Heterogeneity of bronchial endothelial cell permeability

AIGUL MOLDOBAEVA AND ELIZABETH M. WAGNER

Department of Medicine, Johns Hopkins University, Baltimore, Maryland 21224

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Moldobaeva, Aigul, and Elizabeth M. Wagner. Heterogeneity of bronchial endothelial cell permeability. Am J Physiol Lung Cell Mol Physiol 283: L520–L527, 2002.—In vivo models of airway inflammation suggest that most protein transudation occurs from bronchial microcirculation. However, due to technical limitations in the isolation and culture of bronchial endothelial cells, most studies of lung vascular permeability have focused on pulmonary endothelium. Thus conditions for culture of sheep bronchial artery endothelial cells (BAEC) and bronchial microvascular endothelial cells (BMVEC) were established. The bronchial artery and the mainstem bronchi, stripped of epithelium, were dissected, and endothelial cells were isolated by enzymatic treatment. BAEC and BMVEC demonstrated positive staining for factor VIII-related antigen, 1,11-dodecyl-3,3',3'-tetramethylindocarbocyanine perchlorate-labeled low-density lipoprotein, and PECAM-1. Radioligand binding studies confirmed equivalent numbers of bradykinin B2 receptors on BAEC and BMVEC. Permeability of BAEC and BMVEC was determined after treatment with bradykinin and thrombin by comparing the translocation of FITC-dextran (mol wt 9,500) across confluent monolayers (n = 10–12). Bradykinin caused a maximal increase in permeability in BAEC (165% increase) and BMVEC (144% increase) by 15 min compared with vehicle controls. Thrombin treatment altered BMVEC permeability only, reaching a maximal response at 60 min (109% increase). These results demonstrate bronchial endothelial cell heterogeneity and establish methods to determine intracellular mechanisms contributing to airway disease in relevant cell systems.

bradykinin; bronchial microvasculature; fluorescein isothiocyanate-labeled dextran; sheep; thrombin

THE VASCULAR ENDOTHELIUM forms a selective barrier that is critical for limiting passage of plasma proteins and circulating cells between blood and the underlying tissues. Decreased endothelial cell barrier function is an important pathological alteration that leads to a variety of disease states. Within the airway wall, enhanced permeability of the dense bronchial vascular network results in airway edema, a prominent feature of inflammatory airway disease. Airway edema has been shown to contribute to airway narrowing (6), decreased clearance of bronchospastic agents (43), and hyperreactivity observed in asthma (26). A host of inflammatory mediators has been shown to cause fluid extravasation from the airway microvasculature in vivo (26). However, little is known regarding the specific mechanisms of endothelial barrier dysfunction in airways. Most studies exploring airway endothelial properties have utilized in vitro systems with endothelial cells derived from human umbilical veins or other organs. However, several recent studies have documented unique endothelial phenotypes based on the site or organ from which the cells were obtained. Both organ-specific and blood vessel size-specific endothelial cell heterogeneity have been demonstrated for proliferation in the presence of a variety of growth factors (3, 21), release of smooth muscle agonists (13), sensitivity to injury by activated neutrophils (25), ultrastructural differences in morphology (8), and barrier properties of confluent endothelial cell monolayers (7). Additionally, endothelial cells derived from large conduit pulmonary arteries show enhanced permeability relative to pulmonary microvessels (9). Whether the results from the pulmonary vasculature are representative of the systemic endothelial cells that comprise the airway vasculature is unknown. In vivo, airway mucosal microvessels demonstrate increased plasma extravasation when challenged with inflammatory mediators, whereas the large conduit arteries primarily regulate vascular tone (42). In a previous study, we demonstrated the feasibility of obtaining bronchial artery endothelial cells (BAEC) for primary culture (33). The goals of the present study were to further refine and extend these techniques to bronchial microvessels and to determine monolayer barrier properties of sheep BAEC and bronchial microvascular endothelial cells (BMVEC). The sheep was selected because the bronchial artery is easily accessible and large enough to collect adequate numbers of cells for culture. In this study, we compared the permeability of conduit endothelial cells relative to the microvascular endothelial cells that line the bronchial mucosa in response to the inflammatory agonists bradykinin and thrombin.

METHODS

Materials. Dulbecco's phosphate-buffered saline (DPBS), Dulbecco's modified Eagle's medium (DMEM), 10 mM MEM nonessential amino acids solution (100×), 10,000 U/ml penicillin/streptomycin, 25 μg/ml amphotericin B, 0.05% trypsin, 0.53 mM EDTA, and lamb serum were purchased from Life...

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Technologies (Gaithersburg, MD). 1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate-labeled low-density lipoprotein (Dil-AC-LDL) was obtained from Molecular Probes (Eugene, OR). FITC-10 dextran, FITC-70 dextran, antibody to human factor VIII/von Willebrand-associated antigen (vWF), 10 mg/ml of collagenase, 0.2% gelatin, 10 mM thiomarin, 1 μM bradykinin, and 0.1% crystal violet were obtained from Sigma (St. Louis, MO). Endothelial cell growth supplement (ECGS) was purchased from Upstate Biotechnology (Lake Placid, NY). Transwell cell culture inserts (6.5-mm diameter, 0.4-μm pore size) were from Costar (Cambridge, MA). Antibodies to platelet endothelial cell adhesion molecule (PECAM)-1 were from Santa Cruz Biotechnology (Santa Cruz, CA). Cloning disks were from Scineceware (Fisher, Pittsburgh, PA). [3H]bradykinin was from NEN (Boston, MA).

Isolation of BAEC. The bronchial branch of the bronchosophageal artery was cannulated with an 18-gauge angiocatheter (Criticath, Tampa, FL) using methods previously established (41). The maximum length of vessel that could be dissected from the lung was clamped and the vessel was gently flushed with 3 ml of DPBS to remove residual blood. Next, the vessel was filled with collagenase and clamped at both ends. After a 10-min incubation period at room temperature, the vessel was perfused with DMEM. The perfusate was collected in a tube and centrifuged at 500 g for 7 min. The cell pellet was resuspended in 2 ml of culture medium (DMEM with 20% lamb serum, 150 μg/ml ECGS, 100 U/ml penicillin/streptomycin, 0.25 μg/ml amphotericin B, and 0.1 mM MEM nonessential amino acids) and placed in a 0.2% gelatin-coated 35-mm tissue culture dish. After being incubated overnight at 37°C in 5% CO2-95% air, any nonadherent cells were removed, and 2 ml of fresh medium were added to the adherent cells. The medium was changed every 2 days thereafter. The BAECs were identified by their cobblestone morphology. After 5–7 days, areas of cells with this endothelial morphology were marked and, with the use of cloning disks, were digested in trypsin-EDTA and plated on 0.2% gelatin-coated 24-well plates. When cells were grown to confluence, cells were transferred to T-25 flasks and subcultured.

Isolation of BMVEC. The mainstem bronchi and subtended airways were dissected from sheep. The epithelial cell layer was removed, and the underlying tissue with microvessels to the level of cartilage was dissected free. These tissue pieces were placed in 1 ml of collagenase and incubated for 10 min at room temperature. After being incubated, 15 ml of DMEM were added, and cells were filtered through a nylon mesh. The collected cells were pelleted by centrifugation (500 g for 7 min), resuspended in 2 ml of culture medium (DMEM with 20% lamb serum, 150 μg/ml ECGS, 100 U/ml penicillin/streptomycin, 0.25 μg/ml amphotericin B, and 0.1 mM MEM nonessential amino acids), and placed in a 0.2% gelatin-coated 35-mm tissue culture dish. The remaining procedures were the same as those applied in the isolation of BAEC.

Growth curve. To establish growth curves for each of the bronchial endothelial subtypes, 3 × 10^5 cells were seeded onto 96-well plates. The medium was changed every 2 days. To assess optimal growth conditions, we studied six different media: 1) DMEM with 20% fetal calf serum (FCS) and endothelial cell growth factor (ECGF); 2) DMEM with 20% FCS, ECGF, and 100 μg/ml heparin; 3) DMEM with 20% FCS; 4) MCDB 131 with 20% FCS and ECGF; 5) MCDB 131 with 20% FCS, ECGF, and 100 μg/ml heparin; and 6) DMEM with 20% lamb serum and ECGF. Endothelial cell proliferation was determined by a modification of the procedure described by Kueng and colleagues (20). Cells were fixed by addition of formalin (3.7%), air-dried, and stained with 0.1% crystal violet in PBS for 20 min. Excess dye was removed by rinsing the cells (3× with PBS); the nuclear dye was eluted (33% acetic acid), and the optical density was measured (540 nm on Vmax Kinetic Microplate Reader; Molecular Devices, Sunnyvale, CA). Population doubling time was calculated in exponentially growing cells as described by Patterson (27).

Bradykinin binding assay. To confirm the presence of bradykinin B2 receptors on endothelial cells in culture, bradykinin binding assays were performed on confluent monolayers of endothelial cells in 12-well culture plates at 4°C using protocols described by Faussner and colleagues (11). Cells were washed three times with 2 ml of cold PIPES buffer (pH 7.4) containing 40 mM PIPES, 109 mM NaCl, 5 mM KCl, 0.1% dextrose, 0.05% BSA, 2 mM CaCl2, 1 mM MgCl2, 0.1 mM bacitracin, 0.01 mM phosphoramidon, and 0.1 mM captopril. The cells were equilibrated in this buffer for 30 min, and the binding reaction was subsequently initiated by the addition of [3H]bradykinin (0.1–30 nM) in the presence or absence of 5 μM unlabeled bradykinin. The reaction was terminated after 1 h by removal of the supernatant and washing of the cells (3×) with 2 ml of incubation buffer. A 0.5-ml aliquot of 0.3 M NaOH was added to each well, lysed cells were transferred to vials containing 9.5 ml of the scintillation fluid Aquazol (Packard Instrument, Meriden, CT), and the radioactivity was quantified by scintillation spectrometry (Beckman LS6000SC; Beckman Coulter, Somersett, NJ). Specific binding of [3H]bradykinin was calculated by subtracting nonspecific binding (determined in the presence of 5 μM unlabeled bradykinin) from total binding. Saturation studies of [3H]bradykinin were analyzed by the nonlinear curve-fitting program Graphpad Prism (San Diego, CA). In each experiment, two additional wells were used for determination of cell counts.

Permeability assay. Permeability assays to evaluate barrier function of confluent monolayers were performed using a modification of the protocol described by Kelly and colleagues (19). BMVEC and BAEC monolayers were seeded (40,000–50,000 cells/insert) on Transwell insert polycarbonate filters. The filters were treated for 1 h with 0.2% gelatin and air-dried before seeding endothelial cells. Medium was changed every day in the wells. Typically, monolayers were studied 3–4 days postseeding and after serum was removed 24 h before the studies of monolayer permeability. The transport was initiated by adding 1 mg/ml of FITC-labeled dextran in DMEM to the top chamber (in vol of 100 μl). The top chamber was removed and attached to a fresh bottom chamber (in a 24-well plate) containing 0.7 ml of DMEM. Transport across endothelial monolayers was assessed by measuring the flux of two different sized FITC-labeled dextrans (mol wt 9,500, 23A; mol wt 77,000, 60A). Aliquots (35 μl) were removed from the bottom chamber at the times specified for each experiment. The cells were kept at 37°C in a water bath and allowed to equilibrate for a period of 1 h before the addition of an agonist. α-Thrombin (100 nM) and bradykinin (1 μM) were dissolved in DMEM and added to the top chamber. Drug concentrations selected were based on the work of Ehringer and colleagues (10) and Aschner and colleagues (1). Measurements were made every 10 min during the equilibration time and at 5, 15, 30, 60, and 90 min after the addition of agonists. FITC-labeled dextran was measured in a fluorimeter (Aminco-Bowman series 2 spectrometer; SLM Instruments, Rochester, NY) using 480 and 530 nm as the excitation and emission wavelengths, respectively. The permeability coefficient (Pc) was calculated according to the method of Chang and colleagues (7). The transeendothelial flux (Jv) was determined for each monolayer as follows

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where \( \Delta C_t/\Delta t = \text{rate change of FITC-dextran concentration in abluminal medium and } V_a = \text{volume of abluminal medium}. \) PC was determined by

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PC = J_s/S \Delta C
\]

where \( S = \text{surface area of monolayer and } \Delta C = \text{concentration differential of FITC-dextran across monolayer}. \)

Statistical analysis. Data are expressed as means ± SE; \( n = \text{number of experiments}. \) Statistical evaluation was performed by nonpaired \( t \)-tests. \( P < 0.05 \) was accepted as significant.

RESULTS

Characterization of bronchial endothelial cells. BAEC and BMVEC maintained their morphological and functional characteristics throughout 10–15 passages. Cultures of BAEC and BMVEC showed the typical endothelial morphology of polygonal cells forming a contact-inhibited monolayer and cobblestone-like appearance. Figure 1 compares the general morphological characteristics of BAEC (Fig. 1, A–C) and BMVEC (Fig. 1, D–F). Both types of endothelial cells exhibited intense cytoplasmic staining after a 4-h incubation in DiI-Ac-LDL-supplemented media (Fig. 1, B and E) and were positive for endothelial marker staining for vWF (Fig. 1, A and D). Staining for PECAM-1 (Fig. 1, C and F) showed reactivity at the intercellular junctions, particularly when the cells were confluent.

Cell growth characteristics for BAEC and BMVEC in six different media are presented in Fig. 2, A and B, respectively: 1) DMEM with 20% FCS and ECGF; 2) DMEM with 20% FCS, ECGF, and 100 \( \mu \)g/ml heparin; 3) DMEM with 20% FCS; 4) MCDB 131 with 20% FCS and ECGF; 5) MCDB 131 with 20% FCS, ECGF, and 100 \( \mu \)g/ml heparin; and 6) DMEM with 20% lamb serum and ECGF. As shown from these growth curves, only the BMVEC demonstrated a substantially lower rate of growth in MCDB 131 media. Overall, BAEC exhibited a greater rate of cell proliferation than BMVEC. The optimal medium, DMEM with 20% lamb serum and ECGF, was chosen for subsequent permeability studies. Heparin did not alter the growth rate of the two cell types. Population doubling times during log-phase growth for BAEC was 39 h; for BMVEC, it was 65 h.

To confirm the expression of bradykinin \( B_2 \) receptors in cultured BAEC and BMVEC, we performed bradykinin receptor binding assays in three different experiments for both endothelial cell types. Figure 3 shows the results of receptor binding studies from one representative experiment for each endothelial cell type. BAEC revealed binding sites for \([^{3}H] \text{bradykinin} \) with a dissociation constant (\( K_d \)) of 5.60 ± 0.04 nM and a

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Fig. 1. Cultured bronchial artery endothelial cells (BAEC; A–C) and bronchial microvascular endothelial cells (BMVEC; D–F). Both types of endothelial cells were positive for factor VIII/von Willebrand-associated antigen (vWF; A and D), exhibited intense cytoplasmic staining after incubation in 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate-labeled low-density lipoprotein (DiAc-LDL)-supplemented media (B and E), and showed positive platelet endothelial cell adhesion molecule-1 (PECAM-1) staining at intercellular junctions (C and F).
maximum binding site density (B_max) of 35,389 ± 206 sites/cell (109 ± 1 fmol/mg of protein). Studies in BMVEC demonstrated a K_d of 6.00 ± 0.34 nM and a B_max of 35,701 ± 2,031 sites/cell (118 ± 12 fmol/mg of protein). Neither parameter differed between cell types (P > 0.05).

BAEC and BMVEC formed confluent cell monolayers on the semipermeable membrane within 3 days as evidenced histologically as well as by measurement of a stable transmembrane electrical resistance of 8,500–9,500 Ω/cm². Size-dependent differences in basal permeability were evaluated for BAEC and BMVEC. The basal permeability coefficient for BAEC determined for 9,500 mol wt FITC-dextran was 3.2 × 10^{-6} ± 3.4 × 10^{-7} cm/s. For 77,000 mol wt FITC-dextran it was 1.2 × 10^{-6} ± 2.6 × 10^{-7} cm/s. Basal permeability of BMVEC for 9,500 mol wt FITC-dextran was significantly greater than that for BAEC, demonstrating a permeability coefficient of 4.5 × 10^{-6} ± 4.4 × 10^{-7} cm/s (P = 0.009). However, the basal permeability coefficient determined with 77,000 mol wt FITC-dextran for BMVEC was not different (1.4 × 10^{-6} ± 1.7 × 10^{-7} cm/s) from BAEC (P > 0.05).

The time course of changes in barrier properties is presented for bronchial conduit endothelial cells (Fig. 4) and BMVEC (Fig. 5) in culture after bradykinin (Figs. 4A and 5A) and thrombin treatment (Figs. 4B and 5B). Bradykinin caused an increase in the PC of both cell types to 9,500 mol wt FITC-dextran, reaching a maximum at 15 min after treatment. The PC 15 min after bradykinin treatment for BAEC was 5.6 × 10^{-6} ± 0.9 × 10^{-6} cm/s (P = 0.027); for BMVEC, it was 7.8 × 10^{-6} ± 1.8 × 10^{-6} cm/s (P = 0.008). Despite different basal PCs, these two cell types did not differ in the magnitude of response to bradykinin (P > 0.05). These changes were reversed by 30 min, and the PC was not different from the basal level. However, no changes in the PC were seen after bradykinin treatment for 77,000 mol wt dextran in either cell type. Thrombin caused a small and more variable increase in the PC.
calculated for 9,500 mol wt FITC-dextran in BMVEC, which reached a maximum at 60 min ($9.6 \times 10^{-6} \pm 1.2 \times 10^{-6}$ cm/s; $P = 0.05$). The PC averaged for all time points after thrombin was greater than the vehicle average ($P = 0.042$). However, the PC for BAEC was not altered by thrombin treatment. Because this result was unexpected, we verified that BAEC expressed thrombin receptors [protease-activated receptor-1 (PAR-1)] by measuring the increase in intracellular calcium after agonist treatment since Ca$^{2+}$ influx and activation of Ca$^{2+}$-dependent signaling pathways have been confirmed to be essential determinants of thrombin-induced endothelial permeability (34). In both BAEC and BMVEC loaded with the Ca$^{2+}$-sensitive fluorescent dye fura 2-AM, a significant increase in intracellular Ca$^{2+}$ concentration was observed after thrombin challenge in both types of cells (data not shown). These results indicate the presence of functional thrombin receptors, although they do not explain the lack of thrombin permeability effects in BAEC. Furthermore, neither cell type demonstrated a change in permeability to 77,000 mol wt FITC-dextran after thrombin treatment.

DISCUSSION

Much of our understanding of the role played by endothelial cells in various physiological and pathological states has come from the in vitro study of endothelial cells in culture. We report the efficient isolation and characterization of sheep endothelial cells derived from the bronchial branch of the bronchoesophageal artery and from microvessels of the bronchial mucosa. Results demonstrate that cultured BAEC and BMVEC exhibit distinct differences in growth rate and responsiveness to inflammatory factors such as bradykinin and thrombin. Despite these differences, the general morphological features of both types of bronchial endothelial cell cultures were consistent with those of other endothelial cells (2). In vitro sheep BAEC and BMVEC displayed the characteristic cobblestone morphology of endothelial cell monolayers. Another distinguishing marker of endothelial cell phenotype is the ability to metabolize Ac-LDL (16). After 4 h of incubation with DiI-Ac-LDL, isolated bronchial endothelial cells showed strong uptake of this marker. Histological assessment of vWF expression on BAEC and BMVEC demonstrated granular cytoplasmic staining in both types of cells. Immunofluorescent staining showed that
BAEC and BMVEC possessed the endothelial-specific marker PECAM-1 (CD31), expressed at cell borders. These data confirm the endothelial nature of the isolates and provide markers to assess the purity of the cultures after isolation from collagenase-digested sheep vessels.

Because it was essential to optimize growth conditions for in vitro culture of the two cell types, we studied growth characteristics using six different combinations of growth factors/media for BAEC and BMVEC. The cell growth study revealed distinct proliferative rates in the two cell subtypes. Overall, BAEC demonstrated a higher proliferative phenotype than BMVEC. The doubling time for BAEC (39 h) was within the range reported for large vessel endothelial cell culture (17–42 h) (32) and similar to peripheral arteries such as the superficial temporal (47 h) and omental artery (43 h) (40). Although growth of BMVEC lagged BAEC, doubling time of BMVEC (65 h) was similar to the growth characteristics of other microvascular endothelial cells. Folkman and colleagues (12) reported a doubling time for bovine capillary endothelium of 67 h. Although the nutrient medium MCDB 131 has been shown to enhance the proliferation of human macrovascular endothelial cells in culture (39), it had a marked attenuating effect on the rate of BMVEC proliferation. Furthermore, heparin, a common mitogen frequently added to growth media, had no obvious effect on BAEC or BMVEC growth characteristics.

To assess barrier function of the bronchial endothelial cell subtypes, we studied two different sizes of labeled dextrans, applying the methods described by Kelly and colleagues (19). Diffusive translocation of FITC-dextran mol wt 9,500 and mol wt 77,000 is thought to occur primarily through intercellular gaps (17). Both basal permeability and agonist-induced changes in monolayer barrier function can be assessed with these fluorescent molecules, and the use of different sized dextrans provides an index of the size of endothelial gaps. Basal permeability of BMVEC was significantly greater than the basal permeability of BAEC. However, the PCs determined using FITC-dextran mol wt 9,500 are within the range of values published for FITC-albumin (mol wt 69,000, 35Å) in monolayers of other systemic arterial endothelial cells, such as bovine aortic endothelial cells, bovine retinal microvascular endothelial cells, and human umbilical vein endothelial cells (2–3 × 10⁻⁶ cm/s) (7). The basal PC derived for pulmonary artery endothelium for FITC-hydroxyethyl starch (23Å) has been reported to be approximately equivalent to that of systemic endothelial cells (1.8–3.0 × 10⁻⁶ cm/s) (35). However, there are significant differences between micro- and macrovascular endothelial cells derived from the pulmonary vascular tree (36). Several studies have shown that pulmonary microvascular endothelium provides a more restrictive barrier than pulmonary macrovascular endothelial cells at baseline (9). Scanning electron micrographs of rat microvascular pulmonary endothelium showed tight intercellular connections, whereas macrovascular endothelium had visible gaps between cells (19). Basal permeability properties of macrovascular and microvascular monolayers from endothelium within an organ have not been documented for many vascular beds. However, in the lung, it appears that basal permeability of systemic macrovascular and microvascular monolayers differs in a manner opposite to the pulmonary vasculature.

Because bradykinin has been shown to be a potent inflammatory substance causing plasma exudation into the airway mucosa/submucosa of asthmatic subjects (4) as well as in several animal models (22, 28, 43), we assessed differential monolayer responsiveness to this agonist. Because BMVEC were obtained from the bronchial mucosa, we expected that confluent monolayers of these cells would be more responsive to bradykinin than macrovascular endothelium derived from the large conduit bronchial artery. The biological effects of bradykinin are mediated through activation of at least two bradykinin receptor subtypes, which have been classified as B₁ and B₂ (31). The two classes of receptors differ in their affinities for bradykinin and responsiveness to specific agonists and antagonists (38). Endothelial cells have both B₁ and B₂ receptors (37). However, bradykinin binds to the B₂ receptor with higher affinity than other related kinin metabolites (18, 23). Our study of B₂ receptor binding characteristics confirmed the presence of B₂ receptors and demonstrated similar numbers of binding sites and bradykinin binding affinity in the two bronchial endothelial cell types. These receptors demonstrated a somewhat lower binding affinity (Kd = 5–6 nM) compared with that reported for bovine systemic and pulmonary endothelium (Kd = 0.5–2.0 nM) (24). However, it was within the range reported for cultured airway epithelial cells (29). The number of binding sites/cell (Bmax) was approximately equal to that which has been reported for bovine aortic endothelium (24).

Bradykinin caused a prompt and substantial increase in the permeability of both BAEC and BMVEC. Permeability increased to a maximum within 15 min and reversed rapidly; however, the magnitude of the response did not differ between BAEC and BMVEC. Although the rapidity of changes in intercellular gap formation was expected on the basis of previous reports (5), the original hypothesis proposed that BMVEC would show greater increases in permeability to inflammatory agents than BAEC. This hypothesis was based on published in vivo reports demonstrating the primary site of fluid extravasation in airways. Additionally, Grafe and colleagues (15) showed that macrovascular and microvascular endothelial cells from human hearts demonstrated differences in bradykinin degradation during inhibition of angiotosin-converting enzyme and neutral endopeptidase (NEP) enzyme activity with specific inhibitors. They also showed that microvascular cells in culture contain less NEP enzyme activity than macrovascular endothelial cells. Thus it was expected that differences in permeability would be observed for the two bronchial endothelial cell subtypes. However, the results of bradykinin re-

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receptor numbers and binding affinity are consistent with the permeability results.

We also studied the effects of thrombin since the difference between macrovascular and microvascular pulmonary endothelial permeability has been observed in response to this mediator and because thrombin-induced permeability alterations have been extensively characterized (1). Thrombin, binding to PAR-1, has been shown to cause rapid (within 10 min) protein kinase C-dependent alterations in endothelial permeability, primarily through interendothelial cell gap formation (14). Differences in the response to thrombin have been observed in different endothelial cell types, with microvascular endothelial cells demonstrating particular sensitivity (30). Permeability of BMVEC monolayers increased after thrombin treatment; however, BAEC were resistant at the applied thrombin concentration. These results might be explained by the observation that PAR-1 has been shown to be poorly concentrated after thrombin challenge in both types of cells. Although these results indicate the presence of functional thrombin receptors, they do not explain the lack of thrombin permeability effects in BAEC. Overall, these results emphasize endothelial heterogeneity with regard to barrier function and underscore the importance of evaluating the appropriate endothelial cell type.

To conclude, this study represents the first report of successful isolation and characterization of vascular endothelial cells derived from the airway microvasculature. While assessing macromolecule permeability across confluent monolayers, BMVEC showed a less restrictive barrier than BAEC. Both endothelial cell types responded similarly to bradykinin; however, only microvascular endothelial cells were sensitive to thrombin. This study establishes an improved methodological approach to the study of mechanisms of airway vascular endothelial barrier dysfunction.

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