Lymphatic Endothelial Cells Adapt Their Barrier Function in Response to Changes in Shear Stress

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Abstract

Background: Lymphatic endothelial cells form an important barrier necessary for normal lymph formation and propulsion. However, little is known about how physical forces within lymphatic vessels affect endothelial barrier function. The purpose of this study was to characterize how laminar flow affects lymphatic endothelial barrier function and to test whether endothelial cells respond to flow changes by activating the intracellular actin cytoskeleton to enhance barrier function.

Methods and Results: Cultured adult human dermal microlymphatic endothelial cells (HMLEC-d) were grown on small gold electrodes arranged within a flow channel, and transendothelial electrical resistance (TER), an index of barrier function, was determined. Laminar flow was applied to the cells at a baseline shear stress of 0.5 dynes/cm², and was increased to 2.5, 5.0, or 9.0 dynes/cm², causing a magnitude-dependent increase in barrier function that was reversed 30 min later when the shear stress was returned to baseline. This response was abolished by blockade of actin dynamics with 10 μ M phalloidin, and significantly inhibited by blockade of Rac1 activity with 50 μ M NSC23766. Blockade of protein kinase A (10 μ M H-89) did not inhibit the response. Mathematical modeling based on our impedance data showed that the flow-induced changes in TER were primarily due to altered current flow between cells and not beneath cells.

Conclusions: These results suggest that lymphatic endothelial cells dynamically alter their morphology and barrier function in response to changes in shear stress by a mechanism dependent upon Rac1-mediated actin dynamics.

Introduction

LYMPHATIC ENDOTHELIAL CELLS FORM A CRITICAL PERMEABLE BARRIER necessary for the normal formation of lymph in initial lymphatics and for propulsion of lymph in collecting lymphatics. However, details about the specific mechanisms by which lymphatic endothelial cells regulate lymph formation in initial lymphatics, and modify the composition of lymph in collecting lymphatics and lymph nodes, remain elusive. Determination of how lymphatic endothelial cells respond and adapt to different kinds of signals and stresses is key for improving our understanding of the mechanisms underlying their normal function in the lymphatic system.

Lymphatic endothelial cells present phenotypes that are distinct from vascular endothelial cells, such as the specialized "oak leaf" shaped one-way primary valves in initial lymphatics that are involved in lymph formation.^{1,2} On the other

hand, lymphatic endothelial cells share certain characteristics with vascular endothelium, such as the ability to sense changes in shear stress (τ) and produce nitric oxide (NO) as a signal to cause relaxation of smooth muscle when τ is relatively high,^{3,4} much like the NO-dependent flow-induced vasodilation in arterioles.⁵ This particular mechanism has been proposed as a way for collecting lymphatic vessels to optimize the forward flow of lymph during periods of high flow.⁶

Another aspect that our laboratory has been investigating is how lymphatic endothelial cells alter their barrier function to facilitate optimum lymph formation and flow.⁷ In blood vessels and in cultured vascular endothelial cell models, changes in the laminar fluid flow rate can influence barrier function.^{8–11} For example, increasing the fluid flow through isolated, perfused coronary venules not only increases filtration of solutes, but also increases their diffusive permeability coefficient to albumin through a NO-related mechanism.¹¹ In

cultured vascular endothelial cell models, increasing τ causes an initial rapid increase in barrier function, followed by decrease in barrier function over longer periods of time.^{8–10} In both of the isolated venule and cultured endothelial cell models, the effects of increased τ were reversed upon returning to the baseline τ .^{8–11} These studies demonstrate that shear stress sensing is an important property of vascular endothelium for regulating barrier function.

We considered the possibility that lymphatic endothelial cells may share this property. Using cultured lymphatic endothelial cells, and based on evidence that step increases in τ transiently increase barrier function of vascular endothelial cells,^{8,9} we tested the hypothesis that a step elevation of τ rapidly enhances barrier function. In addition, we evaluated whether protein kinase A (PKA) or the small GTPase Rac1, both of which have been implicated as promoters of enhanced endothelial barrier function,¹² may be involved in shear stress-induced changes in lymphatic endothelial barrier function.

Materials and Methods

Materials

Cryopreserved adult human dermal microlymphatic endothelial cells (HMVEC-dLYAd; abbreviated herein as HMLEC-d), Clonetics microvascular endothelial growth medium (EGM-2MV), and endothelial basal medium (EBM) were obtained from Lonza (Chicago, IL). Phalloidin, NSC23766 and H-89 were purchased from EMD-Calbiochem (Gibbstown, NJ).

Cell culture and impedance measurement

HMLEC-d were routinely maintained on gelatin-coated cultureware in EGM-2MV. Barrier function of HMLEC-d monolayers was determined using an Electrical Cell-Substrate Impedance Sensor (ECIS) Model 1600R equipped with a Heidolph peristaltic pump to regulate laminar flow of medium over the cells (Applied Biophysics, Troy, NY). The system was used to monitor behavior of cells grown in cultureware containing gold film surface electrodes, with

culture medium serving as the electrolyte, as previously described.^{13,14} For most experiments, a $1\ \mu\text{A}$ ac signal at 4 kHz was applied from an approximate constant current source, between the small measuring electrodes ($5 \times 10^{-4}\ \text{cm}^2$) and a large counter electrode ($1\ \text{cm}^2$). The instrument monitored the voltage across the electrodes and its phase relative to the applied current, providing a report of total impedance. As the cells grow and cover the surface area of the electrodes, their cell membranes constrict the current and force it to flow beneath and between cells, resulting in a large increase in impedance. Treating the cell-electrode system as a series RC circuit, the ECIS 1600R converted the impedance data to resistance and capacitance of the cell monolayer, which represent barrier function and membrane capacitance, respectively. Transendothelial resistance (TER) is presented as an index of lymphatic endothelial barrier function.

To assess TER under different laminar fluid flow conditions, we used a closed loop circulation in conjunction with the ECIS 1600R, with some modifications to the previously described prototype.⁸ The configuration of the electrodes was in a single column of a flow channel with a length of 50 mm, width of 5 mm, and height of 0.4 mm, with luer fittings on either end of the channel (8F1E array, Applied Biophysics). Approximately 10^6 cells were initially seeded in a $200\ \mu\text{L}$ volume of EGM-2MV. After approximately 4 h the medium was changed and the cells were left to grow overnight. The next day, the flow array was attached to a sterile closed loop circulation of 1/8" silicone tubing (Cole/Palmer) connected to the flow system, consisting of the Heidolph peristaltic pump to circulate the medium, a 50 mL Corning medium bottle serving as a hydraulic filter to dampen the peristaltic flow, the flow array, and a medium bottle serving as a receiver prior to return to the pump (Fig. 1). The pump was placed next to a 37°C 5% CO₂ humidified incubator with a side port through which the tubing to the rest of the flow system passed. Medium within the flow system was allowed to equilibrate with the 5% CO₂ through a port on the medium receiver with a $0.22\ \mu\text{m}$ syringe filter connected. 50 mL of EGM-2MV or EBM was sufficient to maintain constant flow over the cells. Two tubing sizes were used with the pump to generate various flow rates

FIG. 1. Diagram of the ECIS flow system.

FIG. 2. Step increases in fluid shear stress cause rapid enhancement of barrier function in lymphatic endothelial cell monolayers. Measurements of TER (y-axis) of HMLEC-d monolayers were initially taken under no-flow conditions, after which a step increase in laminar flow was applied, generating a shear stress of 10 dynes/cm². The flow was then turned off after 30 min. (A) A rapid increase in TER occurred after the step increase was applied. TER then gradually decreased but remained elevated until the flow was turned off, which caused a rapid drop. (B) This response was similar when the cells were bathed with EBM rather than EGM2-MV. (C) Application of a pulsatile flow (2 s on, 2 s off) with a peak shear stress of 10 dynes/cm² also caused a similar initial increase in TER. The dotted horizontal line indicates the initial baseline TER level just prior to turning flow on. $N = 8$ electrodes for each group.

(Masterflex LS/13 1.6 mm internal diameter, and LS/14 0.8 mm internal diameter). In experiments where pharmacologic inhibitors were added to the system, two three-way stopcocks were placed in this system: one on the flow array to add the inhibitor directly over the channel with cells, and the other at the entry point to the medium receiver to allow sufficient mixing within the remainder of the system.

In some experiments, we used a modified system to allow observation of the cells by time-lapse microscopy during TER measurement. The electrode array was attached to a custom-

built stage adapter mounted on a Leitz Laborlux D upright microscope, equipped with a 10X objective (0.30 NA), Photometrics CoolSnap cf camera (Roper Scientific, Tucson, AZ), and Micro-Manager 1.2 software (University of California, San Francisco). Brightfield images were acquired once per minute during experiments.

The relative contributions of the adhesions between cells (R_b) versus the adhesion of the cells to the substratum (α) to the overall TER were resolved using the ECIS modeling software, which has been discussed previously in detail.¹⁵ For

FIG. 3. Live cell imaging of HMLEC-d during TER measurement under baseline and elevated shear stress. (A) TER from a single electrode during baseline τ (0.5 dynes/cm²) and during elevated τ (9 dynes/cm²). The images in (B), (C), and (D) were taken at the time points indicated on the tracing. The circle (250 μm diameter) in the center is the cell-covered measuring electrode. The images are representative of 4 experiments.

FIG. 4. The laminar flow-induced increase in TER is dependent upon the magnitude of the shear stress. **(A)** Time-course of changes in TER in response to increased laminar flow at varying magnitudes of shear stress. A baseline level of 0.5 in dynes/cm² was initially applied, followed by step increases to 2.5, 5.0, and 9.0 dynes/cm² for 30 min. The increases to 5.0 and 2.5 dynes/cm² were repeated. Each step increase caused a rapid elevation of TER, followed by a gradual decrease in TER, and finally a rapid decrease in TER when shear stress was returned to 0.5 dynes/cm². The tracing shown is the average of 14 electrode measurements. **(B)** displays the mean changes in resistance from baseline 6 min after the corresponding step increases shown in **(A)**. **(C)** shows the ratio of the peak TER to baseline TER observed during various levels of elevated shear stress. **(D)** shows the time to reach the peak TER after various step increases in shear stress. **p* < 0.01 vs. baseline (0.5 dynes/cm²). †*p* < 0.01 vs. 2.5 dynes/cm² group. §*p* < 0.01 vs. 2.5 dynes/cm²-repeat group. *N* = 14 for all groups.

these experiments, readings were taken at multiple frequencies on cell-free electrodes (prior to cell seeding), and then on cell-covered electrodes over time during the experimental protocol. The software then took this data to construct the model of the specific impedance of the cell-covered electrodes as a function of frequency (0.4–40 kΩ), from which R_b and α were solved.

Experimental protocols

After attachment of the flow electrode array to the rest of the flow system, the cells were allowed to equilibrate for 12–6 h prior to the start of the experiment. In the initial experiments, TER was first measured under no flow conditions, after which we applied a step increase in laminar flow to produce a shear stress (τ) of 10 dynes/cm², which was maintained for 30 min and then flow was turned off. This protocol was performed with either EGM-2MV or EBM as the bathing medium. We also performed this protocol, but with a pulsing flow at 2 s on, 2 s off.

In subsequent experiments we applied a relatively low, baseline flow that produced a shear stress of 0.5 dynes/cm². We chose this protocol because we planned to eventually add chemical inhibitors to the system to test various signaling pathways, and a continuous exchange of medium over the cells would allow any additives to the system to reach the cells prior to testing step changes in shear stress. We applied the baseline laminar flow for at least 1 h prior to the initial step increase in fluid shear stress (baseline τ = 0.5 dynes/cm²). One modification in the system that was required to achieve flow rate this low was to reduce the internal diameter of the tubing in contact with the peristaltic pump (Masterflex LS/13 tubing with a 0.8 mm internal diameter). This modification made the applied range for τ to 0.4–9.0 dynes/cm².

We determined whether rapid increases in shear stress cause rapid changes in TER of lymphatic endothelial cell monolayers in a magnitude-dependent fashion. Step increases in τ to 2.5, 5.0, or 9.0 dynes/cm² were applied for 30 min, with 30 min periods of baseline flow (τ = 0.5 dynes/cm²) in between. These levels of τ were chosen because during phasic contractions, the endothelium of normal collecting lymphatics can be exposed to up to τ = 4 dynes/cm² during systole.¹⁶ The range we tested thus reflects both physiological and supra-physiological shear stresses for lymphatic endothelium.

We tested the role of PKA in fluid shear stress-induced lymphatic endothelial barrier enhancement with 1 and 10 μ M H-89.¹⁷ To evaluate the role of the actin cytoskeleton, we applied the actin stabilizer phalloidin at 1 μ M¹⁸ 30 min prior to a step increase in τ . The role of Rac1, which promotes membrane ruffling and stabilization of cortical actin fibers, was tested using 50 μ M of the Rac1-specific inhibitor NSC23766.¹⁹

Data analysis

For each experiment, a tracing of the mean TER vs. time, obtained from multiple electrodes is shown. To assess the short-term changes in TER after a step change in τ , we used the change in TER obtained 6 min after a step change (Δ TER = TER 6 min after step change – TER just before step change). We also evaluated the peak Δ TER and the time to reach a peak Δ TER during elevated τ . These data were averaged and are presented as means $\pm$ S.E. The significance be-

FIG. 5. Normal actin cytoskeleton dynamics are needed for shear stress-induced enhancement of lymphatic endothelial barrier function. Laminar flow was applied to HMLEC-d monolayers initially to produce a baseline shear stress of 0.5 dynes/cm². Application of a step increase to 9 dynes/cm² for 30 min caused an increase in TER until the shear stress was reduced back to 0.5 dynes/cm². Application of 1 μ M phalloidin, an actin stabilizer, caused a slight, initial drop in TER. In addition, the shear stress-induced increase in TER in the presence of phalloidin was significantly inhibited (inset; * $p < 0.05$; $N = 16$).

tween groups was determined by *t*-tests, or one-way ANOVA followed by Tukey's multiple comparisons test where appropriate. Significance was accepted at $P < 0.05$.

Results

TER during laminar fluid flow

Imposing laminar flow to HMLEC-d monolayers, resulting in a step increase in τ from 0 to 10 dynes/cm², caused a rapid

increase in TER (Fig. 2). After the initial increase, the TER remained elevated, but gradually decreased until flow was turned off 30 min later, which caused a rapid drop in TER (Fig. 2A). To determine whether this flow-induced elevation in TER may be due in part to the introduction of fresh growth medium (EGM-2MV), which contains several additives and growth factors, we performed the same protocol with serum-free EBM (Fig. 2B). Application of the same laminar flow protocol when HMLEC-d were bathed with EBM caused a

FIG. 6. Rac1 mediates shear stress-induced changes in HMLEC-d barrier function. Laminar flow was applied to HMLEC-d monolayers initially to produce a baseline shear stress of 0.5 dynes/cm². Application of a step increase to 9 dynes/cm² for 30 min caused an increase in TER until the shear stress was reduced back to 0.5 dynes/cm². Application of the Rac1 specific inhibitor NSC23766 (50 μ M) caused a transient drop in TER by itself, which recovered within 20 min. NSC23766 also significantly inhibited the increase in HMLEC-d TER in response to a step increase in shear stress (inset; * $p < 0.05$; $N = 16$).

FIG. 7. PKA does not mediate the shear stress induced rapid increase in lymphatic endothelial barrier function. Laminar flow was applied to HMLEC-d monolayers initially to produce a baseline shear stress of 0.5 dynes/cm². Application of a step increase to 9 dynes/cm² for 30 min caused an increase in TER until the shear stress was reduced back to 0.5 dynes/cm². Application of the specific PKA inhibitor H-89 (10 µM) caused a marked drop in TER, however, did not prevent the elevated shear-stress-induced increase in TER. A comparable increase in TER was observed both in the absence and presence of H-89 (inset; NS = not significant; N = 8).

similar increase in TER as seen with EGM-2MV. Considering that flow in lymphatics *in vivo* is pulsatile in nature due to the active pumping of collecting lymphatics,²⁰ we also tested this protocol with a pulsatile flow, in which flow was turned on for 2 s and then off for 2 s over the 30 min period, with a peak τ of 10 dynes/cm² (Fig. 2C). This protocol produced a similar pattern of elevated TER as continuous flow. These experiments show that lymphatic endothelial cells are capable of altering their barrier function in response to changes in τ .

In subsequent experiments we used a baseline flow ($\tau = 0.5$ dynes/cm²), allowing continuous exchange of medium without causing significant changes in TER. A step increase in τ caused a rapid increase in TER of HMLEC-d with this protocol (Fig. 3A) in a similar fashion as the experiments in which flow was turned on and off (Fig. 2).

To make certain that the changes in TER caused by altering τ were not due to detachment and re-spreading of cells, we performed a series of ECIS experiments in which the cells were monitored by time-lapse microscopy (Fig. 3). When flow was elevated, any cells or debris that were loosely attached to the substratum could easily be detached by increasing flow. However, the HMLEC-d monolayer remained intact during baseline, the 0.5 h period of elevated τ , and the return to baseline, indicating that the observed changes in TER were not due to disruption and reformation of the cell monolayer.

The magnitude of the $\Delta\tau$ determines the degree of barrier enhancement

We next evaluated whether the magnitude of the increase in TER was dependent upon the degree of the $\Delta\tau$ applied. After a period of 1 h at baseline flow ($\tau = 0.5$ dynes/cm²), we applied step increases in laminar fluid flow lasting 30 min, with 30 min periods at baseline flow in between. We tested

increases in τ to 2.5, 5.0, and 9.0 dynes/cm², followed by repeats of the increases to 5.0 and 2.5 dynes/cm² (Fig. 4A). The degree of the increase in TER was dependent upon the magnitude of the change in τ , with increases to 5.0 and 9.0 dynes/cm² eliciting significantly higher rapid changes in TER levels (measured at 6 min after the step increase) compared to baseline (Fig. 4B). Both the peak increase in TER (Fig. 4C), and the time to reach peak TER (Fig. 4D) were related to the magnitude of the change in τ . These results indicate the ability of lymphatic endothelial cells to dynamically tailor their barrier function to different levels of τ .

Rac1-mediated actin dynamics mediate the shear stress-induced barrier enhancement

We next evaluated what intracellular signaling pathways may be involved in the response by lymphatic endothelial cells to changes in τ . The actin cytoskeleton is an important structure for sensing mechanical stresses and determining cell shape,²¹ so we investigated its potential role in this response. To block actin dynamics, we used the actin stabilizer phalloidin (1 µM), which inhibited the shear stress-induced increase in HMLEC-d TER (Fig. 5). These results indicate the importance of normal actin cytoskeleton dynamics for shear stress-induced enhancement of lymphatic endothelial barrier function.

Rac1, a small Rho family GTPase, promotes membrane protrusion and ruffling, and stabilization of cortical actin fibers, and can enhance barrier function of vascular endothelial cells. We used a specific Rac1 inhibitor, NSC23766, to test its role (Fig. 6). Addition of NSC23766 caused a transient drop in TER that recovered to baseline within 30 min. In addition, Rac1 blockade significantly attenuated the lymphatic endothelial barrier enhancement caused by elevated τ . The overall shape of the curve changed somewhat, with an initial dip in

TER followed by an increase that resembled the response seen in the absence of NSC23766, however much smaller. These data suggest that Rac1 participates in the signaling that generates the normal response to an increase in τ .

PKA does not mediate the shear stress-induced barrier enhancement

We next considered the cAMP/PKA signal transduction pathway, which has an established role in promoting enhanced endothelial barrier function and has also been shown to activate Rac1.^{12,17,22–26} We tested the role of this pathway by inhibiting PKA activity with the specific inhibitor H-89 (Fig. 7). Addition of 10 μ M H-89 caused a rapid drop in TER during the baseline τ . However, blockade of PKA with 10 μ M H-89 (Fig. 7) or 1 μ M H-89 (data not shown) did not inhibit shear stress-induced increases in HMLEC-d TER. These data indicate that although PKA signaling is important for normal lymphatic endothelial barrier maintenance, it is not involved in the enhanced barrier function that is caused by step increases in τ .

Contribution of intercellular junctions to the overall changes in TER

Rac1-mediated membrane extensions are important for forming tight intercellular junctions.^{27–29} If this mediates the observed increase in TER in response to elevated shear stress, we would expect that the resistance between cells (R_b) would make a more significant contribution to the overall increase TER than the resistance between cells and the underlying matrix (α). Evaluation of these two parameters during the time course of a step increase in τ for 30 min showed that R_b increased in the same fashion as the overall TER, while α remained at the baseline level during the changes in τ (Fig. 8). This data shows that the initial, rapid increase in lymphatic endothelial barrier function caused by elevating τ involves tightening of intercellular junctions and not enhanced cell-matrix binding.

Discussion

To our knowledge, this is the first study to investigate shear stress mechanosensing in cultured lymphatic endothelial cells. Lymphatic endothelial cells responded to step increases in τ by rapidly enhancing their barrier function in a similar manner as reported for endothelial cells from porcine pulmonary trunks (PSEC),⁹ human umbilical vein endothelial cells (HUVEC),¹⁰ and bovine aortic endothelial cells (BAEC).^{8,30} In addition, the magnitude of the elevation of HMLEC-d TER was directly related to the degree of the increase in τ , similar to a previous finding with PSEC.⁹ These data suggest a universal sensing, transduction, and structural mechanism for this aspect the response to elevated τ among mammalian lymphatic and vascular endothelial cells.

Shear stress sensing by vascular endothelium has been extensively studied, although the mechanism is not completely understood. Specific mechanosensing of τ is a complex integrative process, involving structural elements like the cell membrane, cytoskeleton, intercellular junctions, and focal adhesions, as well as signal transduction events including potassium channel activation, mobilization of calcium, NO production, GTPase signaling, and activation of protein ki-

FIG. 8. Shear stress-induced enhancement of HMLEC-d barrier function is due mainly to the tightening of junctions between cells. The *top tracing* shows HMLEC-d TER, and *arrows* show when step changes in fluid shear stress were performed. The relative contributions of cell-matrix adhesion (α , *middle tracing*) and cell-cell adhesion (R_b , *bottom tracing*) to the overall TER (*top tracing*) were resolved using a mathematical model (see Materials and Methods; $N = 8$ electrodes).

nases, including focal adhesion kinase (FAK).^{5,21,26,31,32} In the current study with HMLEC-d we focused on pathways relevant to endothelial barrier enhancement, starting with Rac1-mediated effects on the actin cytoskeleton. Rac1, a Rho family small GTPase which promotes actin-mediated membrane ruffling and protrusions,³³ was shown to be activated shortly after applying a step increase in τ to human pulmonary artery endothelial cells (HPAEC).²⁶ In addition, the increase in τ also caused translocation of cortactin to intercellular junctions within 15 min, and this was prevented by transfection of the cells with N17Rac1, a dominant negative form of Rac1.³⁴ In a different study, transfection of HUVEC with N17Rac1 prevented the initial increase in TER caused by a step increase in τ .¹⁰ In the current study, the actin stabilizer phalloidin inhibited the shear stress-induced barrier enhancement, demonstrating the importance of normal actin dynamics (Fig. 5). In addition, the pharmacological inhibitor NSC23766 attenuated the shear stress-mediated elevation of HMLEC-d TER (Fig. 6). It is worth noting that we used a slightly lower dose of NSC23766 (50 μ M) than other studies (200 μ M) of endothelial barrier function to achieve this inhibition.^{22,35} In addition, our finding that the enhancement of R_b was largely responsible for the overall elevation in TER (Fig. 8) supports the concept that

Rac1-mediated membrane protrusions and tightening of intercellular junctions are key features of the HMLEC-d response to elevated τ . Combined, these data suggest that Rac1 mediates the shear stress-induced increase in endothelial barrier function, including in lymphatic endothelial cells. In the current study, we focused mainly on the early response of HMLEC-d to changes in τ , which because of the short time span probably excludes any significant alteration of the gene expression profile of the cells.

Inhibition of PKA has been reported to block shear stress-mediated phosphorylation of endothelial NO synthase in BAEC.³⁶ In addition, activation of the cAMP/PKA in mouse myocardial endothelial cells with forskolin/rolipram can cause activation of Rac1,³⁵ so we tested whether the shear stress-induced increase in HMLEC-d TER may involve upstream PKA activity. Treatment with the PKA inhibitor H-89, including at a high concentration (10 μ M), did not affect the response of HMLEC-d to elevated τ (Fig. 7), suggesting that the shear stress-induced, Rac-1 mediated barrier enhancement of HMLEC-d is independent of PKA activity. It is worth noting, however, that PKA blockade with H-89 caused a drop in HMLEC-d TER. Thus, as with vascular endothelium, cAMP/PKA signaling is important for normal, baseline barrier function of lymphatic endothelial cells.^{12,17,35}

Mechanosensing of changes in flow rate has been documented in isolated collecting lymphatic vessels and is important for modulating the intrinsic pumping activity of lymphatic smooth muscle.^{3,4,6} It is less clear what other effects that τ has on lymphatic vessels, although we speculate that the changes in barrier function that we observe with cultured cells may be part of an adaptive mechanism that prevents excess leakage of fluids and solutes across the collecting lymphatic endothelium. This would in turn optimize lymph flow by ensuring proper delivery to the lymph nodes in prenodal lymphatics, or back to the venous circulation in postnodal lymphatics.

In summary, we demonstrated that cultured lymphatic endothelial cells alter their barrier function in response to elevated shear stress. The response involves a rapid, Rac1-mediated tightening of the endothelial barrier that is independent of PKA. This change may be part of an adaptive response by lymphatic endothelium to optimize lymph flow.

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Disclosure Statement

Dr. Breslin and Ms. Kurtz have no conflicts of interest or financial ties to report.

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