Comparative Evaluation of FGF-2–, VEGF-A–, and VEGF-C–Induced Angiogenesis, Lymphangiogenesis, Vascular Fenestrations, and Permeability

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Abstract—Several endothelial growth factors induce both blood and lymphatic angiogenesis. However, a systematic comparative study of the impact of these factors on vascular morphology and function has been lacking. In this study, we report a quantitative analysis of the structure and macromolecular permeability of FGF-2–, VEGF-A–, and VEGF-C–induced blood and lymphatic vessels. Our results show that VEGF-A stimulated formation of disorganized, nascent vasculatures as a result of fusion of blood capillaries into premature plexuses with only a few lymphatic vessels. Ultrastructural analysis revealed that VEGF-A–induced blood vessels contained high numbers of endothelial fenestrations that mediated high permeability to ferritin, whereas the FGF-2–induced blood vessels lacked vascular fenestrations and showed only little leakage of ferritin. VEGF-C induced approximately equal amounts of blood and lymphatic capillaries with endothelial fenestrations present only on blood capillaries, mediating a medium level of ferritin leakage into the perivascular space. No endothelial fenestrations were found in FGF-2–, VEGF-A–, or VEGF-C–induced lymphatic vessels. These findings highlight the structural and functional differences between blood and lymphatic vessels induced by FGF-2, VEGF-A, and VEGF-C. Such information is important to consider in development of novel therapeutic strategies using these angiogenic factors. **(*Circ Res*. 2004;94:664-670.)**

Key Words: angiogenesis ■ lymphangiogenesis ■ endothelial fenestrations ■ vascular permeability

Angiogenesis contributes to several physiological and pathological processes including embryonic development, wound healing, tumor growth and metastasis, rheumatoid arthritis, diabetic retinopathy, atherosclerosis, and revascularization of ischemic myocardium, hind limb muscles, and brain.[1] Lymphangiogenesis is critically important for tumor spread via the lymphatic system.[2,3] The processes of blood and lymphatic angiogenesis are tightly regulated by several key angiogenic factors. These factors, including the FGF (fibroblast growth factor), VEGF (vascular endothelial growth factor), PDGF (platelet-derived growth factor), and angiopoietin families, modulate both blood and lymphatic vessel growth.[1–5] Thus, blood and lymphatic angiogenesis are coordinately controlled by mitogens with overlapping functions. It is not clear how these factors selectively activate separate pathways of lymphatic or blood angiogenesis when both types of vessels are formed simultaneously.

Although the molecular details of blood angiogenesis are well known, the closely associated lymphatic system has remained poorly characterized due to the lack of specific molecular markers. During the last decade, however, a number of molecules unique to the lymphatic vessels have been identified.[6–9] With these tools available, the lymphatic system and its relevance to disease can be analyzed at a molecular level. Among the known angiogenic factors, VEGF (VEGF-A) was previously thought to act only on blood vessels. However, a recent study reports that VEGF promotes lymphangiogenesis as well.[10]

In contrast to the VEGF family, the FGF family has broader biological functions on a variety of cell types, although the two FGF prototypes, FGF-1 and FGF-2, are potent angiogenic factors in vivo. However, the physiological and pathological relevance of these factors in regulation of angiogenesis needs to be established, because both of them lack a classical signal sequence for secretion.[11] The VEGF family includes at least five structurally related proteins, VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PlGF).[12,13] These molecules interact with a set of cell surface receptors, VEGFR-1, VEGFR-2, and VEGFR-3, that show varying specificity and function. VEGF-C and VEGF-D bind to both VEGFR-2 and VEGFR-3 and promote formation of blood and lymph vessels.[9] VEGF-B and PlGF bind to VEGFR-1 and modulate the effects of VEGF-A, but their roles in stimulation of angiogenesis remain controversial.[13,14]

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In addition to its ability to stimulate angiogenesis, VEGF-A acts as a potent vascular permeability factor (VPF). A large body of work indicates that VEGFR-2 is the receptor that mediates VEGF-A–induced angiogenic and permeability effects. In support of this notion, VEGF-B and PlGF, which only interact with VEGFR-1, lack angiogenic and vascular permeability activity.[13,15]

In most organs, the capillaries are of a continuous type with closely connected endothelial cells surrounded by an intact basement membrane. Endothelial cells typically display numerous 50- to 80-nm plasmalemmal vesicles or caveolae, supposed to be involved in transcytosis of macromolecules.[16–19] Capillary endothelial cells in, eg, endocrine glands, kidney glomeruli, and the choroid plexus are further equipped with 50- to 80-nm diameter transcellular openings, often closed by a thin diaphragm. These fenestrations are labile and dynamic structures that evolve and disappear under different circumstances. In some organs, such as the liver, spleen, and bone marrow, the capillaries may also be discontinuous with large gaps between the cells and an incomplete basement membrane. The build-up of different capillary beds with regard to caveolae, fenestrations, and intercellular gaps is believed to determine their permeability properties.[16–19] The regulatory mechanisms behind the formation of these structures are still not known in detail, but VEGF-A/VPF and other vasoactive mediators are likely to be among the principal actors in this context.

Based on their angiogenic features, FGF-2, VEGF-A, and VEGF-C have entered into clinical trials in the treatment of ischemic heart and hind limb diseases. However, in order to avoid undesired side effects in clinical therapy, it is critically important to understand the organization and functional properties of these growth factor–induced, newly formed vessels. In this study, we report a systematic, quantitative study to compare the fine structure and functions of the newly formed vessels induced by these factors.

Materials and Methods

Reagents

Recombinant human $VEGF_{165}$ (VEGF-A) was prepared as previously reported.[20] A recombinant, mature form of human VEGF-C was expressed in *Pichia pastoris* and purified as described.[21] Recombinant human FGF-2 was obtained from Pharmacia & Upjohn. A polyclonal antibody against mouse LYVE-1 was provided by Douglas Jackson, Institute of Molecular Medicine.

Animals

Male, 5- to 6-week-old C57BL/6 mice (from departmental breeding unit) were acclimated and caged in groups of four or less. The animals were anesthetized using a mixture of Hypnorm: Dormicum:H_2O (1:1:2) intraperitoneally and killed using a lethal dose of CO_2. All animal studies were reviewed and approved by the animal care and use committee of the Stockholm Animal Board.

Mouse Corneal Angiogenesis Assay

The mouse corneal angiogenesis assay was performed as described.[22,23] Corneal micropockets were created with a modified von Graefe cataract knife in the eyes of 5- to 6-week-old mice. A micropellet (0.35×0.35 mm) of sucrose and aluminum sulfate (Bukh Meditec) coated with hydron polymer type NCC (IFN Sciences) containing 80 ng FGF-2, 160 ng VEGF-A, or 160 ng VEGF-C was implanted into each pocket (8 eyes/animals in each group). The pellet was positioned 1.0 to 1.2 mm from the corneal limbus. After implantation, erythromycin ophthalmic ointment was applied to the eye. To study the permeability of the newly formed vessels, 100 μL of a 100 mg/mL suspension of ferritin (Sigma Chemical Company) was injected into the tail vein 60 minutes before the animals were killed.

Electron Microscopy

Five days after implantation of FGF-2, VEGF-A, or VEGF-C pellets into the cornea, the animals were euthanized and the eyes removed and immersed in 3% glutaraldehyde in 0.1 mol/L sodium cacodylate-HCl buffer (pH 7.3) with 0.05 mol/L sucrose. A few hours later, the vessel-containing parts of the cornea (outer third, ie, the parts showing the highest vessel density) were dissected out, cut into small pieces, and put into fresh fixative. After rinsing in buffer, the specimens were postfixed in 1.5% osmium tetroxide in 0.1 mol/L cacodylate buffer (pH 7.3) with 0.7% potassium ferrocyanate for 1.5 hours at 4°C, dehydrated in ethanol (70%, 95%, and 100%), stained with 2% uranyl acetate in ethanol, and embedded in Spurr low viscosity epoxy resin. Thin sections were cut perpendicular to the surface of the cornea with diamond knifes on a Leica Ultracut, picked up on grids, stained with lead citrate, and examined in a Philips CM120 electron microscope at 80 kV. For quantitative evaluation, the specimens were photographed using a Kodak MegaPlus CCD camera and measurements made using the analySIS system (Soft Imaging Software)

Immunocytochemistry

The growth factor–implanted mouse eyes were enucleated at day 5 after the operation and immediately frozen on dry ice and stored at −80°C before use. Frozen sections of 10 μm through the midplane of each cornea were cut using a cryomicrotome, air-dried for 10 minutes, fixed with acetone, and blocked with 30% nonimmune goat serum. Endogenous biotin was blocked using an avidin-biotin reagent (Vector Laboratories). The slides were sequentially stained with rabbit anti-mouse LYVE-1 (1:200), FITC-conjugated anti-rabbit IgG, rat anti-mouse CD31 (1:100, Pharmingen), and streptavidin-conjugated Cy3 (1:2500, Jackson Immuno Research) for 30 to 60 minutes at 20°C. After washing in PBS, slides were mounted in 90% glycerol and examined under a Nikon fluorescence microscope. Images were collected with a digital imaging system and further analyzed using the Adobe Photoshop 6.0 program.

Results

Patterns and Structures of FGF-2–, VEGF-A–, and VEGF-C–Induced Vessels

To compare the angiogenic patterns and the structures of the newly formed blood vessels induced by various factors, we chose the mouse corneal angiogenesis model. Using the cornea, which is an avascular tissue lacking blood and lymphatic vessels, allowed us to compare newly formed vessels induced by individual factors. VEGF-A stimulated the outgrowth of a widespread vasculature with a high density of capillaries and microvessels that fused into primitive vascular plexuses in which individual vessels were indistinguishable. At the leading edge of VEGF-A–induced vessels, the growth of vascular plexuses with fused capillaries resulted in a pseudo-hemorrhagic phenotype (Figure 1A). In contrast to VEGF-A, FGF-2–induced blood vessels consisted of well-defined, separated microvessels growing toward and occasionally penetrating into the implanted FGF-2 pellets (Figure 1A). Similarly, VEGF-C gave rise to well-defined vascular networks with dilated microvessels (Figure 1A). Immunohistochemical analyses of corneal tissues using an anti-PECAM-1 (CD31) antibody confirmed the differences in

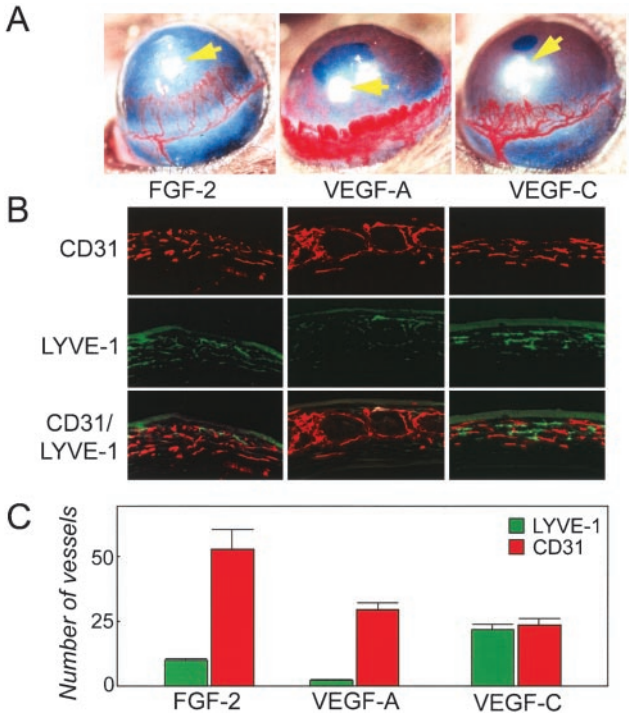

Figure 1. Differences in vascular patterns after induction with FGF-2, VEGF-A, and VEGF-C. Pellets containing the growth factors were implanted into the corneas of C57BL/6 mice. Corneal neovascularization was examined on day 5 after implantation. A, Biomicroscopic pictures of the newly formed vascular networks in the cornea. Arrows point to the implanted pellets. B, Double immunostaining of corneal sections for demonstration of the endothelial cell marker CD31 (red) and the lymph vessel marker LYVE-1 (green). Sections were cut through the midplane of each cornea and photographed in an area with average vessel density. C, Quantitation of blood vessels (CD31-positive) and lymph vessels (LYVE-1–positive). Results are shown as mean with standard deviation indicated as a vertical bar (n=8).

vascular patterns induced by different growth factors. Both FGF-2– and VEGF-C–induced vasculatures were made up of well-separated individual microvessels, and large sinusoidal structures resulting from fusion of multiple capillaries were detectable only in the VEGF-A–induced vasculature (Figure 1B). At day 5 after growth factor implantation, VEGF-C displayed a robust lymphangiogenic response in corneas as detected by an antibody against LYVE-1, a specific marker for lymphatic endothelial cells, whereas FGF-2 exhibited a less potent lymphangiogenic activity (Figure 1B). In contrast, only a few lymphatic vessels were found in the VEGF-A–implanted corneas at this time (Figure 1B). Virtually nonoverlapping patterns of CD31- and LYVE-1–positive signals were detected when images of the same sections were combined digitally (Figure 1B). These findings are consistent with previous reports stating that lymphatic endothelial cells only express CD31 at very low levels.[6] These data demonstrate not only that LYVE-1 is a specific marker for lymphatic endothelial cells but also that CD31 is fairly specific for blood vessel endothelial cells. Quantitative analysis showed that FGF-2 induced the highest density of blood vessels, whereas VEGF-C produced similar numbers of blood and lymphatic vessels (Figure 1C).

Ultrastructure of the Newly Formed Blood and Lymphatic Vessels

Electron microscopy was utilized to gain more detailed information on the fine structure of the corneal vessels induced by various angiogenic factors. This analysis was concentrated on the outer third of the cornea (ie, the part with the highest vessel density) and demonstrated that the blood vessels stimulated by all three factors were capillary in nature. They consisted of a thin wall (VEGF-A<VEGF-C<FGF-2; Table 1) made up of a single layer of intimately connected endothelial cells joined by junctional complexes and surrounded by a few dispersed pericytes but no smooth muscle cells. An incomplete basement membrane was found beneath the endothelial cells. As opposed to these similarities in basic morphological properties, explicit differences were noted between the groups in other respects and especially with regard to the number of fenestrations. These pore-like openings (approximately 50 nm in diameter) in the thinnest parts of the endothelial cells were numerous in the VEGF-A–stimulated vessels, almost nonexistent in the endothelium of FGF-2–induced vessels, and low but fair in counts in the VEGF-C–induced blood vessels (Figure 2; Table 1). The numbers of endothelial cell caveolae did not vary significantly between vessels induced by VEGF-C and FGF-2 but

TABLE 1. Morphological and Permeability Characteristics of Newly Formed Capillaries

Factors	Minimum Width, nm	*P*	Maximum Width, nm	*P*	Fen/μm	*P*	Cav/μm	*P*	AFP/μm^2	*P*
FGF-2	158	<0.001*	419	<0.01*	0.03	<0.001*	3.7	<0.001*	12	0.001*
SD	(47)		(159)		(0.05)		(0.7)		(5)	
VEGF-A	49	<0.01†	241	...	1.33	<0.001†	2.5	<0.05†	50	<0.05†
SD	(6)		(49)		(0.60)		(0.5)		(37)	
VEGF-C	90	<0.01‡	285	<0.05‡	0.25	<0.05‡	3.3	...	29	<0.05‡
SD	(47)		(121)		(0.32)		(0.8)		(24)	

Corneal angiogenesis was stimulated with FGF-2, VEGF-A, and VEGF-C for 5 days as described in Materials and Methods. Eight animals were used in each group. A suspension of anionic ferritin particles (100 μL) was injected into the tail vein 60 minutes before the animals were killed. Corneas were prepared for electron microscopy, and thin-walled segments of individual capillaries were photographed at a final magnification of >×50 000 (5–10 capillaries examined per cornea). Minimum and maximum widths of the capillary wall in these regions, the number of endothelial fenestrations (Fen) and the number of caveolae (Cav) both on the luminal and the abluminal sides were recorded. In addition, the number of anionic ferritin particles (AFP) in a 1-μm-wide zone of the extracellular matrix outside the endothelial cells was counted. Results are given as mean with standard deviation in parentheses. Significance of the differences of the means was calculated using Kruskal-Wallis test followed by Mann-Whitney *U* test (*FGF-2 vs VEGF-A; †VEGF-A vs VEGF-C; ‡VEGF-C vs FGF-2).

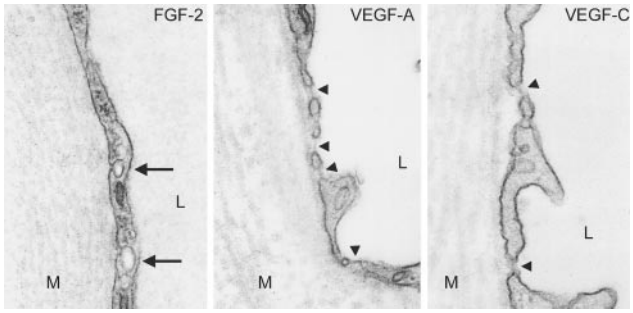

Figure 2. Blood capillaries formed after stimulation with FGF-2, VEGF-A, and VEGF-C for 5 days differ with regard to the existence of endothelial fenestrations. Neovascularization of the mouse cornea was stimulated as described in Figure 1, and the newly formed blood vessels were studied by electron microscopy. Micrographs show thin portions of the capillary wall. Arrowheads mark endothelial fenestrations and arrows caveolae. L indicates capillary lumen; M, extracellular matrix of the cornea.

were about 25% to 30% lower after stimulation with VEGF-A (Table 1).

The lymphatic vessels could be easily distinguished under the electron microscope because their lumen lacked erythrocytes and leukocytes. In contrast, the newly formed blood vessels contained such cells. In addition, the lymphatic vessels were composed of a thin wall with endothelial cells partly different in character from those building up the blood capillaries. Thus, no fenestrations and only few caveolae were found in the lymph endothelial cells (Figure 3). In some places, the latter formed fine valve-like structures bulging into the lumen of the lymph vessels.

Vascular Permeability

To study the permeability properties of the vessels induced by the three growth factors, ferritin was used as a marker. A suspension of this protein was injected intravenously 60 minutes before the animals were euthanized, and the corneas were processed for electron microscopy. Marked differences were observed in the passage of ferritin particles from the lumen of the blood capillaries to the surrounding extracellular matrix of the cornea (Figure 4), increasing in the following order as estimated quantitatively: FGF-2<VEGF-C<VEGF-A (Table 1). These differences in macromolecular leakage out of the vessels were positively correlated to differences in the occurrence of endothelial fenestrations, whereas a negative correlation with the numbers of caveolae was found (Tables 1 and 2). In all experimental groups, ferritin particles were found in caveolae (often seen to be fused into clusters or tube-like structures) both on the luminal and abluminal surfaces of the endothelium, but only few particles had passed into the pericapillary space in these areas (Figure 4A). Likewise, no tracer, or only very limited amounts of it, were noted in the narrow spaces between adjacent endothelial cells. In the thinnest parts of the vessel walls (<0.2 μm), caveolae were rare and the appearance of ferritin particles in the neighboring extracellular matrix appeared for the main part to rely on the existence of endothelial fenestrations (Tables 1 and 2). Thus, the tracer molecules were sparse or absent around capillaries induced by FGF-2 (Figure 4B), abundant around those induced by VEGF-A (Figure 4C), and intermediate in number around those induced by VEGF-C (Figure 4D).

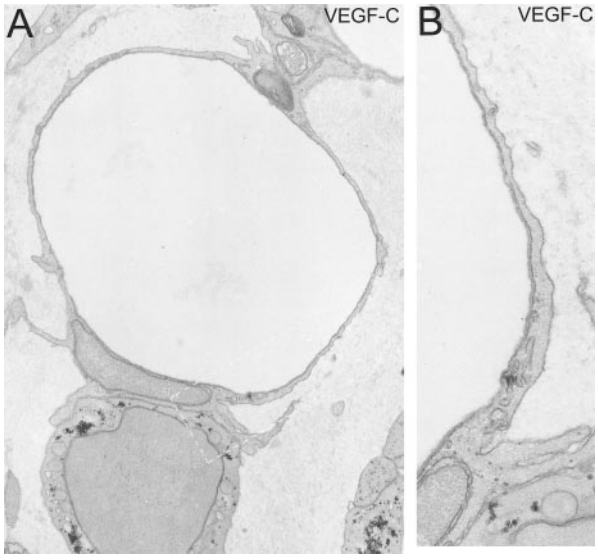

Figure 3. VEGF-C stimulates formation of lymph vessels. Neovascularization of the mouse cornea was stimulated with VEGF-C for 5 days as described in Figure 1, and the newly formed blood vessels were studied by electron microscopy. Left panel shows an overview and the right panel a detail of a lymph capillary. Wall of this vessel is very thin, but in contrast to the blood capillaries induced by VEGF-C (see Figure 2), it lacks fenestrations.

Discussion

Angiogenic therapy aiming to improve blood flow and function of ischemic tissues requires the angiogenic stimuli to establish functional and stable blood vessels. Although clinical evaluation at early stages suggests that treatment with angiogenic factors may be beneficial for the patients, human trials have also encountered several severe complications, including tissue edema, progression of atherosclerotic plaques, and angioma development.[1,24] These problems may be due to the structure and function of various angiogenic factor–induced vessels, a matter that has so far not been examined in detail. In this study, we have systematically compared the fine structure of newly formed blood and lymphatic vessels induced by FGF-2, VEGF-A, and VEGF-C. Our findings provide information that will be essential to consider in the attempts to further advance and refine angiogenic therapy.

Penetration of soluble small and larger molecules across vascular endothelia is mediated by the following structures: (1) caveolae (plasmalemmal vesicles); (2) transendothelial channels (fused caveolae); (3) intercellular gaps; and (4) endothelial fenestrations.[16–19] The formation and functions of these structures are highly regulated by several angiogenic factors. Our data show that blood capillaries induced by various angiogenic factors differed little in the number of caveolae and transendothelial channels, and moderate amounts of tracer particles (ferritin) were found in vesicles on the luminal side and released on the abluminal side of the endothelial cells. Therefore, it seems unlikely that caveolae represented the main route for macromolecular passage across the endothelium. On the contrary, statistical analysis

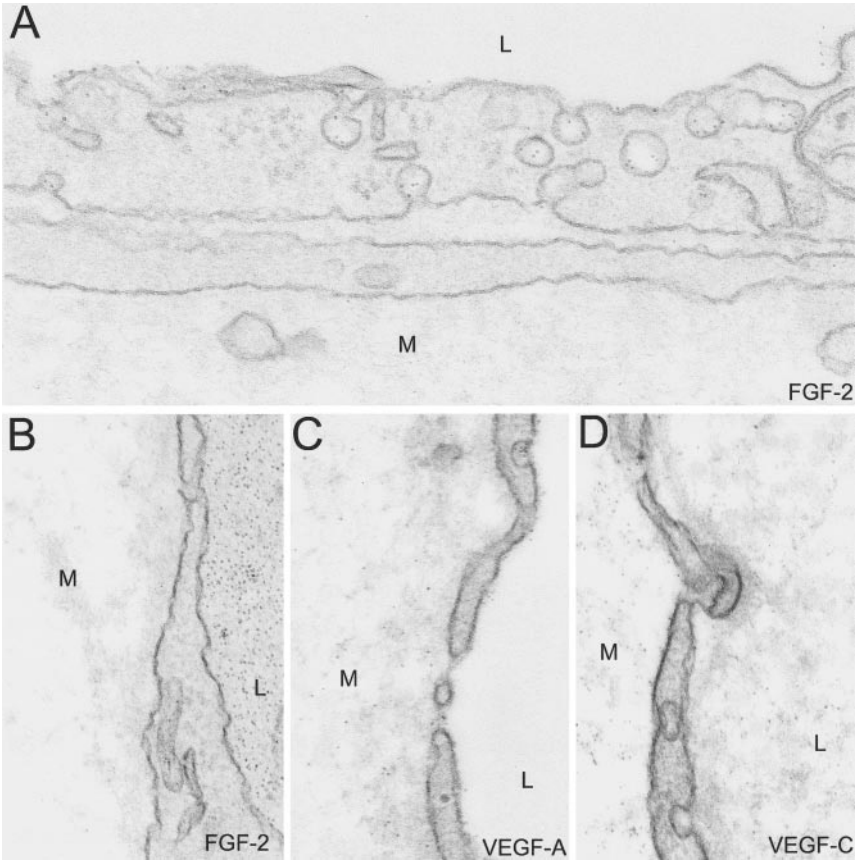

Figure 4. Differences in macromolecular permeability between blood capillaries induced by FGF-2, VEGF-A, and VEGF-C for 5 days. Neovascularization of the mouse cornea was stimulated as described in Figure 1. Suspension of ferritin was injected intravenously 60 minutes before euthanasia of the animals, and the leakage of tracer particles out of the newly formed corneal blood capillaries was analyzed by electron microscopy (see also Table 1). A, Part of an endothelial cell in a capillary formed by stimulation with FGF-2. Ferritin-containing caveolae (plasmalemmal vesicles) are seen both on the luminal (L) and abluminal surfaces of the cell, but only occasional ferritin molecules are detected in the extracellular matrix (M). B through D, Thin-walled parts of blood capillaries induced by FGF-2, VEGF-A, and VEGF-C. No or only a few ferritin molecules are found in the pericapillary matrix (M) in the FGF-2 group compared with the VEGF-A and VEGF-C groups.

revealed a negative correlation between caveolae abundance and transfer of tracer particles from the lumen to the surrounding extracellular space of the blood vessels. Thus, FGF-2–induced capillaries showed the largest number of caveolae but the smallest amount of ferritin leakage, whereas the reverse was true for VEGF-A–induced capillaries. The fact that a certain level of caveolae-mediated vesicular transport of ferritin occurred in all groups and that only VEGF-A and VEGF-C induced a more prominent ferritin leakage further support this notion. Conceivably, caveolae could be the principal route used in FGF-2–induced vessels where few endothelial fenestrations were detected. It should, however, be mentioned that caveolae integrity has been found essential for transendothelial transport and that caveolae contain the machinery required for vesicle budding, docking, and fusion.[16,19] Vascular endothelial cells of mice deficient in caveolin-1 lack caveolae and have defects in uptake/transport of the plasma protein albumin.[25] It should further be noted that the thickness of the endothelial cell layer varied in capillaries induced by the three growth factors (VEGF-A<VEGF-C<FGF-2). Therefore, the average distance for vesicular transport across the endothelial cells was not the same in the different experimental groups. Therefore, although the number of caveolae was lower in VEGF-A– than in VEGF-C– or FGF-2–induced blood vessels, we cannot exclude the possibility that these organelles played an important role in transendothelial transport also in capillaries stimulated with VEGF-A.

As with caveolae, no clear differences in endothelial cell-to-cell junctions were detected between capillaries induced by FGF-2, VEGF-A, and VEGF-C, and no evident transfer of ferritin through these fine clefts were seen. These results are in agreement with the previous finding that only peptides with a MW <3000 are able to pass these sites.[18] Hence, it is improbable that variations in occurrence of wide intercellular gaps explained the variations in ferritin extravasation. Conversely, vasoactive agents like histamine and thrombin have been proposed to increase vessel leakage by stimulating cell contraction and formation of enlarged intercellular gaps, eg, during allergic and inflammatory reactions.[26] We demonstrate that the only structures showing a positive correlation to vascular permeability were endothelial fenestrations. FGF-2–induced capillaries basically lack fenestrations and show a low transfer of ferritin, whereas VEGF-C– and especially VEGF-A–induced vessels have high numbers of fenestrations with higher levels of ferritin translocation detected within the fenestrae. Our findings are consistent with the notion that endothelial fenestrations make up a key route for passage of serum components across vascular walls.[18] In contrast to blood capillaries, lymph capillaries generated by VEGF-C completely lack fenestrations. This may be due to differences in signaling pathways used for angiogenesis and lymphangiogenesis.[9] With regard to uptake of water and macromolecules in lymph vessels, it is believed that gaps between adjoining endothelial cells are of key importance. Still, a vesicle-mediated mechanism for transport of macromolecular material from the extracellular space into the lymph has also been described.[17]

Roberts and Palade showed that local application of VEGF-A induced increased permeability and development of

TABLE 2. Correlation Analysis

Parameters	Minimum Width, nm	Maximum Width, nm	Fen/μm	Cav/μm	AFP/μm^2
Minimum width, nm					
Correlation coefficient	1.00	0.84	−0.83	0.70	−0.41
P	...	<0.001	<0.001	<0.001	0.05
Maximum width, nm					
Correlation coefficient	0.84	1.00	−0.59	0.51	−0.21
P	<0.001	...	<0.002	<0.01	...
Fen/μm					
Correlation coefficient	−0.83	−0.59	1.00	−0.74	0.57
P	<0.001	<0.002	...	<0.001	<0.005
Cav/μm					
Correlation coefficient	0.70	0.51	−0.74	1.00	−0.43
P	<0.001	<0.01	<0.001	...	<0.05
AFP/μm^2					
Correlation coefficient	−0.41	−0.21	0.57	−0.43	1.00
P	<0.05	...	<0.005	<0.05	...

For experimental details and shortenings, see Table 1. Nonparametric correlations between the measured parameters (n=24; 3 experimental groups with n=8 in each group) and significance levels (2-tailed) were determined according to Spearman.

endothelial fenestrations in small venules and capillaries of rat muscles and nude mouse skin.[27] Similar findings were later made in tumors generated in nude mice by CHO cells stably transfected with VEGF-A.[28] Carbon black was used as tracer to study permeability and was most noticeable in open interendothelial junctions. In these studies, muscle, skin, or tumor tissues could produce angiogenic factors other than VEGF-A that contributed to the opening of interendothelial junctions. Alternatively, VEGF-A could upregulate another factor that regulates the opening of tight junctions. The discrepancy between our data and the previously published results could be due to the different systems used in these studies. As the corneal tissue remains avascular under physiological conditions, implantation of an angiogenic factor in the cornea allowed us to analyze the structure of blood vessels induced by this particular factor. Thus, our system excludes the effects displayed by other angiogenic factors. Our observations are also in good agreement with the finding that VEGF-A but not FGF-2 can induce fenestrations in vitro.[29]

Because these pore-like openings in the endothelial cell wall allow the passage of water, small solutes, and macromolecules,[16,18] they can explain the leakiness of VEGF-A–induced vessels.[30] The resulting edema and tissue swelling may cause problems in the clinical use of VEGF-A to promote neovascularization.[31,32] VEGF-C could be an alternative in view of the finding that it induces the production of capillaries with fewer fenestrations and lower permeability. It also induces the growth of lymph vessels and provides for drainage of extravasated fluid, plasma proteins, and leukocytes.[33] Another possibility is that improved knowledge of the signaling mechanisms of different angiogenic factors will make it feasible to direct their action toward the generation of a well-functioning vasculature. We have, for example, observed that stimulation of angiogenesis and vascular permeability by VEGF-A partly are distinct events.[34] Taken together, our findings indicate that different angiogenic factors induce discrete patterns of angiogenesis. FGF-2, VEGF-A, and VEGF-C all induce blood and lymphatic angiogenesis, but to varying extents. The findings not only provide important information for therapeutic angiogenesis but also suggest that blockage of functions of these angiogenic and lymphangiogenic factors may be important not only for primary tumor growth but also for lymphatic metastasis.

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