Vascular Endothelial Growth Factor/Vascular Permeability Factor Enhances Vascular Permeability Via Nitric Oxide and Prostacyclin

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Background—Vascular endothelial growth factor (VEGF), an endothelial cell mitogen that promotes angiogenesis, was initially identified as a vascular permeability factor (VPF). Abundant evidence suggests that angiogenesis is preceded and/or accompanied by enhanced microvascular permeability. The mechanism by which VEGF/VPF increases vascular permeability (VP), however, has remained enigmatic. Accordingly, we used an in vivo assay of VP (Miles assay) to study the putative mediators of VEGF/VPF-induced permeability.

Methods and Results—VEGF/VPF and positive controls (platelet-activating factor [PAF], histamine, and bradykinin) all increased vascular permeability. Prior administration of the tyrosine kinase inhibitors genistein or herbimycin A prevented VEGF/VPF-induced permeability. Placenta growth factor, which binds to Flt-1/VEGF-R1 but not Flk-1/KDR/VEGF-R2 receptor tyrosine kinase, failed to increase permeability. Other growth factors such as basic fibroblast growth factor (FGF), acidic FGF, platelet-derived growth factor–β, scatter factor, and granulocyte macrophage-colony stimulating factor (8 to 128 ng) failed to increase permeability. VEGF/VPF-induced permeability was significantly attenuated by the nitric oxide (NO) synthase inhibitors Nω-nitro-L-arginine (10 mg/kg) or Nω-nitro-L-arginine methyl ester (20 mg/kg) and the cyclooxygenase inhibitor indomethacin (5 mg/kg). The inactive enantiomer Nω-nitro-D-arginine methyl ester (20 mg/kg) did not inhibit VEGF/VPF-induced permeability. In vitro studies confirmed that VEGF/VPF stimulates synthesis of NO and prostaglandin metabolites in microvascular endothelial cells. Finally, NO donors and the prostacyclin analogue taprostene administered together but not alone reproduced the increase in permeability observed with VEGF/VPF.

Conclusions—These results implicate NO and prostacyclin produced by the interaction of VEGF/VPF with its Flk-1/KDR/VEGF-R2 receptor as mediators of VEGF/VPF-induced vascular permeability. Moreover, this property appears unique to VEGF/VPF among angiogenic cytokines. (Circulation. 1998;97:99-107.)

Key Words: endothelium ■ growth substances ■ nitric oxide ■ prostaglandins ■ permeability
Protocol 4: Role of Receptor Tyrosine Kinase in VEGF/VPF-Induced VP

The receptor tyrosine kinases Flk-1/KDR/VEGF-R2 and Flt-1/VEGF-R1 bind VEGF/VPF with high affinity. 28–31 Accordingly, we tested the effect of the tyrosine kinase inhibitors genistein and herbimycin A on VEGF/VPF-stimulated VP. Ten minutes after administration of EBD, saline (control), genistein (100 µmol/L), or herbimycin A (1 µmol/L) was intradermally injected as 0.15 mL volume. Ten minutes later (ie, 20 minutes after EBD), the maximum dose of VEGF/VPF used here to stimulate permeability (128 ng) was injected in 0.1 mL volume into the identical dermal area.

Although VEGF/VPF binds to both Flt-1/VEGF-R1 and Flk-1/KDR/VEGF-R2 receptor tyrosine kinases, PAF has been shown to bind principally to Flk-1/VEGF-R1. 32–34 To further determine which receptor tyrosine kinase is responsible for VEGF/VPF-induced VP, we tested PGF (R&D Systems) (8 to 128 ng) on VP as well.

Protocol 5: Role of NO in VEGF/VPF-Induced Vascular Permeability

Two NO synthase inhibitors, L-NNA (10 mg/kg) or L-NAME (20 mg/kg) were used to investigate the role of NO on VEGF/VPF-induced VP. These were injected through the penile or femoral vein immediately before administration of EBD. Twenty minutes later, (a) saline alone (negative control), (b) VEGF/VPF (8, 16, 32, and 64 ng), or (c) PAF (0.01, 0.1, 1, 10, and 100 µmol/L) was injected intradermally and VP assessed.

To exclude a nonspecific action of L-NAME, the effect of the inactive enantiomer D-NAME (20 mg/kg) on VEGF/VPF-induced VP was also tested. D-NAME was injected through the penile vein before administration of EBD. At 20 minutes after EBD, (a) saline alone or (b) VEGF/VPF (8, 16, 32, 64, and 128 ng) was injected intradermally.

To assess whether NO donors can mimic VEGF/VPF-induced VP, we administered NO donors, SNAP (10, 50, 100, and 500 µmol/L) or S-nitroso-N-acetyl penicillamine (SNAP) (10, 100, and 500 µmol/L in 0.1 mL solution). At 20 minutes after EBD administration, (a) saline alone, (b) SNP or SNAP, or (c) PAF was injected intradermally.

Protocol 6: Role of Prostaglandin(s) in VEGF/VPF-Induced VP

To examine a role for prostaglandins in VEGF/VPF-induced VP, indomethacin (5 mg/kg), a cyclooxygenase inhibitor, was injected intraperitoneally, 26 before EBD administration. At 20 minutes after EBD injection, (a) saline, (b) VEGF/VPF (8, 16, 32, and 64 ng), or (c) PAF was injected intradermally.

To assess whether prostacyclin, a major metabolite of arachidonic acid, can mimic VEGF/VPF-induced VP, we investigated the direct effect of intradermal injection of prostacyclin or its stable analog taspertone. 35 At 20 minutes after EBD, (a) saline, (b) prostacyclin or taspertone (0.01, 0.1, 1, and 10 µmol/L), or (c) PAF was injected intradermally.

Finally, SNP and taspertone were mixed together and injected intradermally to assess the combined activity of NO and prostacyclin in enhancing VP.

Protocol 7: Effect of NO-Independent Vasocostriction on VP

To exclude the possibility that inhibition of VEGF/VPF-induced VP by L-NAME was simply the result of L-NAME–induced vasocostriction, we examined the effects of phenylephrine on VEGF/VPF-induced VP. Phenylephrine was administered intravenously to achieve a comparable increase in systemic blood pressure (BP) to that resulting from L-NAME (20 mg/kg). After administration of sodium pentobarbital (60 mg/kg IP), a 22-gauge cannula was inserted into the left common carotid artery for BP monitoring. After similar increases in

Miles Assay

We used male hairless albino guinea pigs (weight, 200 to 600 g, n = 30) (Charles River Laboratories). These guinea pigs are euthyemic and immunocompetent. Animals were lightly anesthetized with ether (Fisher Scientific). A solution of EBD (Sigma) (0.5% in saline) was filtered through a 0.2-µm microfuge filter (Corning) before use. The animals were then given 0.5 to 1.0 mL of 0.5% EBD through the left femoral vein. EBD binds to circulating plasma proteins and extravasates in response to certain reagents (vide infra), rendering hyperpermeable dermal sites blue. 14 After the animals regained consciousness, various reagents were injected intradermally in volume of 0.1 mL. Intradermal injections were made into the trunk posterior to the shoulder 20 minutes after intravenous injection of EBD with a 30-gauge needle (Becton Dickinson), causing a bleb 9 to 11 mm in diameter. Increase in VP was assessed by the leakage of blue dye into the bleb. 14 As originally described by Miles and Miles, 14 a small area of traumatic blueing 1 to 3 mm in diameter may be seen at the center of the bleb after intradermal injection of saline control. Two persons assessed intensity and area of the blue color changes within blebs.

Protocol 1: Effects of VEGF/VPF on VP

At 20 minutes after EBD injection, the following were injected intradermally: (a) saline alone (vehicle control); (b) VEGF/VPF (8, 16, 32, 64, and 128 ng); and as positive controls, (c) PAF (0.01, 0.1, 1, 10, and 100 µmol/L) or histamine (1, 10, 100, and 1000 nmol/L). At 15 minutes, dermal blueing was assessed.

Protocol 2: Effect of PAF Receptor Antagonist and Histamine H1 Receptor Antagonist on VEGF/VPF-Induced VP

PAF is a potent endogenous stimulator of VP with an unknown mechanism of action. Guinea pigs received the PAF antagonist CV-6209 (Biomol) (2 mg/kg) 27 10 minutes before EBD injection. To confirm the blocking effect of CV-6209, we also examined PAF-mediated (0.1, 1, and 10 µmol/L) VP after administration of CV-6209.

The effects of histamine H1 receptor antagonist diphenhydramine (10 mg/kg) on VEGF/VPF-induced VP was investigated by administration of diphenhydramine (10 mg/kg) 10 minutes before administration of EBD. The blocking effect of diphenhydramine was examined with histamine (1, 10, 100, and 1000 nmol/L) after administration of diphenhydramine.

Protocol 3: Effects of Other Angiogenic Cytokines on VP

At 20 minutes after EBD injection, the following were injected intradermally: (a) bFGF (8 to 128 ng), (b) aFGF (8 to 128 ng), (c) GM-CSF (8 to 128 ng), (d) scatter factor/NGF (8 to 128 ng), (e) PDGF-BB (8 to 128 ng), (f) TGF-β (8 to 128 ng), and (g) PIGF (8 to 128 ng). PAF was the positive control, saline the negative control.
BP were recorded in response to either L-NAME or phenylephrine, EBD was administered to evaluate VEGF/VPF-induced VP.

Protocol 8: Effect of Locally Administered L-NAME or Phenylephrine on VEGF/VPF-Induced VP
To analyze the effects of L-NAME and phenylephrine administered locally rather than systemically on VP, VEGF/VPF was dissolved (8 to 128 ng in 0.1 mL) in saline containing either L-NAME (1 mM/L) or phenylephrine (1 µM/L). We also examined the impact of either L-NAME (0.001 to 1 mM/L) or phenylephrine (0.01 to 10 µM/L) administered alone.

Bovine Microvascular Endothelial Cell Culture
Because modulation of VP involves the microcirculation, we used BMEC (American Type Culture Collection) to study the effect of VEGF/VPF on stimulating release of NO and prostacyclin in vitro. BMEC express mRNA and protein for both constitutive endothelial NO synthase and prostaglandin H synthase-1 and thus may secrete NO and prostaglandins. Cells were grown at 37°C in a humidified atmosphere of 5% CO2+95% air in phenol red-free MEM containing 20% FBS, 0.6 mM/L l-arginine, ampicillin, and kanamycin. Cells were plated at confluence at 10^6 cells/mL (1 mL per well). After attachment to plates, medium was replaced by 1 mL of sterile Krebs/Henseleit (K-H) buffer.

Measurement of Nitrite Production in BMEC
After release, NO reacts with O2 to yield stable nitrate metabolite. Nitrite concentrations were measured by the Griess reaction to estimate total amounts of NO released from the BMEC. At different time points after addition of VEGF/VPF, 300 µL of K-H buffer was removed and mixed with 30 µL of Griess reagent (1% sulfanilic acid, 0.1% naphthalene-ethylene diamine in 5% phosphoric acid; Sigma), incubated for 10 minutes at room temperature, and absorbance measured at 548 nm. For a blank, 300 µL of fresh K-H buffer was mixed with 30 µL of Griess reagent. Concentrations were determined by comparison with sodium nitrate standard. Lower limit of detection was 0.1 µM/L of nitrate.

Measurement of VEGF/VPF-Induced Prostacyclin Production in BMEC
The stable metabolite of prostacyclin, 6-keto PGF1α, was determined in K-H buffer conditioned by BMEC treated with or without VEGF/VPF, was measured by enzyme immunoassay kit (Amersham) at serial time points in response to VEGF/VPF. The lower limit of detection was 6 pg/mL.

Histological Examination After Intradermal Injection of VEGF/VPF
Five-micron-thick sections of skin were cut, stained with hematoxylin-eosin and/or Giemsa, and examined by light microscopy after fixation. Five-micron-thick sections of skin were cut, stained with hematoxylin-eosin and/or Giemsa, and examined by light microscopy after fixation.

Data Analysis
All data are expressed as mean±SEM (n=number of cell culture wells). Comparison of means between two groups was performed by unpaired Student’s t test. If more than two groups were compared, one-way ANOVA was used to evaluate significant differences among these groups; if significant differences were obtained, each difference was further examined by Fisher’s t test. Probability was considered to be statistically significant at P<.05.

Results
Analysis of VP for Angiogenic Cytokines and Putative Mediators

VEGF/VPF Increases VP
Repeated intradermal injection of the vehicle control saline (0.1 mL) did not increase VP. In contrast, intradermal injection of VEGF/VPF (8, 16, 32, 64, and 128 ng) significantly increased VP in a dose-dependent manner (Fig 1A). VEGF/VPF started to render injected area blue at 149±5 seconds (n=5) after intradermal injection. As positive controls, we used PAF (0.01, 0.1, 1, 10, and 100 µM/L), bradykinin (1, 10, 100, and 1000 nM/L), and histamine (1, 10, 100, and 1000 nM/L), all of which increased VP (Fig 1, B, C, and D).

Effects of PAF Receptor Antagonist and Histamine H1 Receptor Antagonist on VEGF/VPF-Mediated Increase in VP
Because PAF and histamine are well-known endogenous mediators of VP, we tested the effect of PAF receptor antagonist CV-6209 and histamine H1 receptor antagonist...
We then tested the effects of PlGF on VP. PlGF binds to only Flt-1/VEGF-R1 but not to Flik-1/KDR/VEGF-R2 receptor tyrosine kinase.\textsuperscript{32,37} PlGF (8, 16, 32, 64, and 128 ng) did not increase VP (Fig 3), suggesting that Flik-1/KDR/VEGF-R2 is the relevant receptor tyrosine kinase for VEGF/VPF-induced VP.

**Effects of the NO Synthase Inhibitors on VEGF/VPF-Induced VP**

We next examined the role of NO in VEGF/VPF-mediated VP by using the NO synthase inhibitors L-NNA (10 mg/kg)

\begin{align*}
\text{A} & \quad \text{bFGF} \\
\text{B} & \quad \text{aFGF} \\
\text{C} & \quad \text{GM-CSF} \\
\text{D} & \quad \text{Scatter Factor (HGF)} \\
\text{E} & \quad \text{PDGF-BB} \\
\text{F} & \quad \text{TGF}\beta
\end{align*}

Figure 2. Effects of other angiogenic cytokines on vascular permeability. A, bFGF; B, aFGF; C, GM-CSF; D, HGF; E, PDGF-BB; and F, TGF-\beta (8, 16, 32, 64, and 128 ng/0.1 mL in each) all failed to increase vascular permeability. Representative photographs of two to three experiments each. Scale bar=10 mm.

\begin{align*}
\text{A} & \quad \text{PlGF (ng)} \\
& \quad 8 \quad 16 \quad 32 \quad 64 \quad 128 \\
\text{B} & \quad \text{Genistein} \\
\text{C} & \quad \text{Herbimycin A}
\end{align*}

Figure 3. Role of Flik-1/KDR/VEGFR-2 receptor tyrosine kinase in VEGF/VPF-mediated vascular permeability. A, PlGF (8, 16, 32, 64, and 128 ng in 0.1 mL saline) failed to increase vascular permeability. B and C, VEGF/VPF-induced (128 ng) VP was markedly attenuated by previous intradermal injection of the tyrosine kinase inhibitors genistein (100 \( \mu \text{mol/L} \) (B)) or herbimycin A (1 \( \mu \text{mol/L} \) (C)). Genistein (100 \( \mu \text{mol/L} \) or herbimycin A (1 \( \mu \text{mol/L} \) alone did not change vascular permeability. Representative photographs of two to three experiments each. Scale bar=10 mm.
or L-NAME (20 mg/kg). Both attenuated VEGF/VPF-induced VP. The inhibitory effect of L-NAME on VEGF/VPF-induced VP is shown in Fig 4B. In contrast, pretreatment with NO synthase inhibitors did not alter VP induced by PAF (Fig 4B), indicating that PAF does not induce VP through NO. The inactive stereoisomer D-NAME (20 mg/kg), which does not inhibit endothelial NO synthesis, failed to inhibit VEGF/VPF-induced VP (Fig 4C). VEGF/VPF-mediated increase in VP was thus dependent on local NO production.

Effects of the Cyclooxygenase Inhibitor Indomethacin on VEGF/VPF-Induced VP

Pretreatment with indomethacin (5 mg/kg) inhibited VP stimulated by VEGF/VPF (Fig 4D). In contrast, indomethacin pretreatment had no effect on PAF-induced VP. Histological examination disclosed no evidence of VEGF/VPF-associated inflammatory cell infiltrate and/or mast cell degranulation at the site of intradermal injection with or without indomethacin pretreatment. Thus VP due to VEGF/VPF, in contrast to PAF, was also dependent on local prostaglandin(s).

Effect of NO-Independent Vasoconstriction on VEGF/VPF-Induced Increase in VP

In vivo injection of L-NAME (20 mg/kg) increased systemic BP from 96/70 to 132/96 mmHg and inhibited VEGF/VPF-induced VP (Fig 5A). Phenylephrine (0.05 to 0.2 mg/kg) was injected in a cumulative fashion, and 0.2 mg/kg of phenylephrine increased BP from 91/69 (baseline) to 140/92 mmHg, similar to the increase in BP recorded after administration of L-NAME. In contrast to L-NAME, however, phenylephrine did not inhibit VEGF/VPF-induced VP despite similar increases in systemic BP (Fig 5B). These results support the notion that VEGF/VPF-induced VP is mediated by NO but is unaffected by NO-independent vasoconstriction.

Effect of Locally Administered L-NAME and Phenylephrine on VEGF/VPF-Induced Increase in VP

Neither L-NAME (0.001, 0.01, 0.1, and 1 mmol/L) nor phenylephrine (0.01 to 10 μmol/L) alone increased VP (Fig 5C, D). VP was reduced after administration of local VEGF/VPF with L-NAME (1 mmol/L) (Fig 5C). Local administration of VEGF/VPF with phenylephrine (1 μmol/L) resulted in increased VP similar to that seen with VEGF/VPF alone (Fig 5D).

Effects of NO Donors, Prostacyclin, and Prostacyclin Analogue Taprostene on VP

Since VEGF/VPF-induced VP was significantly attenuated by inhibitors of either NO synthase or cyclooxygenase, we then tested two NO donors and prostacyclin, a major metabolite of arachidonic acid by action of cyclooxygenase, for increased VP. NO donors, SNP (10, 50, 100, and 500 μmol/L) and SNAP (10, 100, and 500 μmol/L) alone each failed to increase VP (Fig 6A). Similarly, prostacyclin (0.1 and 1 μmol/L) and
taprostene (0.01, 0.1, 1, and 10 μmol/L) both failed, when administered alone, to increase VP (Fig 6B). When SNP and taprostene were mixed and injected intradermally together, however, VP increased in a dose-dependent manner (Fig 6C). (Histological examination of Giemsa-stained sections disclosed no evidence of mast-cell degranulation.) These results indicate that interaction between NO and prostaglandin (possibly prostacyclin) is required for increased VP.

Measurement of Nitric Oxide and Prostaglandin Metabolites in Cultured Microvascular Endothelial Cells

VEGF/VPF Stimulates NO Release From Microvascular Endothelial Cells

Because increased VP implies functional alteration in the microcirculation, we investigated the effects of VEGF/VPF on NO production in BMEC. Cross-reactivity of rhVEGF/VPF with BMEC was previously confirmed in our laboratory. As shown in Fig 7A, VEGF/VPF (50 ng/mL) stimulated NO synthesis, with peak NO production 5 to 15 minutes after the addition of VEGF/VPF.

VEGF/VPF Stimulates Prostacyclin Release From BMEC

To assess the effects of VEGF/VPF on prostacyclin production, we measured 6-keto-PGF1α, a stable metabolite of prostacyclin. VEGF/VPF (50 ng/mL) significantly stimulated 6-keto-PGF1α production in cultured BMEC in a time-dependent manner (Fig 7B).

Effect of Inhibitors on VEGF/VPF-Mediated Nitrite/Nitrate and 6-keto-PGF1α Production

We tested the effects of the NO synthase inhibitor L-NAME (100 μmol/L), the cyclooxygenase inhibitor indomethacin (10 μmol/L), and the tyrosine kinase inhibitor genistein (100 μmol/L) on VEGF/VPF-mediated NO and 6-keto-PGF1α production. L-NAME significantly inhibited both NO and 6-keto-PGF1α production (Fig 8). Indomethacin significantly inhibited 6-keto-PGF1α production but not NO release. Tyrosine kinase inhibitor genistein significantly attenuated both 6-keto-PGF1α and NO production significantly, consistent with the results of the Miles assay.

Role of Flt-1/VEGF-R1 Receptor Tyrosine Kinase in VEGF/VPF-Induced NO Synthesis

Because PIGF failed to increase VP in Miles assay, we tested whether PIGF stimulates NO synthesis in vitro. Consistent with the results of the Miles assay, PIGF (100 ng/mL) could not be shown to increase NO production from cultured BMEC (Fig 9). Because PIGF ligates only Flt-1/VEGF-R1, these findings suggest that Flk-1/KDR/VEGF-R2 but not Flt-1/VEGF-R1 receptor tyrosine kinase mediates VEGF/VPF-induced NO synthesis.

Discussion

The present study demonstrates that (a) VEGF/VPF-induced VP is attenuated by NO synthase inhibitors, the cyclooxygenase inhibitor indomethacin, and tyrosine kinase inhibitor genistein; (b) PIGF, known to bind Flt-1/VEGF-R1 but not Flk-1/KDR/VEGF-R2, did not increase VP; (c) other angiogenic cytokines including aFGF, bFGF, scatter factor (HGF), PDGF-BB, GM-CSF, and TGF-β all failed to increase VP; (d) VEGF/VPF stimulated NO and prostacyclin production in cultured microvascular endothelial cells, whereas PIGF failed to release NO; (e) neither NO donor (SNP or SNAP) nor prostacyclin administered alone increased VP, but combined administration of SNP and taprostene, a stable prostacyclin analogue, did lead to an increase in VP. These findings thus provide evidence that NO and prostacyclin production in microvascular endothelial cells mediate VEGF/VPF-induced VP. Although histamine and PAF are known endogenous mediators of VP, our studies using receptor antagonists indicate that these molecules are not mediators of VEGF/VPF-induced increase in VP. Furthermore, histological examination revealed neither inflammatory cell infiltration nor degranulated mast cells on Giemsa-stained sections (data not available).
shown) after intradermal injection of VEGF/VPF, consistent with previous data from Collins et al.39

Our studies using genistein and herbimycin A confirmed essential role of receptor tyrosine kinase(s) in VEGF/VPF-mediated VP. The demonstration that PlGF fails to augment VP confirms recent findings by Park et al32 and implies that the Flt-1/VEGF-R1 tyrosine kinase receptor alone cannot mediate VEGF/VPF-induced VP32–34; this effect instead appears to be mediated wholly or in part by Flk-1/KDR/VEGF-R2. Furthermore, this feature is not shared by other growth factors that nevertheless stimulate angiogenesis in vivo.

VEGF/VPF stimulates transient accumulation of cytoplasmic calcium in cultured endothelial cells.41 VEGF/VPF-induced increase in endothelial cytosolic Ca2+ probably activates calcium-calmodulin–dependent enzymes such as endothelial constitutive NO synthase. In fact, Ku et al39 previously showed that VEGF/VPF stimulates NO production in isolated canine coronary arteries. The present study extends these previous observations by demonstrating that VEGF/VPF also stimulates NO release from cells regulating VP at the microvascular level.

Previous studies have implicated NO as a factor regulating VP.19–21 Nguyen et al21 demonstrated that substance P, a potent endothelium-dependent vasodilator, increases micro-VP through NO-dependent pathways. More recent findings suggested that NO-mediated alteration of VP may depend on its local concentration.20

VEGF/VPF-induced VP appears dependent on not only NO but also prostaglandin production. It has been suggested that prostaglandins are involved in the regulation of both angiogenesis and VP.25,26 Yamamoto et al32 recently reported that a stable prostacyclin analogue, SM-10902, accelerated wound healing in a murine model of diabetic skin ulcers. Fujii et al36 have demonstrated that lipopolysaccharide-induced VP is attenuated by either an NO synthase inhibitor or indomethacin. These results collectively support the notion that prostaglandins produced from arachidonic acid by cyclooxygenase can regulate VP.

Our data indicate that NO synthase inhibitor L-NAME inhibited not only nitrite production but also 6-keto PGF1α production from microvascular endothelial cells. This finding suggests a possible interaction between NO and prostaglandin. In this context, recent studies demonstrated that either endogenous or exogenous NO can stimulate prostaglandin production through cyclooxygenase activation.36,42 Thus increased production of prostacyclin in response to VEGF/VPF observed in our study might derive in part from NO stimulated by VEGF/VPF.

VP often precedes and/or accompanies angiogenesis, particularly tumor-associated angiogenesis.5,13,43,44 However, angiogenic growth factors other than VEGF/VPF failed to increase VP. This suggests that VEGF/VPF, in contrast to other angiogenic cytokines, directly enhances VP of an established mature vascular bed. Because most angiogenic cytokines have been shown to increase VEGF/VPF expression,45–48 VP associated with nascent vascular development stimulated by other angiogenic cytokines51 may or may not be mediated by VEGF/VPF.

Endothelial cell-to-cell interaction and related adhesion molecules such as vascular endothelial cadherin, platelet endothelial cell adhesion molecule-1, and/or gap junction proteins have been implicated in the regulation of VP.25 In contrast, Dvorak et al13 and Kohn et al53 found that vascular leakage could not be attributed to passage of molecules through interendothelial cell junctions or injured tumor endothelium.
but instead involved transendothelial transport through a novel cytoplasmic organelle that they termed the vesicular-vacuolar organelle. Others have reported VEGF/VPF-induced ultrastructural features consistent with endothelial fenestration. The downstream details by which NO/prostacyclin-mediate VP may involve these routes remains to be fully elucidated.

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References
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