DNase I inhibition assay (9), which is based on the ability of monomeric G-actin to inhibit the hydrolysis of DNA by DNase I into its component nucleotides. Monolayers cultured in six-well plates were washed and rendered permeable in Hanks' balanced salt solution containing 1% Triton X-100, 2 mM $MgCl_2$, 2 mM EGTA, 0.2 mM ATP, 0.5 mM dithiothreitol, and 0.1 mM PMSF, and the supernates containing G-actin were tested in a DNase I inhibition assay calibrated for G-actin. Activities within a range of 30–70% inhibition were interpolated to G-actin concentrations expressed in μg/mg of total EC protein.

Statistical Methods. The mean response of each experimental group was compared with its simultaneous control by *t* test. Analyses of variance were used to compare the mean responses among experimental and control groups.

RESULTS

Effects of SPARC on EC Shape and Transendothelial ^{14}C-BSA Flux. A SPARC exposure of 6 h increased ^{14}C-BSA flux in a dose-dependent manner (Fig. 1*A*). The mean (± SEM) baseline flux of ^{14}C-BSA was 0.013 ± 0.003 pmol/h ($n = 137$), and the mean (± SEM) transfer of ^{14}C-BSA across naked filters (without endothelial monolayers) was 0.215 ± 0.015 pmol/h ($n = 16$). The lowest SPARC concentration that increased ^{14}C-BSA flux compared with the medium control was 0.5 μg/ml. At 15 μg/ml, only SPARC exposures ≥ 1 h increased ^{14}C-BSA flux compared with controls, with further increments throughout the 6-h period (Fig. 1*B*). There were no significant differences in ^{14}C-BSA fluxes across control monolayers over 6 h. Although brief exposures to medium containing SPARC at 15 μg/ml or medium alone for 10 min [0.022 ± 0.002 ($n = 16$) vs. 0.015 ± 0.002 ($n = 11$); $P = 0.0421$] and 30 min [0.024 ± 0.002 ($n = 16$) vs. 0.015 ± 0.002 ($n = 11$); $P = 0.0068$] increased the ^{14}C-BSA flux measurable at 6 h,

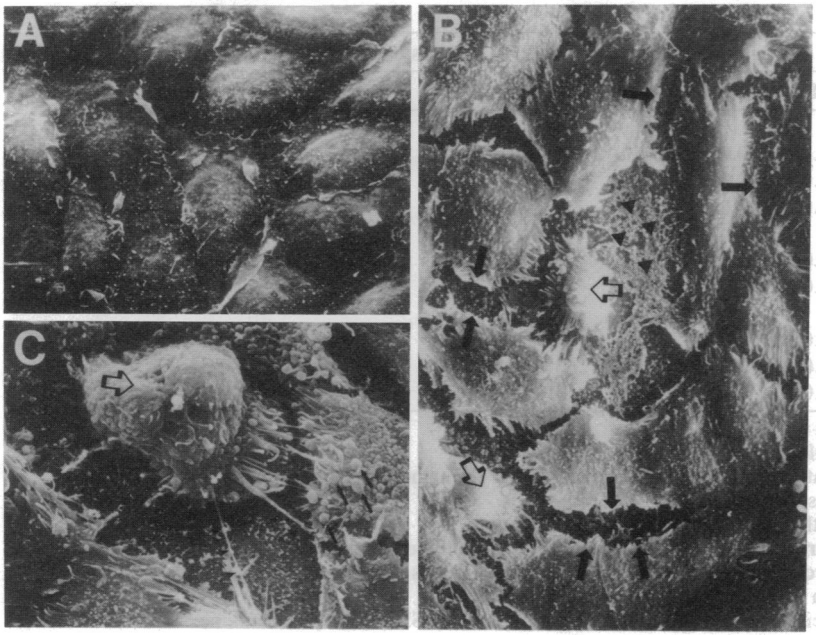

FIG. 2. Effects of SPARC on EC morphology. Scanning EM of postconfluent monolayers grown on filters in assay chambers after exposure to SPARC at 15 μg/ml for 6 h (*B* and *C*) or to medium alone (*A*). (*A*) Control monolayers were in tight apposition and displayed the typical cobblestone appearance. (×1450.) (*B*) Monolayers exposed to SPARC remained attached to matrix-coated filters (closed arrowheads) but exhibited extensive interendothelial gaps (closed arrows). Occasional cells assumed an extremely rounded phenotype (open arrows). (×1450.) (*C*) Selected cells (open arrows) became rounded with marked separation from neighboring cells and displayed numerous slender processes (small arrowheads) and extensive blebs (small closed arrows). (×2900.)

Table 1. Specificity of the SPARC effect on transendothelial ^{14}C-BSA flux

Treatment*	Flux, pmol/h Mean ± SEM	*n*	Significance
Pretreatment baseline	0.013 ± 0.003	75	
Medium control	0.017 ± 0.002	10	
Anti-SPARC antibody†	0.027 ± 0.007	5	NS
Trypsin/TI‡	0.024 ± 0.002	11	NS
SPARC (15 μg/ml)	0.095 ± 0.019	8	P=0.0005§
SPARC + antibody†	0.028 ± 0.002	9	P=0.0024¶
Heat-treated SPARC‖	0.025 ± 0.002	6	P=0.0081¶
SPARC + trypsin/TI‡	0.030 ± 0.002	12	P=0.0001¶
LPS (0.3 ng/ml)**	0.015 ± 0.003	7	NS
LPS (30 ng/ml)**	0.019 ± 0.003	7	NS

NS, not significant; TI, trypsin inhibitor; *n*, no. of measurements.
*Exposure time for all experimental and control groups was 6 h.
†Rabbit anti-murine SPARC polyclonal antibody was used in 100-fold excess relative to SPARC (18).
‡Trypsin was used at 32.5 units/ml for 30 min followed by soybean trypsin inhibitor at 2 μg/ml for 30 min.
§Comparison is with simultaneous control.
¶Comparison is with exposure to SPARC at 15 μg/ml.
‖Treatment was with SPARC that had been heated to 70°C for 20 min.
**_E. coli_ 0111:B4 LPS at 10 and 100 times the concentrations that could be present in preparations of SPARC (0.005–0.03 ng of LPS per μg of SPARC; ref. 19).

these increases were less than the effect seen after a continuous 6-h exposure.

The effect of SPARC on EC morphology in postconfluent monolayers grown on filters was studied by scanning EM (Fig. 2 *A–C*). In control monolayers, the cells were in tight apposition with no intercellular gaps and exhibited a cobblestone appearance (Fig. 2*A*). In monolayers exposed to SPARC at 15 μg/ml for 6 h, cells remained attached to the substrate but retracted from each other, with resultant intercellular gaps bridged by cell processes (Fig. 2*B*). Some of the cells assumed a rounded morphology, displayed numerous slender processes and extensive blebbing, and were clearly separated from neighboring cells (Fig. 2*C*).

To exclude contribution from endotoxin or other contaminants, transendothelial ^{14}C-BSA flux was assayed after 6-h exposures to fresh SPARC (15 μg/ml), heat-treated SPARC, SPARC preincubated with neutralizing antibody, proteolyzed SPARC, *Escherichia coli* lipopolysaccharide (LPS; 0.3 and 30 ng/ml), or medium alone (Table 1). Treatment with anti-SPARC antibody decreased the SPARC-induced effect, whereas heated or trypsin-digested SPARC or LPS failed to increase ^{14}C-BSA flux compared with medium controls.

The SPARC-induced increments in ^{14}C-BSA flux were not temperature-dependent (Table 2). Exposure to SPARC increased ^{14}C-BSA flux compared with the isothermal medium controls at 37°C, 25°C, and 4°C. Although the endothelial response to SPARC was maximal at 37°C, the endothelium clearly responded at both 25°C and 4°C. The SPARC-induced effect could not be blocked by prior inhibition of protein synthesis by cycloheximide at 50 μg/ml (Table 2). In fact, treatment with cycloheximide enhanced the increments in ^{14}C-BSA flux induced by SPARC, compared with an equivalent exposure to SPARC in the absence of cycloheximide. SPARC also increased the flux of ^{14}C-BSA across aortic and retinal EC monolayers compared with their respective controls (Table 2).

Effect of SPARC on EC Detachment and Cytotoxicity. Total numbers of viable and nonviable EC from monolayers exposed to SPARC and to medium alone were not significantly different at 6 h (Table 3). Although the percent detachment for the EC exposed to SPARC at 15 μg/ml for 6 h was increased compared with the medium control, the mean percent detachment was ≤ 3.5% for each group (Table 3). Experimental and control monolayers were not significantly different with respect to trypan blue exclusion and/or LDH release (Table 3).

Role of Actin Reorganization in SPARC-Induced Changes in Endothelial Barrier Function. EC exposed to medium with SPARC at 15 μg/ml or medium alone were stained with fluorescein-phalloidin as shown in Fig. 3 *A–C*. All control EC contained continuous transcytoplasmic actin filaments and exhibited tight cellular apposition without intercellular gaps (Fig. 3*A*). After an exposure of 2 hr or more to SPARC, increases in the peripheral actin bands and isolated ellipsoid disruptions within the F-actin lattice could be seen (Fig. 3 *B* and *C*). These disruptions occurred principally at the cell-to-cell interface. EC cultured in assay chambers were preincubated with the specific F-actin stabilizing agent NBD-

Table 2. Effects of temperature, protein synthesis inhibition, and type of endothelia on SPARC-induced changes in transendothelial flux of ^{14}C-BSA

	Flux, pmol/h: Medium control Mean ± SEM	*n*	SPARC exposure Mean ± SEM	*n*	Significance
Temperature‡					
4°C	0.023 ± 0.004	10	0.055 ± 0.002	18	$P < 0.0001$§
22°C	0.020 ± 0.003	9	0.059 ± 0.003	14	$P < 0.0001$§
37°C	0.018 ± 0.003	6	0.080 ± 0.004	6	$P < 0.0001$§
Protein synthesis inhibition					
No cycloheximide	0.014 ± 0.002	11	0.065 ± 0.004	11	P = 0.0415‖
With cycloheximide¶	0.022 ± 0.003	11	0.091 ± 0.008	12	
Types of endothelia					
Pulmonary artery	0.018 ± 0.002	22	0.088 ± 0.013	22	$P < 0.0001$§
Aorta	0.018 ± 0.002	19	0.087 ± 0.009	20	$P < 0.0001$§
Retinal microvessel	0.020 ± 0.001	6	0.112 ± 0.010	11	$P < 0.0001$§

*Medium controls were assayed at 6 h.
†SPARC exposure was at 15 μg/ml for 6 h.
‡For these experiments, appropriate temperatures were maintained throughout both the treatment period and the barrier function assay.
§SPARC exposure is compared with simultaneous medium control.
¶Cycloheximide (50 μg/ml) was introduced 0.5 h prior to and throughout the exposures to SPARC or medium. This concentration of cycloheximide blocked EC protein synthesis >95% as measured by [^{35}S]methionine incorporation into trichloroacetic acid-precipitable protein.
‖SPARC exposure with prior protein synthesis inhibition is compared with SPARC exposure alone.

Table 3. Effect of SPARC on EC detachment and cytotoxicity

Measurements	Medium control Mean ± SEM	*n*	SPARC exposure Mean ± SEM	*n*
Total EC × 10^{-6} per monolayer	1.11 ± 0.07	11	1.05 ± 0.09*	12
EC detachment, %	2.0 ± 0.17	6	3.5 ± 0.37†	6
Trypan blue exclusion, % nonviable cells	2.45 ± 0.26	6	2.56 ± 0.26*	6
LDH release, %	3.58 ± 0.55	8	3.65 ± 0.85*	8

Medium controls were assayed after 6 h, and SPARC exposure was at 15 μg/ml for 6 h. Measurements (mean ± SEM) were as follows: cell counts and percent detachment determined for post-confluent monolayers cultured on 25-mm filters suspended in six-well plates; percentage of cells failing to exclude trypan blue; and percent LDH release calculated as (LDH in medium)/(LDH in medium + LDH in cell lysate) × 100.

*Not significant compared with medium control.

†Significantly increased compared with medium control (P = 0.0028).

phallicidin (0.3 μM) for 3 h prior to and throughout exposure to medium with or without SPARC (Fig. 3*D*). Uptake of NBD-phallicidin by EC was confirmed by epifluorescence microscopy. Prior F-actin stabilization protected endothelial monolayers against SPARC-induced increments in the transendothelial flux of ^{14}C-BSA. Neither the mean F-actin content nor the mean G-actin concentration for monolayers exposed to SPARC differed significantly from their respective controls throughout the 6-h period (data not shown).

DISCUSSION

The cell-matrix interactions required for diverse cellular functions are regulated by adhesive and antiadhesive extracellular proteins (13, 17, 21). SPARC is a highly conserved, antiadhesive glycoprotein that can inhibit EC spreading and induce partial detachment from substrata *in vitro*; this antiadhesive influence promotes a rounded cell morphology (13, 17). There is experimental evidence to suggest that SPARC is operative during processes that require cell disengagement from the ECM (e.g., migration and proliferation) and tissue remodeling under normal conditions or in response to injury (13, 15, 18, 21). In the present report, we have demonstrated that exogenous SPARC induces dose- and time-dependent increments in the flux of ^{14}C-BSA across bovine pulmonary artery EC monolayers. This experimental system does not reproduce the permeability characteristics of blood vessels *in vivo* (22); it precludes hydrostatic and osmotic pressure gradients, circulating effector cells, and numerous nonendothelial-derived host mediator systems (4). A 6-h exposure to SPARC at concentrations as low as 0.5 μg/ml increased ^{14}C-BSA flux; a minimal exposure time ≥ 1 h was required in the presence of SPARC at 15 μg/ml. Since the effect of SPARC on EC did not require serum, it is unlikely that the changes in barrier function induced by SPARC could be ascribed solely to contamination by LPS (3). However, these findings do not exclude the possibility of synergistic action between SPARC and trace amounts of LPS.

The dose and time requirements for the changes in barrier function mediated by SPARC are compatible with those described by Sage *et al.* (17) for the changes in EC shape observed in the presence of this protein. Bovine aortic EC plated on collagen-coated or plastic substrata in the presence of murine or bovine SPARC attached but did not spread and assumed a rounded morphology. At 18 h the rounded phenotype was a function of the concentration of SPARC; at 15 μg/ml, ≈50% of the cells were not spread. SPARC at 0.5 μg/ml also induced a rounded morphology in confluent cells (17). In the present report, the changes in barrier function were temporally coincident with changes in EC shape. Scanning EM as well as F-actin fluorescence microscopy revealed widespread cell rounding associated with retraction between adjacent cells. Some cells displayed marked rounding, extended processes, and extensive blebbing of the plasma membrane. However, the response to SPARC within the same monolayer was not uniform.

The mechanism(s) by which SPARC regulates the endothelial barrier are not well understood. The changes in barrier function that we observed could not be ascribed to cell injury as measured by trypan blue exclusion or LDH release. Failure to block the effect by inhibition of protein synthesis, together with a 1-h stimulus-to-response lag time, indicates that SPARC might not induce synthesis of a second protein mediator by EC. Prior inhibition of protein synthesis not only failed to block the SPARC-induced effect but also was found to enhance it. In fact, Lane and Sage (16) have shown that the endothelial cell rounding induced by either SPARC or SPARC peptides was also enhanced in the presence of cycloheximide.

SPARC interacts with both the EC and its ECM and is thought to regulate their association (15, 17). Murphy-Ullrich

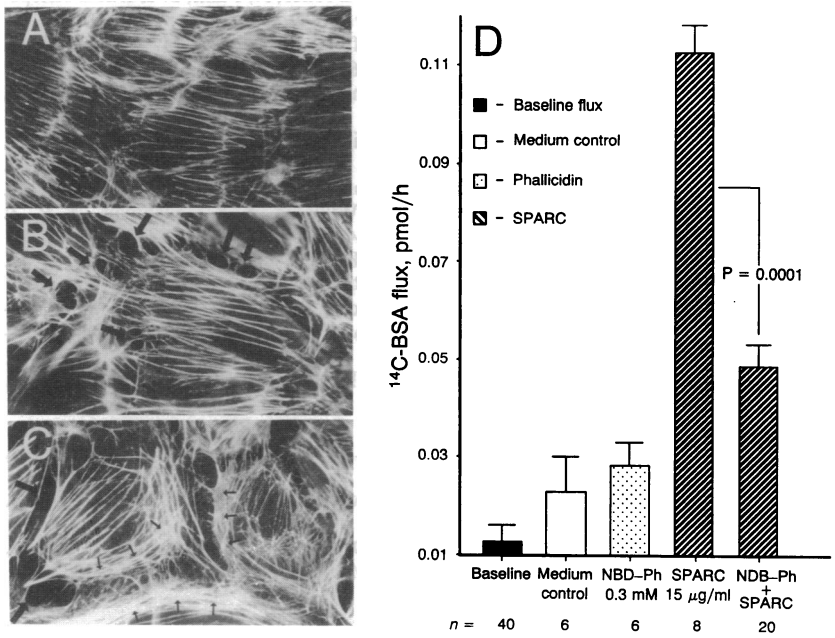

FIG. 3. SPARC-Induced actin reorganization and changes in endothelial barrier function. Endothelial monolayers grown on filters were exposed to medium (*A*) or SPARC at 15 μg/ml for 2 hr or more (*B* and *C*). The monolayers were fixed, rendered permeable, and stained with fluorescein-phalloidin. (*A*) Monolayers exposed to medium alone contained continuous transcytoplasmic actin cables and exhibited tight cellular apposition without intercellular gaps. (*B* and *C*) Exposures of SPARC for 2 h or more induced intercellular gaps (closed arrows). In *C*, note the circumferential F-actin redistribution (small closed arrows). (*D*) EC monolayers grown on filters were pretreated with 0.3 μM NBD-phallicidin (NBD-Ph) or medium alone for 3 h prior to and throughout a 6-h exposure to either medium with SPARC or medium alone in the absence of FBS. Vertical bars represent the mean (± SEM) transendothelial flux of ^{14}C-BSA. Baseline (± SEM) ^{14}C-BSA flux is shown by the closed bar. (*A–C* = ×600.)

et al. (21) recently demonstrated that SPARC diminished the number of focal adhesions in cultured bovine aortic EC. These structures provide an array of specialized actin binding and anchoring proteins for attachment of actin filaments to the plasma membrane (23); such proteins are also linked to transmembrane glycoproteins that communicate with the ECM. A 2-h exposure to SPARC was associated with the loss of focal adhesions from ≈50% of cells and a circumferential distribution of F-actin. These data are compatible with our findings that SPARC induces EC detachment and a redistribution of F-actin to the cell periphery. Preloading with the specific F-actin stabilizing agent phallicidin protected against barrier dysfunction. This result is compatible with the suggestion that depolymerization of F-actin is causally related to the changes in barrier function induced by SPARC. However, we were unable to demonstrate changes in either the F- or G-actin pools or any shift between these pools over time. The measurements of F- and G-actin reflect mean concentrations for entire monolayers. If a minority of cells were affected, heterogeneous changes in actin organization within a monolayer might not be apparent. It is possible that F-actin destabilization occurs secondary to the disruption of focal contacts, and F-actin disassembly localized to the focal contact site would be exceedingly difficult to detect. Actin reorganization as the final common pathway for receptor-mediated changes in endothelial barrier function is a primary event associated with obvious changes in the F- and G-actin pools (9, 11). Although we did not observe such changes in this study, loss of focal contacts with secondary actin reorganization restricted to focal contact sites could explain the changes in EC shape, intercellular gap formation, and barrier dysfunction as mediated by SPARC in the absence of detectible changes in the F- and G-actin pools.

One possible mechanism for the decrease of focal contacts by SPARC could involve activation of intracellular proteases that selectively cleave actin binding or anchoring proteins (24). Such a process could generate the disruptions within the filamentous actin network that we observed in this study. The redistribution of vinculin from focal contacts to other sites in EC exposed to SPARC (21) is also concordant with this concept.

The ability of SPARC to regulate the transendothelial paracellular pathway may be enlisted for the EC response to acute host injury. Cultured EC basally secrete 1–3% of total secreted protein as SPARC, which can be augmented up to 3-fold by exposure to LPS (15). SPARC is secreted from EC in 1.5 h or more (15), and we found a stimulus-to-response time for SPARC-induced changes in barrier function of ≈1 h. That EC can both produce and respond to SPARC (14, 15, 17) is suggestive of an autocrine or a paracrine pathway of regulation. Another potential tissue source for SPARC in the context of acute EC injury is the activated platelet (25), the α granules of which release abundant amounts of SPARC (26). EC injury can involve platelet–endothelial interaction and platelet activation (27). By promoting intercellular gap formation, SPARC might actively mediate the extravasation of circulating macromolecules (e.g., acute phase proteins, complement components, and immunoglobulins) into tissues. These gaps might also facilitate leukocyte diapedesis. Alternatively, if the changes in barrier function become dysregulated, vascular leak syndromes deleterious to the host might ensue. A prototypic pathological correlate for SPARC-induced barrier dysfunction might be pulmonary vascular endothelial injury in endotoxin-challenged animals (28) or in the high-mortality adult respiratory distress syndrome in humans (27). SPARC, which is found in high concentrations in the lung (18, 29), is elevated in response to LPS (15). LPS also induces disseminated intravascular coagulation and platelet activation (28). Another clinical antecedent to life-threatening edema formation is head injury. In the present report, retinal microvascular EC that form part of the blood–brain barrier were highly responsive to exogenous SPARC. Further studies are required to determine whether other injurious stimuli enhance expression of SPARC and whether SPARC is operative *in vivo* during the physiological and/or pathological regulation of endothelial barrier function.

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