VEGF induces hyperpermeability by a direct action on endothelial cells

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Hippensiel, S., M. Krüll, A. Ikemann, W. Risauf, M. Clauss, and Norbert Suttorm. VEGF induces hyperpermeability by a direct action on endothelial cells. Am. J. Physiol. 274 (Lung Cell. Mol. Physiol. 18):1778-1784, 1998.—Vascular endothelial growth factor (VEGF) is a key regulator of vasculo- and angiogenesis. Earlier studies demonstrated a permeability-increasing effect of VEGF in skin tests, leading to its other name, vascular permeability factor. We wondered whether VEGF-induced hyperpermeability was a direct effect of VEGF on endothelial cells and studied the permeability of human and porcine endothelial cell monolayers in a well-characterized in vitro system. VEGF increased the hydraulic conductivity up to 20-fold and simultaneously decreased the albumin reflection coefficient. This effect occurred after a delay of 150 min, although VEGF-induced early endothelial cell activation was verified by enhanced inositol phosphate accumulation within 5 min and increased P-selectin expression within 15 min. Platelet-derived growth factor and granulocyte-macrophage colony-stimulating factor, two endothelial cell nonspecific mitogens, were without effect on endothelial permeability. The increase in intracellular cyclic nucleotide levels of human endothelial monolayers abolished VEGF-induced endothelial hyperpermeability. In summary, VEGF increased endothelial permeability by a direct action on endothelial cells. Based on the pattern of endothelial cell activation by growth factors, VEGF appears to be a unique stimulus.

vascular endothelial growth factor; cultured human endothelial cells; hydraulic conductivity; adhesion molecules; platelet-derived growth factor; granulocyte-macrophage colony-stimulating factor

VEGF induces hyperpermeability by a direct action on endothelial cells. On intradermal injection or topical application to muscle preparations, VEGF indeed caused a hyperpermeability that occurred within minutes (9, 18, 21). In the case of sustained hypoxia, VEGF action, therefore, would be twofold: a rapid increase in endothelial permeability for enhanced substrate supply and, in the long run, formation of new capillaries.

VEGF, the best-studied VEGF variant, is secreted as a 46-kDa, heparin-binding homodimeric glycoprotein by a variety of cells and acts almost exclusively on endothelial cells by binding with high affinity to the cognate tyrosine kinase receptors VEGFR-1 (flt-1) and VEGFR-2 (KDR/flk-1) (7, 14). At least four isoforms of human VEGF mRNA encoding VEGF proteins of 121, 165, 189, and 206 amino acids are produced as a result of alternative splicing from a single gene (26).

The endothelium provides a major permeability barrier of the vessel wall. Under resting conditions, extravasation of macromolecules is highly restricted, whereas small molecules use para- and transcellular pathways to cross capillaries rapidly. In inflammed tissues, stimulation of endothelial cells with thrombin, hydrogen peroxide, or bacterial toxins will result in cell retraction accompanied by opening of intercellular gaps, thereby allowing enhanced paracellular fluid flux (15, 22).

The mechanisms that underlie VEGF-induced vascular hyperpermeability are unclear. The in vivo studies mentioned did not differentiate between VEGF-induced alterations of the local hemodynamics, with possibly increased filtration pressures; VEGF-related activation of inflammatory cells; and/or a direct effect of VEGF on the endothelium itself. The demonstration of a rapid fenestration of the endothelium in cremaster vessels as well as the recent description of vesiculovascular organelles (VVO) in skin endothelial cells after VEGF exposure points to the endothelium as a possible direct target of the VEGF action in vivo (9, 18).

We therefore analyzed the effects of VEGF on the permeability of human endothelial cell monolayers in a well-characterized in vitro system. The results indicated that VEGF increased the hydraulic conductivity up to 20-fold and simultaneously decreased the albumin reflection coefficient (RC) of human endothelial monolayers, suggesting that VEGF increases permeability by direct action on endothelial cells. Platelet-derived growth factor (PDGF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), two endothelial cell nonspecific mitogens, were without effect in this system. Interestingly, VEGF-related hyperpermeability occurred after a delay of 150 min. To verify early endothelial cell activation, we also studied stimulation of phosphatidylinositol metabolism and P-selectin expression in cultured human endothelial cells.
METHODS

Materials. Tissue culture plasticware was obtained from Becton Dickinson (Heidelberg, Germany). Medium 199, FCS, Hanks’ balanced salt solution (HBSS), PBS, trypsin-EDTA solution, Puck’s A, HEPES, and antibiotics were from Gibco (Karlsruhe, Germany). Excell 400 medium was from Biochrom (Munich, Germany). Collagenase (CLS type II) was purchased from Worthington Biochemical (Freehold, N J). Gelatin from porcine skin type I, glutaraldehyde grade II, sodium nitroprusside (SNP), borax (Na2B4O7·10H2O), thrombin, GM-CSF, PDGF-AB, and cholera toxin were purchased from Sigma (Munich, Germany). Polycarbonate micropore filter membranes (25-mm diameter, 5-µm pore size) were purchased from Nuncopore (Tübingen, Germany). 3H2O (1 mCi/mg), [methyl-3H]albumin (0.026 mCi/mg), [methyl-3H]inositol (535 mCi/mg), and Na251CrO4 (1 mCi/ml) were from Amersham Buchler (Braunschweig, Germany). All other chemicals used were analytic grade and obtained from commercial sources.

Purification of VEGF. Human VEGF 165 cDNA, expressed in the Pichia pastoris expression system, was kindly provided by Dr. B. Hafen (Institut für Biochemie und Biophysik, Universität zu Köln, Germany). Pichia pastoris transfer vector pVL 1393, was used to infect SF9 insect cells. Human recombinant VEGF was purified from conditioned serum-free Excell 400 medium of the infected SF9 cells as previously described (4). Briefly, 100 ml of conditioned medium were applied to a heparin high trap (Pharmacia, Freiburg, Germany) equilibrated with 50 mM phosphate buffer (pH 7.0) and 120 mM NaCl. The column was eluted with high-performance liquid chromatography (SmartSystem Pharmacia, Freiburg, Germany) with an ascending salt gradient (0–1.5 M NaCl), and fractions were assessed for purity by SDS-PAGE with the PhastGel apparatus (Pharmacia) and subsequent staining as previously described (4). For final purification (a single band in SDS-PAGE), it was sufficient to repeat the liquid chromatography one or two times.

Monoclonal antibodies. Purified freeze-dried monoclonal antibodies (MAbs) directed against P-selectin (CLB/thromb6) were obtained from Dianova (Hamburg, Germany), and MAbs directed against intercellular adhesion molecule (ICAM-1; RR1/1) and E-selectin (H18/7) were from Serva (Heidelberg, Germany). Horseradish peroxidase-conjugated polyclonal sheep anti-mouse IgG antibodies were purchased from Amersham (Dreieich, Germany). All antibodies used were azide free. To further characterize the adhesion system, endothelial cells were preincubated with 20 µg/ml of inhibitory MAb for 30 min.

Preparation of human umbilical cord vein endothelial cells. Cells were isolated from umbilical cord veins and identified as previously described (12). Isolated endothelial cells were seeded in tissue culture flasks (80 cm2) or on 6-, 24-, or 96-well plates (Becton Dickinson). Confluent primary cultures of human umbilical cord vein endothelial cell (HUVEC) monolayers were used for quantification of adhesion molecules (cell-surface ELISA), polymorphonuclear neutrophil (PMN) adhesion assay, and determination of inositol phosphates. Endothelial monolayer permeability (hydraulic conductivity and albumin RC) was determined using HUVECs in their third and, in selected experiments, first passages.

Preparation of porcine endothelial cell monolayers. Endothelial cells were isolated from pulmonary arteries of freshly slaughtered pigs by exposure to 0.1% collagenase in Puck’s saline for 12–15 min at 37°C. Cells were dispersed, characterized, and maintained in medium 199–10% FCS in a humidified atmosphere (37°C, 5% CO2) as previously described (11, 22–25). Permeability studies performed were done using confluent porcine endothelial cell monolayers in their third and, in selected experiments, first passages.

Isolation and labeling of human PMNs. Heparinized human donor blood was centrifuged in a discontinuous Percoll gradient to yield a PMN fraction of >97% purity as previously described (12). Freshly isolated neutrophils were radiolabeled with Na251CrO4 (1 mCi/ml). Briefly, after isolation, PMNs were incubated with 100 µCi of 51Cr at 37°C for 1 h in RPMI 1640 medium containing 10% FCS. Subsequently, the cells were washed twice in HBSS (with calcium, without magnesium) to remove unincorporated 51Cr.

Determination of hydraulic conductivity and albumin RC. Endothelial cell monolayers were grown on polycarbonate filter membranes as previously described (11, 22–25). A confluent monolayer on a filter membrane was mounted in a modified chemotaxis chamber, and a hydrostatic pressure of 10 cmH2O was applied to the “luminal” side of the cell monolayer. The filtration rate across the endothelial monolayer was continuously determined, and the hydraulic conductivity was calculated and expressed as 10−5 cm/sec per centimeter of water. For calculation of the selectivity of the endothelial monolayer, 3H2O (1 mCi/g) and [methyl-3H]albumin (0.026 mCi/mg) were added to the upper compartment. The amount of 3H2O and [methyl-3H]albumin in the lower compartment was continuously measured with a Ramona L-5 radioactivity monitor (Raytest, Heidelberg, Germany). The albumin RC was calculated on the basis of 3H2O and [methyl-3H]albumin in the upper and lower compartments of the filter system as previously described (11, 22–25).

Experimental protocol. Only monolayers that showed a final hydraulic conductivity of <0.5×10−5 cm·s−1·cmH2O−1 in the presence of a hydrostatic pressure of 10 cmH2O were used (“sealed” filters; see Ref. 24 for details). In all experiments, VEGF was added as a bolus into the upper compartment at time 0. SNP was applied as a bolus at time point −5 min. SNP was also added to the fluid reservoir that provided the hydrostatic pressure to the upper compartment. Thus fluid filtrated from the upper into the lower compartment was replaced by SNP-containing buffer from the reservoir. Cholera toxin was given 60 min before the addition of VEGF.

PMN adhesion to HUVEC monolayer. The medium was aspirated, and the endothelial cells were washed twice with HBSS. 51Cr-labeled PMNs (1×106 in 1 ml of buffer) were added to each well (24-well plate). The cell mixture was stimulated with VEGF or thrombin for 3–30 min. Subsequently, unbound PMNs were removed by gentle aspiration, and each well was washed twice with HBSS. Adherent PMNs and endothelial cells were lysed with 2 H2SO4 for 30 min. Radioactivity of the lysate was quantitated with a γ-counter (Cobra Autogamma B5003, Canberra Packard, Frankfurt, Germany). The percentage of PMN adhesion was calculated as the 51Cr fraction in the lysate in relation to the total radioactivity added (12).

Cell-surface ELISA for P-selectin, ICAM-1, and E-selectin expression on HUVECs. Expression of adhesion molecules on monolayers of human endothelial cells was determined with a cell-surface ELISA technique (12). Briefly, confluent HUVEC monolayers in 96-well flat-bottom microtiter plates were washed; stimulated with VEGF, GM-CSF, PDGF, thrombin, or lipopolysaccharide as indicated; and finally fixed with 4% paraformaldehyde. Human Ig was used to reduce nonspecific binding, and primary antibodies were added for 30 min. Thereafter, the cells were washed three times and exposed to a horseradish peroxidase-conjugated rabbit anti-mouse IgG antibody for 30 min. After the cells were washed, o-phenylenediamine was added for 5 min. Data are given as optical density at 492 nm.

Total inositol phosphates. The sum of inositol mono-, di-, and triphosphate was determined, with minor modifications,
RESULTS

Sealed endothelial cell monolayers of human and porcine origin displayed a hydraulic conductivity of 
\(<0.5 \times 10^5 \text{ cm} \cdot \text{s}^{-1} \cdot \text{cm} \cdot \text{H}_2\text{O}^{-1}\) and an albumin RC of 
\(>0.75\). These in vitro data compare very well to corresponding values in the microcirculation (16). A bolus addition of VEGF (0.1–100 pg/ml) to HUVECs time and dose dependently increased the hydraulic conductivity of the cell monolayers up to 20-fold. The effect occurred after a delay of ~150 min. In contrast, thrombin (1 U/ml) increased the permeability of HUVEC monolayers maximally within 30 min (Fig. 1). Control cell monolayers were stable throughout the experimental period and reacted promptly on addition of staphylococcal α-toxin (10 µg/ml), a well-known permeability-increasing agent (23, 24) (Fig. 1). VEGF-induced hyperpermeability was accompanied by a reduction in the selectivity of the HUVEC monolayer as indicated by the drop in the albumin RC from 0.8 to 0.4 in the presence of 100 pg/ml of VEGF (Fig. 2). In control monolayers, this parameter was stable for at least 5 h. VEGF (and thrombin) also increased the hydraulic conductivity of porcine pulmonary artery endothelial cell monolayers with similar kinetics, although the VEGF (and thrombin) dose-response curve was shifted to the right in the porcine system (Table 1).

Studies applying electron microscopy revealed the presence of VVO and fenestrae in VEGF-treated HUVECs on filter membranes after 150 min of exposure to 100 ng/ml of VEGF (S. Hippenstiel, H. Wolburg, and N. Suttrop, unpublished observations).

We wished to extend the observations made to endothelial cell nonspecific growth factors and exposed endothelial cell monolayers to GM-CSF (10 pg/ml to 100 ng/ml) and PDGF (10 pg/ml to 100 ng/ml). Both mitogens had no effect on endothelial monolayer permeability within the time frame tested (5 h; Table 1).

Previous studies (23, 25) have shown that endothelial hyperpermeability provoked by thrombin, hydrogen peroxide, or bacterial toxins was blocked by increased endothelial nucleotide levels. In detail, activation of adenyllyl or guanylyl cyclase with cholera toxin or nitric oxide, respectively, proved to be a very effective measure to antagonize enhanced endothelial monolayer permeability (23, 25). Similarly, pretreatment of HUVEC monolayers with \(10^{-6} \text{ M} \) cholera toxin or \(10^{-6} \text{ M} \) SNP abolished VEGF-induced endothelial hyperpermeability (Fig. 3). These pretreated cell mono-

![Fig. 1. Vascular endothelial growth factor (VEGF) time and dose dependently increased hydraulic conductivity (used as a measure of monolayer permeability) of cultured human umbilical venous endothelial cell (HUVEC) monolayers. VEGF effects are shown in comparison to thrombin. Stimuli (VEGF and thrombin) were added as a bolus at time 0 (arrow). Control monolayers were stable throughout experimental period and responded promptly on addition of staphylococcal α-toxin, an established permeability-increasing agent. Data are means ± SE of 4 separate experiments.](http://ajplung.physiology.org/)

![Fig. 2. VEGF decreased selectivity of endothelial cell monolayers. Resting (sealed) cell monolayers displayed a hydraulic conductivity (HC) of \(<0.5 \times 10^5 \text{ cm} \cdot \text{s}^{-1} \cdot \text{cm} \cdot \text{H}_2\text{O}^{-1}\) and an albumin reflection coefficient (RC) of >0.8. VEGF increased HC substantially and decreased RC to 0.4. Both permeability parameters were determined simultaneously on the same endothelial monolayer. Data are means ± SE of 4 separate experiments.](http://ajplung.physiology.org/)
and porcine endothelial cell monolayers on hydraulic conductivity of human Table 1. Effect of VEGF, GM-CSF, and PDGF

<table>
<thead>
<tr>
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<th>Hydraulic Conductivity, (10^{-5} \text{cm} \cdot \text{s}^{-1} \cdot \text{cmH}_2\text{O}^{-1})</th>
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<tr>
<td></td>
<td>(n)</td>
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<tr>
<td>HUVEC</td>
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<td>VEGF (100 pg/ml)</td>
<td>4</td>
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<td>PDGF (100 pg/ml)</td>
<td>4</td>
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<tr>
<td>GM-CSF (100 pg/ml)</td>
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<tr>
<td>Thrombin (1 U/ml)</td>
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<tr>
<td>Control</td>
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<tr>
<td>Porcine pulmonary artery endothelial cells</td>
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<tr>
<td>VEGF (pg/ml)</td>
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<td>10</td>
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<tr>
<td>GM-CSF (100 pg/ml)</td>
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Values are means ± SE; \(n\), no. of experiments. VEGF, vascular endothelial growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; PDGF, platelet-derived growth factor; HUVEC, human umbilical vein endothelial cells; ND, not determined. Thrombin rapidly increased endothelial permeability in both endothelial cell types, whereas VEGF acted with a delay. GM-CSF and PDGF had no effect on endothelial permeability in either cell type within time frame tested. *Significantly different from time-matched control cells, \(P < 0.05\).
system now clearly established the capability of VEGF to increase endothelial monolayer permeability by a direct action on endothelial cells. It is important to point out that our in vitro assay determines the permeability of sealed endothelial cell monolayers under convective conditions (22). A study (16) in isolated, ventilated, and perfused rabbit lungs indicated an RC of 0.7 for albumin in the pulmonary microvasculature, a value similar to our in vitro data (Fig. 2). Hydraulic conductivity in our permeability system also compares very well with corresponding values in the microcirculation in situ.

The mechanisms of the VEGF effect at the cellular level are unclear. One interesting feature was the pronounced delay of VEGF-related hyperpermeability. Compared with the fast thrombin effect at 15 min, VEGF-induced hyperpermeability occurred after 150 min as the earliest time point. This discrepancy was noted, although similarly rapid cell activation could be demonstrated after both stimuli as indicated by the prompt phosphoinositol accumulation and P-selectin expression.

A pronounced delay in VEGF-related hyperpermeability was also observed in cultured brain microvessel endothelial cells (29). In these cell preparations, VEGF increased permeability after 5–50 h of VEGF incubation (29). Bates and Curry (2) studied frog mesenteric microvessels using the Landis technique and noted a rapid increase in permeability within 30 s and a second substantial permeability peak after 24–48 h. Taken together, VEGF appears to be a

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**Table 2. Effect of VEGF, GM-CSF, and PDGF on expression of P-selectin, E-selectin, and ICAM-1 in HUVEC monolayers**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Optical Density at 492 nm</th>
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<tr>
<td></td>
<td>P-selectin (5 min)</td>
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<tr>
<td>VEGF (25 pg/ml)</td>
<td>0.13 ± 0.05*</td>
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<tr>
<td>PDGF (0.1 pg/ml)</td>
<td>0.14 ± 0.03*</td>
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<tr>
<td>GM-CSF (100 pg/ml)</td>
<td>0.18 ± 0.04*</td>
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<tr>
<td>Thrombin (0.1 U/ml)</td>
<td>0.20 ± 0.04*</td>
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<tr>
<td>LPS (10 ng/ml)</td>
<td>0.01 ± 0.01</td>
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Values are means ± SE of 4 separate experiments. Δ, Change in.

Expression of P-selectin, E-selectin, and intercellular adhesion molecule-1 (ICAM-1) was determined using cell-surface ELISA techniques as described in Methods. All stimuli (besides lipopolysaccharide (LPS)) induced a rapid increase in P-selectin cell-surface expression, whereas only LPS upregulated E-selectin and ICAM-1 expression. *Significantly different from time-matched control cells that displayed an absolute optical density of 0.09 ± 0.02 at 492 nm, P < 0.05.

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**Fig. 4.** VEGF dose dependently increased expression of P-selectin on HUVECs (left) and P-selectin-mediated polymorphonuclear neutrophil (PMN) adhesion to HUVECs (right). Effect was comparable to thrombin and occurred rapidly within 3–5 min. VEGF-induced PMN adhesion was almost completely blocked by preincubation of endothelial cells with 20 µg/ml of anti-P-selectin-antibodies. Data are means ± SE of 4 separate experiments.

**Fig. 5.** Exposure of HUVECs to VEGF resulted in a time- and dose-dependent accumulation of total inositol phosphates (consisting of inositol mono-, di-, and trisphosphate). Twenty-five picograms of VEGF per milliliter turned out to be as active as 0.3 U/ml of thrombin. Platelet-derived growth factor (PDGF; 25 pg/ml) and granulocyte-macrophage colony-stimulating factor (GM-CSF; 25 pg/ml) also increased inositol phosphates. Data are means ± SE of 3 separate experiments.
unique stimulus with respect to the induction of endothelial permeability by growth factors.

Only little is known about VEGF-related signal transduction. VEGF acts by binding with high affinity to the receptors VEGFR-1 (flt-1) and VEGFR-2 (KDR/flk-1) (7, 14). Receptor occupation results in dimer formation, activation of the tyrosine kinase domains, and autophosphorylation of the receptors (6). Multiple proteins such as p125FAK and paxillin are tyrosine phosphorylated (1). Phospholipase C-γ is also tyrosine phosphorylated and activated, leading to inositol phosphatase formation, an intracellular Ca²⁺ rise, and protein kinase C activation (3, 30). VEGF also increased phosphatidylinositol 3-kinase activity (30). Moreover, activation of a 44- and 42-kDa mitogen-activated protein kinase was recently demonstrated in VEGF-stimulated bovine brain capillary endothelial cells (6). Finally, p38 mitogen-activated protein kinase activation and subsequent actin reorganization was noted in human endothelial cells after VEGF stimulation (19).

The signals generated after VEGF addition will ultimately result in enhanced permeability, although the individual steps involved remain to be established. In this context, the inhibition of VEGF-induced hyperpermeability by increasing intracellular cyclic nucleotide levels is of interest. Previous studies (23, 25) have shown that endothelial hyperpermeability provoked by thrombin, hydrogen peroxide, or bacterial toxins was blocked by high endothelial nucleotide levels. Activation of adenyllyl or guanyllyl cyclase with cholera toxin or nitric oxide, respectively, turned out to be a very effective measure to antagonize enhanced endothelial monolayer permeability (23, 25). Protein kinases A and G are important participants in the continuous cross-talk between different second messenger systems, and it is therefore conceivable that adenyllyl or guanyllyl cyclase activation can interfere with VEGF-related signal transduction pathways. Consistent with this notion, a protein kinase A-related inhibition of the mitogenic action of VEGF in capillary endothelial cells as well as a reciprocal relationship between VEGF and nitric oxide in the regulation of endothelial integrity was reported very recently (5, 27).

The mechanisms that finally induce enhanced fluid flux across the endothelium remain unclear. In previous studies (11, 24), disruption of the endothelial cell microfilament system, provoked by two highly selective tools, Clostridium botulinum C2 toxin and C. difficile B toxin, resulted in endothelial cell retraction, thereby opening intercellular gaps that facilitated increased paracellular permeability. These alterations were unchanged by increased endothelial nucleotide levels (11), and these considerations, therefore, do not apply for the VEGF study presented.

In other studies (15, 22, 23, 25) using bacterial exotoxins, thrombin, hydrogen peroxide, or a calcium ionophore, we and others obtained evidence for endothelial cell retraction. In subsequent experiments performed in the presence of high endothelial nucleotide levels, enhanced permeability was reversed, suggesting that endothelial cell relaxation can counteract hyperpermeability (23, 25). Thus there is a parallelism to the VEGF study presented, but morphological information obtained by light microscopy did not provide evidence for VEGF-induced endothelial cell retraction with the occurrence of large gaps between endothelial cells.

A third possibility relates to the formation of small transient pores that are not readily resolved by light microscopy as described by Schaeffer et al. (20) for bradykinin as the stimulus. Moreover, VEGF-related induction of rapid fenestration of the endothelium was shown by Roberts and Palade (18). Topical application or intradermal injection of VEGF increased microvascular permeability and induced endothelial fenestration of postcapillary venules and capillaries in the cremaster muscle and skin (18).

Finally, the recent description of VVO in skin endothelial cells after VEGF injection appears to identify a fundamental mechanism that may be instrumental in understanding the increased permeability (9). According to this study, VVO, which can occupy up to 18% of the endothelial cytoplasm, represent an efficient shuttle system across the endothelium. Initial studies applying electron microscopy revealed the presence of VVO as well as fenestrae in VEGF-treated HUVECs on filter membranes after 150 min of exposure to 100 ng/ml of VEGF, suggesting that these structures provide the basis for VEGF-induced enhanced endothelial monolayer permeability (S. Hippenstiel, H. Wolburg, and N. Suttrop, unpublished observations).

The interpretation of our study is limited because cultured HUVECs and porcine pulmonary artery endothelial cells were used. For an exact analysis of the alterations of endothelial barrier function in clinical disorders, it would be desirable to study human endothelial cells of different vascular levels (arteriole, capillary, and venule) and of different organs. The culture of human pulmonary microvascular endothelium is difficult, and, therefore, the applicability of the data presented to human pulmonary disease is not clear.

In this context, the pronounced temporal discrepancy between in vivo (within 30 min) and in vitro effects (after 150 min) of VEGF on endothelial permeability may be related to differences between endothelial cells of micro- and macrovessels. On the other hand, in vivo systems are complex, and VEGF-induced hyperpermeability may be a combined effect of VEGF action on the endothelium, blood cells, and humoral factors (2, 4, 9, 13, 18, 21).

In conclusion, VEGF, a key regulator of vasculo- and angiogenesis, induced endothelial hyperpermeability by a direct action on endothelial cells. Compared with other growth factors, VEGF appears to be a unique stimulus.

Hypoxia is the strongest stimulus for VEGF synthesis and VEGF-receptor expression as demonstrated in rat lungs exposed to acute and chronic hypoxia (28). Subsequently, VEGF may increase endothelial permeability that enhances substrate supply, which, in turn, provides the basis for vascular remodeling, a process that represents a common feature of many chronic lung diseases.
REFERENCES


