PART I

Biology of Blood Vessels and Mechanisms of Vascular Inflammation

CHAPTER 1
Vascular Development

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Overview

- Vascular formation appears early during embryo development and entails both genetic and epigenetic factors.
- Two fundamental mechanisms are recognizable: vasculogenesis and angiogenesis, the latter including sprouting angiogenesis and intussusceptive microvascular growth.
- During the development of the vascular tree, blood vessels express a precise spatial and temporal hierarchy and form organ- and tissue-specific vascular beds.
- Several cytokines and signaling mechanisms are implicated in arterial, capillary and venous specification, a process which involves endothelial cells (EC) interfacing with pericytes, mural cells and tissue-borne elements.
- Structural and molecular heterogeneity of EC is believed to contribute to the generation of vascular bed diversity.

Development of the cardiovascular system

The circulatory system consists of the heart and an interconnected network of blood vessels which differ in size, structure and function. The heart develops from the precardic lateral folds to form the primitive heart tube. This consists of an inner endothelium, which is separated from the outer myocardial tube by the elastic cardiac jelly. Emergence of cardiac endothelium and cardiomyocytes occurs almost concomitantly and, at first, they develop rather independently from one another [1]. The endocardium is continuous with the endothelium of the major blood vessels, the axial vein and the dorsal aorta.

Blood vessels first appear as the result of vasculogenesis, i.e. the formation of capillaries from endothelial cells (EC) differentiating from groups of mesodermal cells (Figure 1.1). The vascular plexus is established before the onset of heart beat. Vasculogenesis leads to the formation of the first major intraembryonic blood vessels and to the set up of the primary vascular plexus in the yolk sac. Development of the vascular network of certain endodermal organs, including liver, lung, pancreas, stomach, intestine and spleen, occurs by vasculogenesis. Otherwise, in the developing brain and kidney the formation of the vascular tree occurs by angiogenesis.

Several factors are critical for vasculogenesis. Angioblasts begin to differentiate into EC and assemble into tubes, principally as the result of a series of inductive cues:

1 Vascular endothelial growth factor (VEGF)
2 Signals from surrounding tissues, and
3 The expression of intercellular and cell-matrix adhesion molecules.

EC tubes are soon stabilized by pericytes recruited from the surrounding mesenchyme to form early capillaries. In microvessels, platelet-derived growth factor (PDGF) and transforming growth factor β1 (TGF-β1) signals are involved in the recruitment of pericytes. In larger vessels, arterioles and venules, the vascular wall is made up of EC and smooth muscle cells, which are recruited mainly through the Tie-2 and angiopoietin-1 (Ang-1)

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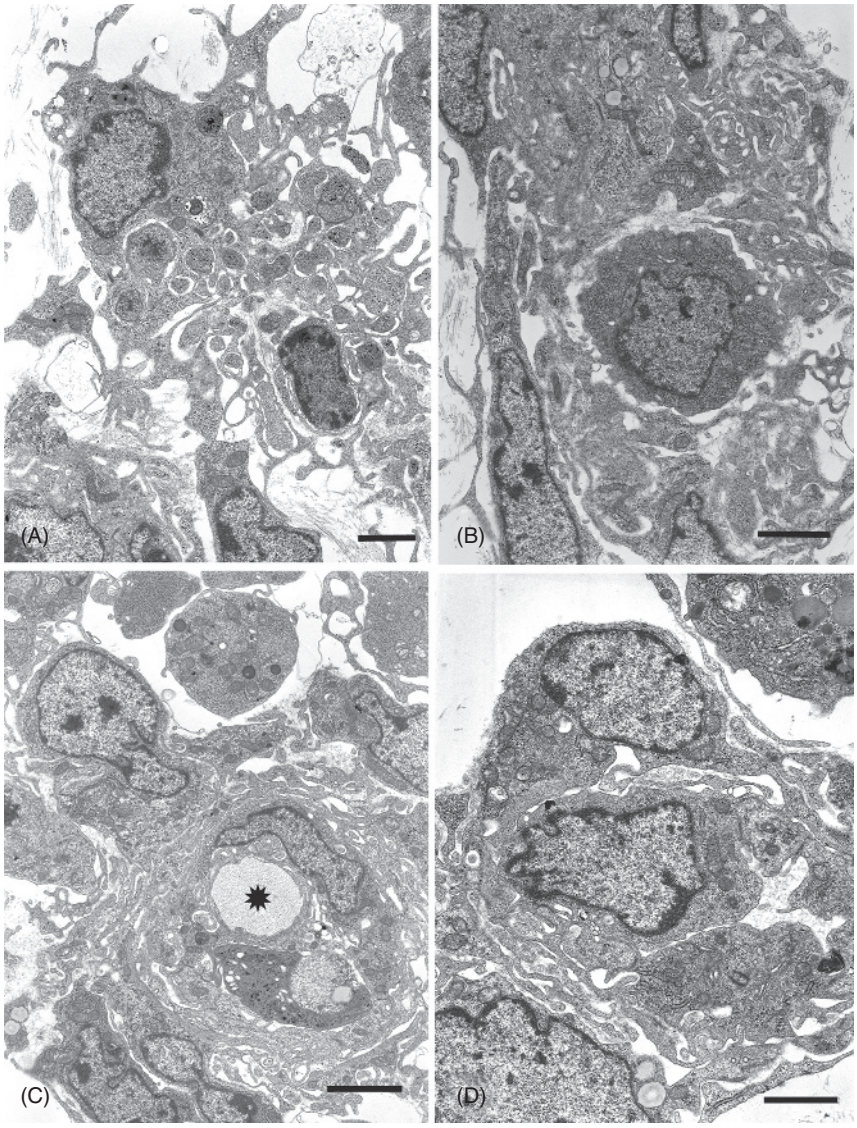

Figure 1.1 Electron micrographs of vasculogenic areas in the chick embryo chorioallantoic membrane (CAM). Poorly differentiated mesenchymal cells exhibiting highly irregular surface profiles and cytoplasmic processes closely interdigitating with similar projections of neighboring cells are documented at day 8 of incubation. In C, an initial vascular lumen (asterisk) is observable. Micrograph in A is taken from an erythropoietin-stimulated CAM. Part A reproduced from Crivellato et al. [21] with permission from Nature. Bars = 2 μm.

receptor–ligand pair, although neuropilins and the Notch pathway are also involved in mural cell formation.

VEGF-A deficient embryos die *in utero* between days 8.5 and 9.5 postcoitum and their primitive vascular structures are severely defective, while VEGF receptor-2 (VEGFR-2) deficient mice die early as a result of blocked migration of angioblasts to the initial sites of vasculogenesis. Embryos lacking VEGFR-2 die *in utero* between days 8.5 and 9.5 postcoitum and show no development of any blood vessels or hematopoietic cells. The loss of both lineages suggests that VEGFR-2 is required for hemangioblast development.

It has been established that vasculogenesis occurs also in postnatal life, as "postnatal vasculogenesis," which is de novo vessel formation by *in situ* incorporation, differentiation, migration and/or proliferation of bone marrow-derived endothelial precursor cells (EPC).

The term angiogenesis, applied to the formation of capillaries from pre-existing vessels, is based on endothelial sprouting or intussusceptive microvascular growth (IMG) (Figure 1.2). With IGM, the capillary network increases its complexity and vascular surface by inserting a

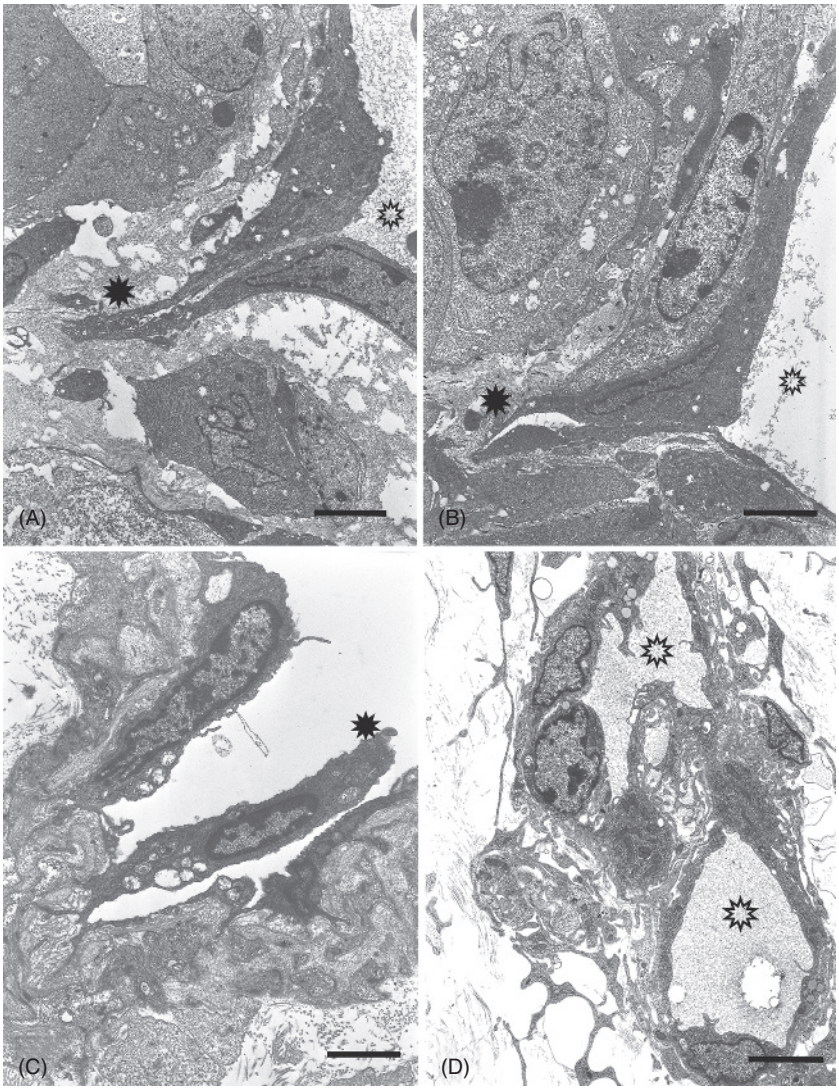

Figure 1.2 Electron micrographs of angiogenic areas in the chick embryo CAM. In A and B, sprouting processes penetrate into the perivascular mesenchyme at day 10 of incubation and show slit-like lumina (solid asterisks). Empty asterisks indicate the vascular lumen. The growing front of the vessel (sprouting endothelial tip) is devoid of pericytes. C and D depict the angiogenic process of intussusceptive growth. In C, an intravascular endothelial pillar (solid asterisk) is observable. In D, taken from an erythropoietin-stimulated CAM, the process of longitudinal segmentation of the original capillary into two newly formed blood vessels (empty asterisks) is completed. The perivascular stroma is penetrated deeply into the original lumen, pushing the endothelial lining inward and causing the formation of two distinct new vessels. Part D reproduced from Crivellato et al. [21] with permission from Nature. Bars, A–C = 1 μm; D = 3 μm.

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multitude of transcapillary pillars, through four consecutive steps:

1 Creation of a zone of contact between opposite capillary walls;

2 Reorganization of the intercellular junctions of the endothelium, with central perforation of the endothelial bilayer;

3 Formation of an interstitial pillar core; and

4 Subsequent invasion of the pillar by cytoplasmic extensions of myofibroblasts and pericytes, and by collagen fibrils.

It is thought that the pillars then increase in diameter and become a capillary mesh. The majority of vessels of the developing embryo are formed through angiogenesis.

Sprouting capillaries are guided by specialized EC called tip cells, which express vascular VEGFR-2, and are located at the leading front of growing vessels and continually extend and retract numerous filopodia, thus defining the direction in which the new vascular sprout grows.

The gross anatomy of the vascular system is characterized by highly reproducible branching patterns, with major and secondary branches forming at precisely designed sites and with organ-specific vascular architectures. For example, in lung development, there is a close structural expansion of lung parenchyma and lung vascularization. Developing lung vessels come into increasing proximity to the epithelial cells, which leads to the formation of the functional exchange regions of the alveolus. Otherwise, in brain development vascular endothelial cells penetrate in the brain anlagen recruited by endothelial cell mitogens released by neuroblasts. EC, in turn, release factors that support neuron development. Flow is critical to maintain vessel branches and a process termed intussusceptive branching remodeling has been described and shown to operate in changing branching angles.

Pruning involves the removal of supernumerary blood vessels from redundant channels. It results in reduction of the number of vascular branches and vascular density. This is one of the mechanisms allowing the vascular system to adapt to the changing hemodynamic and metabolic influences and to create a more efficient angioarchitecture.

Blood flow generally ceases in these excess capillaries, the lumens are obliterated and the EC retract toward adjacent capillaries. Remodeling is known to involve the growth of new vessels and the regression of others as well as changes in the diameter of vessel lumen and vascular wall thickness. Some vessels may fuse to form a larger one, such as fusion of the paired dorsal aortae, or they may establish new connections like the coronary vessels which connect to the aorta. It is likely that only a smaller number of embryonic blood vessels persist into adulthood, with most capillaries of the embryonic plexus regressing at some time in development (Figure 1.3).

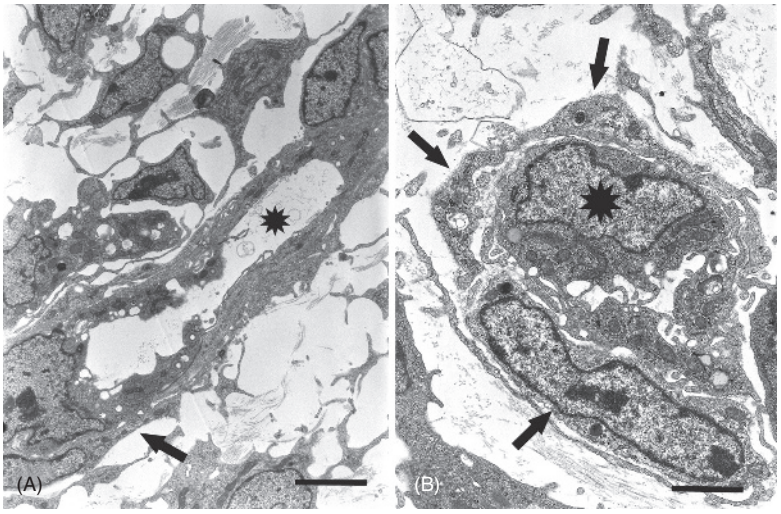

Figure 1.3 Electron micrographs of two capillaries in the chick embryo CAM. In A and B, two well-developed capillaries are observable at day 12 of incubation. Both capillaries present a continuous endothelial lining and are surrounded by cytoplasmic envelops from pericytes (arrows). A is taken from an erythropoietin-stimulated CAM and the solid asterisk points to the vascular lumen. Part A reproduced from Crivellato et al. [21] with permission from Nature. Bars = 2 μm.

Endothelial cell heterogeneity and organ specificity

EC form a continuous monolayer between the blood and the interstitial fluid. The EC surface in an adult human is composed of approximately 6×10^{13} cells and covers a surface area of approximately 7 m^2 [2].

The endothelial lining synthesizes, metabolizes, and releases a number of humoral and hormonal substances which act on adjacent cell systems or on some further distant structures. Quiescent EC generate an active antithrombotic surface through the expression of tissue factor pathway inhibitors: heparan sulfate proteoglycans that can interfere with thrombin-controlled coagulation, and thrombomodulin that facilitates transit of plasma and cellular constituents throughout the vasculature. Perturbations induce EC to create a prothrombotic and antifibrinolytic microenvironment.

Cessation of blood flow into a capillary segment causes vessel regression, whereas an increase in pressure may induce local recruitment of smooth muscle cells and lead to a differentiation of a capillary into an artery or vein.

There are differences between the endothelium of different species, between large and small vessels, and between EC derived from various microvascular beds and/or organs. Such differences have been ascribed to genetic predisposition and microenvironmental influences [3]. These latter include extracellular matrix components and locally produced growth factors, interactions with neighboring cells and mechanical forces. Interactions between the different microvascular cells and surrounding stromal cells have a major role in determining vascular structure and function. These interactions may occur through the release of cytokines and the synthesis and organization of matrix proteins on which the endothelium adheres and grows. The organ microenvironment can directly contribute to induction and maintenance of the angiogenic factors. The different angiogenic stages of the vasculature are precisely regulated by microenvironmental balance of proangiogenic and antiangiogenic molecules. Moreover, EC release in a paracrine fashion and express on the cell surface many signaling molecules that can affect the density of developing tissue cells intimately associated to them. This might be of crucial significance during organ formation. It has been speculated that EC–tissue interactions may "offer the opportunity to control organ development and growth systematically, rather than individually for each organ" [4].

EC and organ-specific cells interact with each other continuously, and this interaction is mutual in that EC and organ-specific cells exchange signals, allowing the generation of a functional organ provided with an endothelium adjusted to the needs of the adjacent tissue cells.

The introduction of electron microscope in the 1950s revealed that EC lining the capillaries of different organs are morphologically distinct. For instance, the vasculature of liver, spleen and bone marrow sinusoids is highly permeable because vessels are lined by discontinuous EC that allow cellular trafficking between intercellular gaps. Conversely, EC capillaries in the brain and retinal capillaries, dermis, bone tissue, skeletal muscle, myocardium, testes and ovaries are continuous. In the brain capillaries, the endothelium participates in the formation of the blood–brain barrier (BBB). EC in endocrine glands and kidney are fenestrated. Fenestration in the capillary endothelium seems to depend on VEGF-A secretion [5]. EC heterogeneity is also evident in individual organs. For example, the kidney contains fenestrated EC in its peritubular capillaries, discontinuous EC in its glomerular capillaries and continuous EC in other regions.

A novel angiogenic factor selective for endocrine gland endothelium known as EG-VEGF has been shown to induce fenestrations in capillary vessels [6]. EG-VEGF is unrelated to the VEGF family and acts via G-protein-coupled receptors.

The phenotype of EC is unstable and likely to change when they are removed from their microenvironment. The principal problem in defining organ-specific endothelial markers is the impurity of the EC used for *in vitro* analysis and the lack of organ-specific markers of EC in culture.

The presence of a unique organelle discovered by Weibel and Palade in 1964 was found to be an important marker to identify bona fide EC. Antigens are differentially expressed on EC of certain organs and tissues [7]. The von Willebrand factor (vWF) marker is widely but not uniformly expressed on EC. It is expressed at higher levels on the venous rather than on the arterial side of the capillary circulation, and in human tissues in the endothelium of larger vessels and in the adult endocardium. It is largely absent from sinusoidal EC.

Microvascular endothelium is more prone than large vascular endothelium to form capillary-like structures when seeded on extracellular matrix preparations and to respond to certain cytokines.

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EC alter their morphology in response to angiogenic factors. There is an increase in the expression of endoplasmic reticulum and Golgi apparatus, together with changes in mitochondrial size and number. The EC surface forms finger-like protrusions on the abluminal side adjacent to the basement membrane, intercellular gaps appear and the complex of EC and pericytes retracts.

☞ Key Concepts: Microvascular beds

- Endothelium derived from various microvascular beds and organs displays tissue-specific differences.
- Such differences depend on genetic and microenvironmental factors, including extracellular matrix components, locally produced cytokines and growth factors, interactions with neighboring cells and mechanical forces.
- EC express histogenetic and organogenetic properties. They release in a paracrine fashion and express on the cell surface many signaling molecules that can affect the destiny of developing tissue cells intimately associated with them.

Arterial and venous endothelial cell distinctions

Arteries and veins are structurally and functionally distinct. Classically, it was believed that EC of the primary capillary plexus constitute a rather homogeneous group of cells and that differentiation into arteries and veins occurred because of the influence of hemodynamic forces.

Labeling experiments in zebra fish indicate that the arterial and venous fate of endothelial precursors may be determined before the formation of the blood vessels. The discovery that members of the ephrin Efn family are differentially expressed in arteries and veins from very early stages of development was one of the first indications that artery–vein identity is intrinsically programmed. Efn-B2 is expressed in arterial EC, large arteries within the embryo and in the endocardium of the developing heart. The principal receptor for Efn-B2, Eph-B4, displays a reciprocal expression pattern in embryonic veins, large veins and also in the endocardium. This is the first evidence for a molecular difference between arterial and venous EC. Mutations of the Efn-B2 and of Eph-B4 both lead to early embryonic lethality. Remodeling of the primary vascular plexus into arteries and veins was arrested in both mutants, suggesting important roles for Efn-B2–Eph-B4 interactions on arterial and venous EC differentiation, respectively. Moreover, Efn-B2–Eph-B4 signaling participates in the formation of arterovenous anastomoses through arresting VEGF-A and Ang-1-induced EC proliferation–migration at the arterial–venous interface.

Other specific markers for the arterial system include neuropilin-1 (NRP-1) and members of the Notch family, Notch-3, DDL4 and GRIDLOCK (Grl). Venous markers include NRP-2 which, at later developmental stages, becomes restricted to lymphatic vessels in chick and mouse. Herzog et al. [8] examined the expression patterns of NRP-1 and NRP-2 during the early stages of vasculogenesis and concluded that, before the initiation of flow, the primitive vessels of the extraembryonic vascular plexus are already segregated into veins and arteries. Efn-B2 expression cannot be seen in the arterial part of the extraembryonic vascular plexus of 13-somite chick embryos even though the expression of NRP is already segregated. These observations suggested that Efn-B2 is a relatively late marker of arteries.

Notch signaling is required for remodeling the primary plexus into the hierarchy of mature vascular beds and maintaining arterial fate, and is essential for the homeostatic functions of fully differentiated arteries. Genetic studies have suggested a key role for Notch signaling, downstream of VEGF-A, in specifying arterial versus venous fate. During vascular development, defects in signaling through the Notch pathway, which comprises ligands such as Jagged-1, Jagged-2 and Delta-like-4, and receptors, such as Notch-1, Notch-2 and Notch-4, disrupt normal differentiation into arteries or veins, resulting in loss of artery specific markers [9].

Shear stress is considered to be the driving force behind arteriogenesis, which operates to increase the diameter of those vessels forced to handle more flow and hence subjected to an elevated shear stress. Le Noble et al. [10] used a time-lapse video microscopy system and examined arterial–venous differentiation in the developing yolk sac of the chick embryo. They observed that prior to the onset of flow, EC expressing arterial and venous specific markers are localized in a posterior–arterial and anterior–venous pole. Ligation of one artery by means of a metal clip, lifting the artery, and arresting arterial flow distal to the ligation site could morphologically transform the artery into a vein. When the arterial flow was restored by removal of the metal clip, arterial makers was reexpressed, suggesting that the genetic fate of arterial EC is plastic and

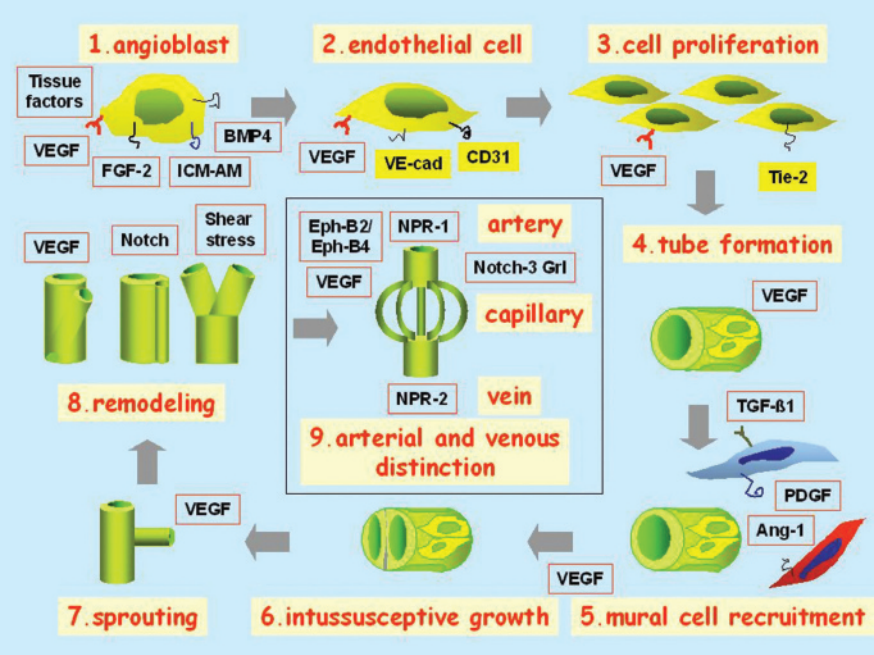

Figure 1.4 General scheme of blood vessel formation. Early capillaries establish through vasculogenesis. Endothelial cells arise from mesoderm via the differentiation of hemangioblasts and/or angioblasts (1). Cytokines such as vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), bone morphogenic protein-4 (BMP-4), intercellular matrix adhesion molecules (ICM-AM) and other tissue factors are crucial in driving this step. Once established, the endothelial cell lineage expresses VE-cadherin (VE-cad), PECAM-1 (also known as CD31) and Tie-2 receptor, and recognizes VEGF as its main growth and survival factor (2). Endothelial cell proliferation and migration as well as the formation of early vascular tubes are under the main control of VEGF (3 and 4). Capillaries are stabilized by pericytes and smooth muscle cells (5). The former are recruited by transforming growth factor β1 (TGF-β1) and platelet-derived growth factor (PDGF), the latter by the angiopoietin-1 (Ang-1)–Tie-2 signaling pathway. Vessels further grow by angiogenesis in the forms of either intussusceptive microvascular growth (6) or sprouting angiogenesis (7). Then, the early vascular networks undergo extensive pruning and remodeling (8) under the influence of driving forces such as VEGF and Notch signals as well as hemodynamic conditions (shear stress). The arterial and venous distinction is established by Ephrin-B2 (Efn-B2)–Eph-B4 signals, neuropilin-1 (NPR-1) and NPR-2 gradients, and members of the Notch family, including Notch-3 and GRIDLOCK (Grl) (9).

controlled by hemodynamics. A general schema of blood vessel development is represented in Figure 1.4.

☞ Key Concepts: Arteries and veins

- In zebra fish, arterial and venous fate of endothelial precursors may be determined before the formation of the blood vessels.
- Efn-B2, a member of the ephrin family, is expressed in arterial endothelial cells and the principal receptor for Efn-B2, Eph-B4, displays a reciprocal expression pattern in embryonic veins.
- In the chick and mouse, specific markers for the arterial system include neuropilin-1 (NRP-1) and members of the Notch family, Notch-3, DDL4 and GRIDLOCK (Grl).
- Notch-1, Notch-2 and Notch-4 receptors bind to ligands such as Jagged-1, Jagged-2 and Delta-like-4.
- Shear stress is considered to be the driving force behind arteriogenesis.

Lymphatic capillaries

Structural features of lymphatic capillaries include:
- Their endothelium has an extremely attenuated cytoplasm, except in the perinuclear region;
- 5′-nucleotidase activity of the endothelium;
- Tight and adherent junctions are not frequently seen;

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• A discontinuous basement membrane, expressing collagen type IV and laminin;
• The absence of pericytes;
• Lymphatic endothelial cells (LEC) are closely linked to surrounding connective tissue by fine (10–12 nm) anchoring filaments. These filaments are attached to the cell's abluminal surface and extended deeply into the connective tissue, firmly attaching endothelium to extracellular matrix fibres.

More than 10 years ago, highly specialized and specific antibodies against LEC have been identified. Prospero-related homeobox-1 (*Prox-1*) is a homeobox gene, expressed only by LEC. Studies of mice deficient in *Prox-1* revealed that these mice were unable to develop a lymphatic vascular system and that *Prox-1* was required for a subset of venous EC in the embryonic cardinal veins to migrate out and to form the initial lymphatic vessels during early embryogenesis. Sox-1 has been identified as a novel protein that trans-activates *Prox-1* expression in LEC of mice. Lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) is a homolog of the CD44 glycoprotein expressed by LEC. In normal tissues, LYVE-1 is highly expressed in lymphatic vessels of the intestinal villi, dermis, lymph nodes, vermiform appendix and stomach. Podoplanin is an integral plasma membrane protein primarily found on the surface of rat podocytes expressed by LEC, but not by blood vessels. Podoplanin was first used to identify lymphatic vessels, but it was later shown that it is a useful marker for some malignant tumors. D2-40, which recognizes the formalin-resistant epitope of podoplanin, is the most specific and sensitive marker of LEC. Desmoplakin, a protein of the junctional system, connecting very flat LEC, is a marker for small lymphatic vessels and is not expressed by larger lymphatic collecting ducts, including the thoracic duct.

Comparative microarray analyses of specific transcriptomes of LEC versus vascular EC have revealed a number of novel differentially expressed genes, although approximately 98% of genes are expressed at comparable levels in those genetically closely related cell types. Transcriptional profiling studies revealed increased expression of several extracellular matrix and adhesion molecules in vascular EC, including versican, collagens, laminin and N-cadherin, and of the growth factor receptors endoglin and VEGFR-1. Among several genes with specific expression in LEC, VEGFR-3, Prox-1, LYVE-1 and podoplanin should be mentioned.

VEGF-C and VEGF-D have a crucial role in lymphangiogenesis through the activation of VEGFR-3. Selective activation of VEGFR-3 in transgenic mice expressing VEGF-C or VEGF-D is sufficient to induce lymphangiogenesis without major effects on angiogenesis. Ang-2 is expressed by LEC and is involved in the normal development of the vascular system. Ang-2 null mice show disorganization and hyperplasia of the lymphatic capillaries associated with changes in the media of collecting ducts and lymphedema.

There was accumulated evidence that supports the proliferative activity of LEC in prenatal and/or postnatal life, both in physiologic and pathologic conditions. Based on these observations, it was hypothesized that lymphatic vessel growth and/or growth factors that induce lymphangiogenesis, such as VEGF-C and VEGF-D, platelet-derived growth factor-BB (PDGF-BB) and hepatocyte growth factor may be inhibited by specific antibodies.

Tumor lymphangiogenesis is stimulated by VEGF-C and VEGF-D, and both lymphangiogenesis and lymph node metastases are inhibited by VEGF-C and VEGF-D antagonists. Numerous studies have demonstrated a direct correlation between VEGF-C and VEGF-D expression in human cancer and tumor metastasis, suggesting that lymphangiogenesis has an important role in promoting tumor metastasis.

LEC-specific markers have multiple functions in physiologic and pathologic conditions, are helpful to identify tumor tissue changes related to lymphangiogenesis and to search for a rational therapeutic approach. Some questions regarding tumor lymphangiogenesis remain unanswered, including the mechanisms of migration and invasion of tumor cells into the lymphatic vessels, which is the key factor for tumor metastasis, and the differences between pre-existing and newly formed lymphatic vessels. The immunohistochemical application of podoplanin has been used to investigate the relationship between lymphatic vessel density and lymph node metastasis, for tumor cell detection in lymphatic vessels and for the diagnosis of some vascular tumors.

☞ Key Concepts: Lymphatic capillaries

- Lymphatic capillaries have an extremely attenuated, 5′-nucleotidase-positive endothelium which exhibits few tight and adherent junctions and lines a discontinuous basement membrane, expressing collagen type IV and laminin.

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- Pericytes are absent and anchoring of lymphatic endothelial cells (LEC) to the surrounding connective tissue is effected by fine (10–12 nm) filaments.
- Specific gene and molecular marker profile is expressed by LEC. The homeobox gene prospero-related homeobox-1 (*Prox-1*) is expressed only by LEC.
- Molecular markers for LEC include the lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1), podoplanin, D2-40 and desmoplakin.

Blood–brain barrier

The existence of a specialized barrier at the level of cerebral vessels was first postulated in 1900 by Lewandowsky, based on the observation that the intravenous injection of cholic acids or sodium ferrocyanide had no pharmacologic effects on the central nervous system, whereas neurologic symptoms occurred after intraventricular application of the same substances. Lewandowsky introduced the term blood–brain barrier (BBB) to describe this phenomenon.

The BBB is a complex cellular system of EC, astroglia, pericytes and neurons, to establish a functional "neurovascular unit." Brain EC have particularly complex tight junctions (comprising several classes of transmembrane molecules, including occludins and claudins, which interact with transmembrane proteins of adjacent EC), and few pinocytotic vesicles that together act as a physical barrier. Moreover, they are endowed with a variety of transport proteins, such as transferrin receptors, gamma-glutamyl transpeptidase, Glut-1 and P-glycoprotein. Establishing the barrier is accompanied by further changes in the phenotype of the brain EC, such as upregulation of the HT7-antigen/basigin, or downregulation of the MECA-22 antigen.

Astrocytes project their endfeet tightly to cerebral EC, and both influence and conserve the barrier function. Early tissue culture studies have demonstrated that conditioned medium by astrocytes can induce tight junction formation in capillary EC. Subsequent characterization of astrocyte–endothelial interactions have identified a number of factors that can modulate the expression of tight junctions and/or transendothelial permeability, such as TGF-β1, fibroblast growth factor-2 (FGF-2), glial-derived neurotrophic factor (GDNF) and Ang-1, which induce BBB properties such as high electrical resistance and reduced permeability in EC. The effects between EC and astrocytes are reciprocal, with alterations between the two cell types leading to alterations in astrocyte shape and growth.

Pericytes limit the transport across the endothelial barrier and release Ang-1 and TGF-β1 which induce and maintain critical BBB functions. More recently, it has been demonstrated in the human fetal telencephalon that growing microvessels are formed by a pericyte-driven angiogenic process in which EC are preceded and guided by migrating pericytes. The basement membrane lies beneath EC, envelops pericytes and comes in contact with the subjacent and tightly adherent glial processes.

The features of barrier vessels are acquired during the embryonic development by progressive decrease in their permeability, by structural modifications involving both endothelial tight junctions and glial perivascular endfeet differentiation, and by expression of specific endothelial transporters and antigens. It is well accepted that the vessel morphofunctional maturation is coupled with the expression of tight junction proteins, such as zonula occludens-1 (ZO-1), and of the glial end-feet proteins, such as aquaporin 4 (AQP4) and glial fibrillary acidic protein (GFAP).

The loss of the BBB is commonly observed when tumors invade and grow into the brain. It has been attributed to the generation of neovasculature with fenestrated endothelium, opened intercellular junctions and incomplete basement membrane.

☞ Key Concepts: The blood–brain barrier

- The blood–brain barrier (BBB) is a neurovascular unit shaped by neurons, endothelial cells, astrocyte end-feet and pericytes.
- Brain EC express distinct transmembrane molecules, such as occludins and claudins, as well as transport proteins, such as transferrin receptors, gamma-glutamyl transpeptidase, Glut-1 and P-glycoprotein.
- BBB properties are molded by astrocyte–endothelial interactions which modulate the expression of tight junctions and/or transendothelial permeability, through TGF-β1, FGF-2, GDNF and Ang-1 signaling.
- Ang-1 and TGF-β1 are also released by pericytes that function as endothelial tube guides.
- Maturation of the BBB is coupled with the expression of tight junction proteins, such as zonula occludens-1 (ZO-1), and of the glial end-feet proteins, such as aquaporin 4 (AQP4) and glial fibrillary acidic protein (GFAP).

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Implications of vascular diversity for disease expression and therapy

Demonstrated or accepted

The characteristics of the endothelium – in addition to environmental factors, which also include the type of local blood flow – are of critical relevance in determining disease susceptibility. For instance, it has long been recognized that systemic vasculitides impact distinct segments and branches of the vascular tree. New findings indicate the importance of smooth muscle cells and dendritic cells in the pathogenesis of systemic vasculitides. Dendritic cells are localized at the adventitia-media border of the normal medium-sized arteries and expressed a series of Toll-like receptors in a vessel-specific pattern. Whereas necrotizing sarcoid granulomatosis, Takayasu's arteritis, and giant cell arteritis cause macrovascular compromise, cryoglobulinemic vasculitis affects microcirculation. The pulmonary vascular bed has intensely been investigated in relation to its structural and functional differentiation into segmental compartments. Remarkably, selective location of Weibel–Palade bodies within EC of arteries and arterioles but not capillaries has been recognized. Thus, the different EC subpopulations and their surrounding microenvironment may represent important factors in pulmonary vasculitides. Selective involvement of the skin vascular bed has been recognized in mouse models of diffuse sepsis [11]. In these studies, polymerase chain reaction (PCR) analysis evidenced increased mRNA levels of EC activation markers, such as P-selectin, endothelial intercellular adhesion molecule-1 (ICAM-1) and plasminogen activator inhibitor-1 (PAI-1), which were restricted to the skin vasculature whereas brain, heart and lung vessels appeared unaffected. Even thrombotic or hemorrhagic states recognize specific vascular beds as the sites of disease occurrence. It is the endothelium, indeed, that synthesizes a large number of anticoagulants and procoagulants, which are unevenly expressed in the vasculature. The prothrombotic factors include tissue factor; vWF; protease-activated receptors (PAR-1 and PAR-4), serving as thrombin receptors; thromboxane A2 and platelet-activating factor; PAI-1, which inhibits fibrinolysis; and adhesive molecules, which attract leukocytes to the endothelial surface. In contrast, there are antithrombotic factors consisting of tissue factor pathway inhibitor; protein C and protein S; thrombomodulin; heparan; nitric oxide synthetase; prostacyclin; and members of the plasminogen–plasmin system. Data indicate that hemostasis is differentially regulated between different vessel types and organs, and most hyper- and hypocoagulable states – including those associated with a systemic imbalance in anticoagulants and procoagulants – lead to local thrombotic lesions or hemorrhagic complications.

The tumor growth-stage-specific efficacy of drugs suggests that qualitative differences exist in the tumor vasculature at different stages [12]. Distinct tumor vessels may need specific vascular growth factors and cytokines at defined tumor stages. Remarkably, only the mother vessel and the glomeruloid microvascular proliferation types of tumor vessels require VEGF-A for their maintenance, whereas the other types of tumor vessels have acquired VEGF-A independence. This fact may explain the limited success of anti-VEGF-A/VEGFR therapy in human cancer.

Finally, there are vascular tumors that derive from EC and express unique autonomous properties. In infantile hemangioma, a benign vascular lesion of EC origin, molecular profiling has provided evidence for a placental derivation of EC [13]. Kaposi's sarcoma, an AIDS-defining vascular tumor, involves a phenotypically unique spindle cell that appears to derive from lymphatic EC [14].

Hypothetical

In speculative terms, the phenotypic heterogeneity of EC in the different vascular beds may have profound implications in disease natural history. As previously mentioned, vWF, P-selectin and factor VIII are located within Weibel–Palade bodies in pulmonary arteries and arterioles, but these prothrombotic and proinflammatory organelles are absent in capillaries. Despite the absence of Weibel–Palade bodies, pulmonary capillaries express vWF, P-selectin and factor VIII. This distinct segmental organization may have important implications in the mechanisms of pulmonary thrombosis and neutrophil trafficking during pneumonia.

Coronary artery disease is an example of a disease that targets the arterial EC. In response to hypercholesterolemia, myocardial EC increase the expression of adhesion molecules, which leads to intimal thickening and plaque formation. Indeed, atherogenic oxidized low density lipoprotein (LDL) preferentially induces cellular proliferation and adhesion pathway genes in human coronary artery EC, whereas in human saphenous vein EC, focal adhesion, inflammatory response, apoptosis and NFκB pathway genes are downregulated [15]. Furthermore, molecular signals, such as tumor necrosis factor

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α (TNFα) and interleukin 1β (IL-1β) activation, induce apoptosis and downregulate anti-inflammatory genes in human coronary artery EC, whereas both antiapoptotic and antiatherogenic genes are induced in human saphenous vein EC. Long-term exposure to systemic disease conditions can also alter the basal gene expression pattern and functional behavior of EC in an EC subset-specific manner. This may have important implications in metabolic diseases such as diabetes. Using the type 2 diabetic Goto-Kakizaki rat model, it has been demonstrated that myocardial microvascular EC express decreased protein levels of VEGF, VEGFR-1 and VEGFR-2, and exhibit decreased phosphorylation of the receptors compared with their healthy controls, whereas aortic EC from the diabetic rats do not exhibit such an altered phenotype [16]. Selective EC activation may be responsible for the development of some brain pathologies. For instance, BBB dysfunction may be linked with Alzheimer's disease. Brain microvessels appear thin and tortuous and their basement membrane is thickened and vacuous.

Local blood flow is an important factor for EC stability in a given vascular segment. EC lack preferential cell alignment and often show a polygonal morphology in zones of disturbed vascular flow in regions susceptible to atherogenesis such as the aortic arch or heart valves. Analysis of EC gene expression at such locations exhibits an upregulation of genes associated with endoplasmic reticulum processing of proteins, endoplasmic reticulum stress and unfolded protein response. This genetic profile may, in turn, contribute to enhanced endothelial permeability via focally increased EC proliferation in these regions [17,18]. Studies performed in the swine aortic valve have shown that the endothelium of the normal aortic side was phenotypically distinct from that of the ventricular side, expressing a balance of pro- and anti-inflammatory transcripts and a procalcified profile [19]. Transcript profiling of valve endothelial populations demonstrated that the susceptible aortic side was much more sensitive to 2 weeks of hypercholesterolemic diet than the ventricular side [20].

Clinical implications

- EC diversity has crucial implications for the susceptibility to vascular disease.
- Smooth muscle cells and vascular dendritic cells contribute to vascular diversity.
- Systemic vasculitides and diffuse septic reactions target distinct segments and branches of the vascular tree as well as selective vascular beds.
- Thrombotic or hemorrhagic conditions recognize specific vascular beds.
- Vascular diversity has potential implications for the pathogenesis of metabolic diseases like atherogenesis and diabetes.
- EC heterogeneity is recognizable in the tumor vasculature at different stages, a situation that may profoundly affect the efficacy of tumor treatment.

Conclusions

Blood vessels develop early during embryo life by vasculogenesis and represent an essential component of all organs. Both genetic and epigenetic factors are involved in blood vessel formation. They arrange into a sophisticated, highly branched sequence of vascular channels lined by EC that express a precise spatial and temporal hierarchy. This segmental heterogeneity implies a local multiplicity of structural and functional diversifications. Recent data are consistent with the assumption that EC phenotypes differ in space and time providing a foundation for the identification of specific molecular signatures to a given microvascular bed. As clearly expressed by Barnes et al. [13], "at any given point in time, no two ECs in the body are phenotypically identical." In addition, a unique EC type, expressing the basic structural and molecular profile of vascular EC but also exhibiting distinct morphologic and functional characters, lines lymphatic vessels.

EC diversity has crucial implications for the development of vascular diseases. Systemic vasculitides and diffuse septic reactions target distinct segments and branches of the vascular tree as well as selective vascular beds. Even thrombotic or hemorrhagic conditions recognize specific vascular beds as the sites of disease occurrence. Potential implications for the pathogenesis of vascular metabolic diseases like atherogenesis and diabetes are also strong. EC differences exist in the tumor vasculature at different stages, a situation that may profoundly affect the efficacy of tumor treatment.

In conclusion, understanding how early, basic EC can differentiate into a specialized assortment of organ- and tissue-associated EC is essential for appreciating the complexity of vascular disorders and for establishing critically designed strategies of treatment for vascular diseases.

Indeed, identification of vascular-bed-specific molecular profiles should facilitate the development of molecular imaging for diagnosis and surveillance as well as the improvement of "intelligent" molecules targeting selected vascular districts.

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