Role of the Endothelium in Neonatal Diseases

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Abstract

In both fetal and neonatal physiologic and pathologic processes in most organs, endothelial cells are known to play critical roles. Although the endothelium is one of the most ubiquitous cell type in the body, the tight adherence to the blood vessel wall has made it difficult to study their diverse function and structure. In this article, we have reviewed endothelial cell origins and explored their heterogeneity in terms of structure, function, developmental changes, and their role in inflammatory and infectious diseases. We have also attempted to evaluate the untapped therapeutic potentials of endothelial cells in neonatal disease. This article comprises various peer-reviewed studies, including ours, and an extensive database literature search from EMBASE, PubMed, and Scopus.

Keywords

Angiogenesis; Bronchopulmonary dysplasia; Endothelium; Necrotizing enterocolitis; Neonate; Retinopathy of prematurity

Introduction

Endothelial cells are metabolically active cells bordering the blood vessels inner lining, where they have a crucial function in both physiology and pathology. Due to their critical anatomic location, these cells have always been believed to have unlimited therapeutic potential, but the relative inaccessibility of the endothelium in intact organs has curtailed detailed *in vivo* studies. Recent advances in diagnostic microtechnology have provided

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some solutions to this problem, at least in larger blood vessels, and have renewed the scientific interest in these cells. These cells are important regulators of trans-vascular blood-to-tissue barrier to macromolecules and nutrients, trafficking of leukocytes between blood and inflamed tissues, and of tissue respiration via both hemodynamic homeostasis and neoangiogenesis. With the dispersive, arboreal vascular arrangements, endothelial cells are distributed throughout our body.

Abnormalities in the function of endothelial cells are depicted in several neonatal conditions, such as intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), hypoxic-ischemic encephalopathy (HIE), bronchopulmonary dysplasia (BPD), acute kidney injury (AKI), and necrotizing enterocolitis (NEC). Endothelial markers may be helpful in the diagnosis, monitoring, prognosis, and clinical management of many neonatal conditions. Therapeutic targeting of microvascular structure and function may also be useful in neonatal conditions. The current article merges peer-reviewed evidence arising from our research as well as extensive literature review from notable databases, such as Scopus, EMBASE, and PubMed.

Origin of Endothelial Cells

The vascular system plays a vital homeostatic role in all vertebrates by promoting nutrient transport, oxygen, waste products and metabolites, immune surveillance, and the autoregulation of perfusion via chemical stimuli and hormones that help in the communication between the blood vessels and underlying tissues. Endothelial progenitor cells (EPCs) were discovered in the late 1990s resulting in a paradigm shift in our understanding of angiogenesis. The endothelium consists of a single layer of cells lining blood vessels in the body and is formed very early in gestation. Angiogenesis refers to the formation of new capillaries from existing vessels, while vasculogenesis refers to *de novo* formation of blood vessels during embryonic development.[1–3] There are several sources of endothelial cells, including the neural crest cells, embryonic mesoderm, and hemangioblasts. These have been summarized in Figure 1.

- *Differentiation from embryonic mesoderm:* These primitive mesodermal cells differentiate into hematopoietic precursors or angioblasts, and these cellular subsets can both develop into endothelial cells.[4,5] The intraembryonic endothelium forms a primitive vascular labyrinth[2,6] shortly after gastrulation in the extraembryonic yolk sac. The endothelial/vascular maturation of mesodermal cells is induced by signals emanating from visceral endoderm,[7] such as increased production of growth factors such as the basic fibroblast growth factor (bFGF) or FGF2, bone morphogenetic protein 4 (BMP4), and the vascular endothelial growth factor (VEGF).[3,8]

The transition of mesenchymal into endothelial cells may be a reversible, bidirectional process. The activation of transforming growth factor-β, bone morphogenetic protein, wingless/integrated (Wnt), and the Notch signaling pathways may be important in a mechanistic sense.[9,10] The "dedifferentiation" and activation of endothelial cells involve a change in appearance from a characteristic cobblestone to a more elongated, "mesenchymal" shape with increased migratory and proliferative capacity. These transformed cells lose

some of the intercellular junctional proteins and related barrier function[7,10] but become pro-inflammatory with higher levels of leukocyte adhesion molecules (intercellular adhesion molecule 1, vascular cell adhesion molecule 1), cytokines, and various growth factors.[11] However, with alterations in function, these changes may also shorten the lifespan of these cells. Such endothelial-to-mesenchymal transitions have been noted in various pathological conditions marked by vascular injury, chronic inflammation, and shear stress.[10,11]

- *Differentiation from hemangioblasts:* Plein et al.[12] showed that nearly a third of all endothelial cells in the brain and up to 60% of those in the liver may originate from hemangioblasts differentiating into erythro-myeloid progenitors (EMPs). These EMP-derived endothelial cells express high levels of the gene *Hoxa*. In another study, Feng et al.[13] showed that these intraembryonic endothelial cells likely do not originate from circulating EMPs that express the cluster differentiation (CD) 45 (protein tyrosine phosphatase receptor type C)+. Csf1r-expressing EMPs may also not consistently differentiate into endothelial cells in the brain, liver, heart, and lungs.

The term "hemangioblast" was coined by Murray[14] early in the 20th century to describe a subset of cells that can differentiate into either endothelial or hematopoietic cells during embryogenesis. This hypothesis found favor in the physical proximity of hematopoietic and endothelial lineages within blood islands,[14,15] but the conclusions were not definitive due to the structural complexities in the developing blood islands and also because of the limited number of cells available to study during these early stages of development. Previous imaging and tissue engineering indicating spatiotemporal associations between these embryological hematopoietic and endothelial lineages[16,17] and studies indicating that human embryonic stem cells are differentiated *in vitro* into both hematopoietic and endothelial cell lineages[18] have largely been refuted.

Recent literature suggests that some of the hemogenic endothelium may be a source of hematopoietic stem cells (HSCs). Lineage-tracing studies, *ex vivo* culture, and time-lapse confocal imaging show that hematopoietic cells, including HSCs herald from a hemogenic endothelium, and form an intermediate endothelial state.[4,19,20] Hemogenic endothelium is a specialized subset of the endothelium with only a transient capacity to produce hematopoietic cells through endothelial-to-hematopoietic transition.[21] In murine models, endothelial cells that lose endothelial characteristics to assume a more hematopoietic phenotype begin to co-express surface markers CD144, CD31, KDR, CD117, and CD34, but not the hematopoietic markers, such as CD41, CD45, CD73, and Ter-119.[21–24] Human hematogenic endothelial cells express the surface markers CD43, CD34, CD144, CD117, CD90, CD45, and CD105, but low CD38, and almost not CD45RA.[22,25]

The HSCs are self-renewing cells with multilineage reconstitution potential following transplantation into a recipient. After birth, HSCs are seen predominantly in the bone marrow and form a self-renewing pool at the apex of the hierarchical network of hematopoiesis. Some HSCs are also known to differentiate into hematopoietic progenitor cells (HPCs).[22,26] The HPCs differ from HSCs with relatively limited self-renewal and engraftment potential.

Bone marrow-derived EPC: EPCs in the bone marrow have been redefined in numerous recent consensus statements to possibly originate from the following:

- Endothelial Progenitor Cells: EPCs express surface markers, such as factor VIII, CD31, CD34, E-selectin (CD62E), intercellular adhesion molecule (ICAM)-1 (CD54), von Willebrand factor (vWF), and VCAM-1 (CD106).[1,27–29] EPCs can migrate to the peripheral blood and express surface adhesion molecules that regulate the movement of these cells to and away from the blood.

- Mesenchymal Stem Cells: Data on mesenchymal stem cells (MSCs) being a source of endothelial cells are controversial. Colony-forming units of fibroblasts (CFU-Fs) in the bone marrow, also known as the MSCs, express CD29, CD71, CD73, CD90, CD144, CD120a, CD105, CD106, and CD 124,[30–33] but no CD34, CD31, vWF, vascular endothelium cadherin (VE-cadherin), VEGFR2, CD62E, VCAM-1, and ICAM-1.[30–33] CD44 was detected in some studies,[31,33] but not in others.[30] MSCs expressing VEGFR2, vWF, and VE-cadherin are most likely endothelial progenitors.[30,32,33] The discovery that mesenchymal cells can rescue damaged endothelial cells was demonstrated using laser scanning confocal microscopy to show that mitochondrial transfer was facilitated by a tunneling nanotube-like structure between human umbilical vein endothelial cells and MSCs.[34]

Endothelial Cell Phenotypes

The phenotypic markers on endothelial cells can vary between various vascular structures in a particular organ and also between different organs. There may also be important structural variations notable within capillaries, veins, and arteries. The endothelium found in veins and arteries may seem to be comprised of an uninterrupted, continuous layer of cells; the capillary endothelium in various tissues can show more obvious differences and may appear continuous, discontinuous, or fenestrated.[35] These spatial and temporal variations have been correlated with differential expression of various messenger RNA (mRNA) and proteins.

The first reported arterial EC marker was a transmembrane ligand ephrinB2 arterial.[36] Notch signaling is vital to arterial EC differentiation. Loss of Notch signaling leads to a loss of the expression of ephrinB2 in the arteries in zebrafish.[37] The first reported marker for venous EC was the ephrin B2 receptor tyrosine kinase EphB4.[36] Venous EC differentiation is recognized as a default EC differentiation pathway, resulting from inadequate activation of Notch signaling during the differentiation of angioblasts to ECs.[38,39] Lymphatic ECs are formed as a result of differentiation from venous ECs. Prox-1 is the most functional and specific EC of lymphatic origin. Disruption of mouse Prox-1 disrupts lymphatic vessel development and budding of lymphatic ECs.[40] Insufficient activation of VEGFR3 signaling leads to hypoplastic lymphatic vessels.[41]

Endothelial cells have diverse microenvironments across vascular beds and display unique structural and morphologic heterogeneity across organs. The EC-translating ribosome affinity purification (TRAP) has emerged as a powerful tool to analyze the *in vivo* EC translatome across several diverse vascular beds to provide greater accuracy, sensitivity,

and cellular resolution instead of whole-tissue RNASeq. TRAP identified 82 gene markers shared by five vascular beds (lung, heart, kidney, liver, and brain), such as *Tek* and pan-EC markers such as *Eng*, *Nos3*, *Cdh5*, and *Robo4*.[42]

Table 1 summarizes various endothelial cell markers; some are expressed after activation of growth factors and inflammatory cytokines, and others refer to specific endothelial cells in different organs or tissues. Endothelial cell phenotypes include the following:

- Endothelial cells precursors: Embryonic ECs as a cell lineage expand without contribution circulating precursors or new angioblasts are the current consensus.[12] The relationship of circulating endothelial progenitors to myeloid cells remains subject to controversy.[43] Cells of myeloid origin are CD14^{+}, while EPCs are CD14^{−}. However, monocytes or macrophages (CD14^{+} cells) can adopt an endothelial phenotype during angiogenesis.[44]

- Brain Endothelium: In the central nervous system, endothelial cells regulate plasma filtration and the movement of circulating cells through the blood-brain barrier, most likely via the assembly of tight junctions.[45,46] Cerebral microvasculature likely originates from the meningeal vessels, but the subsequent angiogenesis involves the whole brain.[47]

Heterogeneity in EPCs

EPCs are a heterogeneous population of mononuclear cells originating in the bone marrow and can be mobilized to the fetal/postnatal circulation.[48–50] EPCs make up 1–5% of all bone marrow cells and about 0.0001–0.01% of monocytes circulating in the peripheral blood.[51] These cells express endothelial antigens, like CD31, vWF, VE-cadherin, endothelial nitric oxide synthase (eNOS), and VEGFR2.[52–55] The differentiation of hemangioblasts into endothelial cells has been studied in greater detail (Fig. 2). Based on phenotypical and biological properties, the EPCs are believed to be comprised of early and late EPC subgroups. Early EPCs give rise to the conventional colony-forming unit-endothelial cells (CFU-Es) and augment angiogenesis in a concentration-dependent or paracrine manner, whereas the outgrowth and differentiation of late EPCs promote the development of vascular networks.[56] Early EPCs are spindle-shaped, CD133 + CD45 +, and have limited proliferative capacity, a relatively short lifespan of about 3–4 weeks, and secrete angiogenic factors, such as VEGF, interleukin-8, and the CXC-ligand 8/CXCL8. Late EPCs are cobblestone-shaped, CD31 + KDR +, appear at 2–3 weeks, may live up to 12 weeks, proliferate rapidly, and express VE-cadherin, Flt-1, and CD45.[56,57] Both early and late EPCs seem to have comparable vasculogenic capacities.

Based on gene expression profiles, endothelial cells increasingly seem to be a heterogeneous population. Endothelial subpopulations have been identified that show differences in the expression of bone morphogenic protein-2, -4; ephrin-4, and neuropilin-1. In the skin, distinct endothelial cells express platelet and endothelial cell adhesion molecule 1 (PECAM-1), notch-1, and leukocyte markers (ICAM-1, L-selectin, notch 2, CD36, and CD163).[55] The aorta shows at least 3 distinct subpopulations, one comprised of lymphatic endothelial cells, whereas the other two seem to be specifically involved in

angiogenesis, lipoprotein processing, and extracellular matrix production.[58] The adult mouse lung contains a distinct subpopulation of endothelial cells that expresses high levels of carbonic anhydrase 4 (Car4) and is distinct from arterial and venous macrovascular, and microvascular endothelial cells.[59] Car4-high endothelium is located throughout the lung periphery, expresses high levels of CD34 and VEGF receptors, and responds to VEGF-A. High numbers of Car4-high ECs can be seen in lung regions regenerating after influenza- or bleomycin-induced injury. The discovery of endothelial subsets with differing capacities for angiogenesis has opened exciting therapeutic possibilities.

Endothelial Cell Function

Endothelial cells show a vast heterogeneity in function. The vascular endothelium is exposed to and responds to numerous tissue microenvironments, resulting in a substantial phenotypic heterogeneity in the vascular system. Epigenetic and non-epigenetic factors are responsible for determining this heterogeneity in the endothelium. Marcu et al.[60] studied endothelial cells isolated from the lungs, heart, liver, and kidneys, and showed organ-specific ECs to have a unique expression of gene clusters, potential for angiogenesis, barrier properties, and metabolic rates, each of which enables their organ-specific functional and development properties. Endothelial cells are known to be highly ubiquitous and one of the most functionally diverse cell systems. Vascular endothelial lining regulates blood flow, nutrient delivery and waste removal; blood coagulation; inflammation; angiogenesis; and vascular remodeling through autonomous and intercellular signaling mediated via neurotransmitters, hormones, and cytokines; and interaction with several cells, such as smooth muscle cells, pericytes, cytokines, and blood cells.[61] Prostacyclins and endothelium-derived nitric oxide (NO) cause vasodilation, while superoxide, endothelin, and thromboxane induce vasoconstriction; both sets of mediators regulate tissue perfusion.[62]

Endothelial Cell Barrier Function

The endothelial lining surface area is large and facilitates the substance exchange between blood and tissues. In humans, the endothelial surface area is estimated to be about 350 m^2.[63,64] Cells in the endothelial cell monolayer are linked to one another via tight, adherent, and gap junctions, which then connect to cytoplasmic proteins and the cytoskeleton.[65] Interestingly, endothelial cells maintain a tight barrier function throughout the process of vascular remodeling; vasculogenesis stimulants, such as VEGF-A, do not change microvascular permeability in the inner blood-retina barrier *in vivo* or *in vitro*, even when specific changes may be seen in transcellular transport or in tight or adherens junctions.[66–68]

The plasma membranes of closely aligned endothelial cells form an important barrier with tight junctions. The main transmembrane constituent of these junctions is the occludins.[69] Below the tight junctions, the adherens junctions are comprised of several proteins, including the surface adhesion glycoproteins, VE-cadherins, which form a zipper-like component at the base of endothelial cells. These proteins connect with their cytoplasmic tail to the underlying actin-based microfilament cytoskeleton.[70,71]

Endothelial Cell Response to Shear Stress

Endothelial cells react actively to blood flow, predominantly to mechanical cues with polarizing changes in conformation, electrical charge, or to the release of biochemical stimuli, such as nitric oxide or prostacyclin.[30,31,72] At rest, endothelial cells typically are shaped like a polygon, but under conditions of stress, they elongate in the direction of flow, thereby reducing the resistance to moving fluids.[30] In response to shear stress, cultured endothelial cells elongate and become oriented along the direction of blood flow[32] by reorganizing the cytoskeleton.[33] Shear stress is known to directly activate the endothelial NO synthetase (eNOS) promoter and increase its expression, and also promote the release of endothelial cell factors that promote endothelial cell survival while inhibiting leukocyte migration, coagulation, and smooth muscle proliferation.[30,72]

Endothelial Cell as Regulator of Vascular Tone

Endothelial lining of vessels regulates vascular tone and function in response to numerous neurotransmitters, hormones, and vasoactive factors.[62] The endothelium releases various vasoactive factors that can be vasodilatory, such as NO, prostacyclin (PGI_2), and endothelium-derived hyperpolarizing factors (EDHF) or vasoconstrictive, such as thromboxane (TXA_2) and endothelin-1(ET-1).[62] Any imbalance of these vasoactive factors leads to dysfunction of the endothelium.

Endothelial Cells in Angiogenesis

The onset of neovascularization or angiogenic switch[73] has several triggers, such as metabolic stress, hypoxia, inflammatory stimuli, and immune response, and may also be related to genetic mutations.[74] During hypoxic conditions, hypoxia-responsive transcription factors regulate the expression of genes that allow tissues and cells to acclimatize to low oxygen conditions.[74] VEGF as an endothelial cell-specific mitogen is unique for its roles in promoting endothelial cell proliferation and vascular permeability.[49,75] VEGF can stimulate blood vessel development through the process of vasculogenesis or angiogenic sprouting, whereas ephrinB2 and Ang1 promote vascular remodeling and maturation of the vasculature.[49,76] VEGF has three major isoforms that originate from alternative splicing, namely VEGF-A_{120}, VEGF-A_{164}, and VEGF-A_{188};[77] these isoforms also exhibit anti- and pro-angiogenic splice variants. VEGF is known to have two transmembrane receptors, VEGFR1, otherwise known as the feline McDonough sarcoma (fms)-related receptor tyrosine kinase 1 (Flt1), and VEGFR2, otherwise known as the kinase insert domain receptor (Flk-1). VEGFR1 is known to be expressed either as a soluble Flt1 receptor (sFlt1) formed through alternative splicing of the Flt1 mRNA[75] or as the membrane-bound Flt1. The two isoforms of VEGFR1 have a binding affinity that is tenfold higher for VEGF-A than VEGFR2.[78] VEGF can prevent apoptosis in umbilical vein endothelial cells and human dermal microvascular endothelial by inhibiting the activity of stress-activated protein kinase/c-junNH2-kinase (SAPK/JNK) and activating the mitogen-activated protein kinase (MAPK) pathway.[79]

VEGF and Notch show synergistic effects to promote the formation of blood vessel branches. VEGFR2, not VEGFR1, stimulates the induction of tip cells and promotes vascular sprouting (Fig. 3).[80] Notch is activated by the delta-like ligand 4 (DLL4) in

neighboring endothelial cells; conversely, DLL4 inhibits tip cell behavior through the upregulation of VEGFR1 and the downregulation of VEGFR2 and VEGFR3 receptors.[80,81] For effective angiogenesis, VEGF acts cooperatively with several factors, such as the angiopoietins (Ang).[82] VEGF and Ang both have receptors on endothelial cells. Ang-1 and -2 bind to tyrosine kinase receptors, Tie 1 and Tie 2[83] (Fig. 3), while Ang-1, -2, and -4 all bind to the Tie 2 receptor.[84] Ang-1 promotes vascular integrity by promoting endothelial cell migration, inhibiting endothelial cell apoptosis, promoting the generation of capillary-like structures, and recruiting pericytes to vascular tissues.[84,85] Ang-1–Tie 2 signaling is shown to assist the maintenance of quiescent endothelial cell phenotype. Tie 2 interacts with the p85 subunit of phosphatidylinositol-3-kinase (PI3K) to activate the PI3K-AKT pathway, leading to increased survival and chemotaxis of endothelial cells.[86,87] AKT activation inhibits the forkhead transcription factor FKHR (FOXO1), which may protect endothelial cells from apoptosis.[88] Ang-1 and its binding to Tie 1 can promote vascular remodeling and are generally considered pro-angiogenic, whereas Ang-2 counteracts these effects and may be anti-angiogenic.[89,90] Ang-2 is regarded as an agonist of Tie 2 and has been shown to stimulate Tie 2/Akt signaling, as well as inhibit the expression of FOXO1-target gene to enable the regulation of transcription and apoptosis. Ang-2 may also inhibit vascular permeability and acts as an autocrine agonist of Tie 2 and protect stressed endothelial cells.[91]

In the brain, Ang-1 can be neuroprotective and inhibit apoptosis in brain neurons by activating phosphatidyl-inositol 3-kinase and also promoting the phosphorylation of Akt and restoring caspase-3 cleavage.[92] Coadministration of VEGF-A and Ang-1 synergistically increased DNA synthesis, cell proliferation, endothelial cell migration, and sprouting more than either agent alone.[93]

Endothelial Cells and Inflammation

Endothelial cells can modulate the recruitment of inflammatory cells to locations of injury and produce cytokines, growth factors, colony-stimulating factors, and chemokines in response to mechanical or chemical stimuli.[52,94,95] These cytokines can then induce a feed-forward cycle by promoting cell–cell interactions and the proliferation and survival of endothelial cells and also by inducing an endothelial cell pro-inflammatory phenotype that produces cytokines [interleukin (IL)-1], chemokines [IL-8, monocyte chemoattractant protein (MCP)-1], tumor necrosis factor (TNF), and adhesion molecules [vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, and endothelial (E)-selectin], all of which recruit leukocytes to sites of injury.[53,54] Activated endothelial cells recruit leukocytes to sites of infection, which is critical to host defense. Upregulation of related adhesion and ligands on these leukocytes by bacterial or host pro-inflammatory mediators promotes adherence to endothelial cells and focused migration to the sites of infection, where these cells may phagocytize and kill the pathogens.[96]

During inflammation, leukocytes migrate across the vascular endothelium into the tissues in a series of steps. The first steps involve relatively weak, adhesive interactions with the rapidly flowing leukocytes to slow these cells down, followed by a few halting, rolling tumbles on the endothelial surface. These interactions are gradually strengthened

with the leukocyte activation and their subsequent adherence in the endothelium. These stationary leukocytes then migrate into the interstitium through spaces between adjacent endothelial cells. As one can imagine, this is an area of intense study. During transmigration across the vascular endothelium, leukocytes can take either the paracellular path to squeeze their way through between adjacent endothelial cells, or less frequently, show transcellular migration across individual endothelial cells.[97] The principal endothelial adhesion molecules engaged in the attachment and transmigration of leukocytes include CD34, intercellular adhesion molecule 1 (ICAM1, CD54), endomucin (a membrane-bound glycoprotein expressed luminally by endothelial cells), ICAM2, the glycosylation-dependent cell adhesion molecule-1 (GLYCAM1), podocalyxin (a member of the sialomucin protein family), mucosal vascular addressin cell adhesion molecule 1 (MADCAM1), P-selectin, junctional adhesion molecule A (JAM-A), JAM-B, CD 99, vascular cell adhesion protein 1 (VAM1), CD106PECAM1, E-cadherin, and single-chain type-I glycoprotein.[98–101]

Endothelial Cells and Coagulation

Endothelial cells are important modulators of coagulation both in the physiological conditions and also during inflammation and infection. Endothelial cells express anticoagulant factors on their outer membrane surface. Loss of surface thrombin-binding proteins, such as thrombomodulin, and downstream protein C-mediated signaling play a vital role in minimizing thrombin activation and clotting in physiology. The loss of these factors leads to decreased ability of endothelial cells to modulate coagulation and inhibits the release of endothelium-derived factors, such as PGI2 and NO.[102]

Endothelial Cells in Neonatal Disorders

Fetal organs, especially the eye, lungs, and kidneys, show important vascular development in the third trimester of gestation. Therefore, impaired vascular development has been implicated in numerous conditions of prematurity, such as retinopathy of prematurity (ROP), bronchopulmonary dysplasia (BPD), and acute kidney injury (AKI). In neonates, endothelial cell function is well regulated in physiology and known to be altered in pathological states. Table 2 lists biomarkers of endothelial cell in various neonatal diseases.

Endothelial Cells in Neonatal Sepsis

The incidence of early-onset neonatal sepsis with positive cultures in newborns is about 0.98/1,000 live births and most likely higher in very-low-birth-weight (VLBW) infants.[103] The incidence of late-onset sepsis is more variable and could be as high as 30% in extremely low-birth-weight (ELBW) infants.[104] A dysregulated immune host response is associated with the pathogenesis of neonatal sepsis. Gram-negative sepsis has high mortality rates, with most mortality occurring in the acute phase, within the first three days of onset of sepsis.[105]

During inflammation, the vascular endothelium expresses a plethora of cytokines with a local chemotactic gradient that recruits the leukocytes into peripheral tissues. Such recruitment responses in neonates may be weaker in most organs when compared to adults and in preterm in comparison to term neonates.[106,107] In other organs such as the intestine, particularly during necrotizing enterocolitis, the recruitment may be enhanced.

Inflammation of vascular endothelium during sepsis leads to altered chemotaxis and leukocyte transmigration because of the impaired endothelial expression of adhesion molecules, such as E-selectin, ICAM-1, and P-selectin.[108] Some of these changes may be related to altered expression of pro-inflammatory ligands such as TNF, which can affect the expression of adhesion molecules VCAM-1 and ICAM-1.[29]

Biomarkers that regulate endothelial cells and reflect their microenvironment may be useful in monitoring sepsis. Angiopoietins stimulate endothelial cells to increase or suppress inflammation. Ang-1 expressed in peri-endothelial cells can suppress inflammatory responses and stabilize the microvasculature by inhibiting nuclear factor κB (NFκB) activation. In contrast, Ang-2, which is expressed preferentially in endothelial cells, is pro-inflammatory and can increase the permeability of vessels and destabilize them. Ang-1 binds to Tie 2, the tyrosine kinase receptor to maintain the endothelial resting state, thereby suppressing vascular permeability during inflammation (Fig. 3).[109]

Endothelial Cells in the Neonatal Brain

Endothelial cells in the brain microvasculature have an intricate relationship with neuronal development and function, suggestive of a neurovascular crosstalk. Endothelial cells stimulate the proliferation and differentiation of neuronal precursors toward neuronal lineage.[50] During postnatal development, endothelial cells promote excitatory synaptogenesis through upregulation of VEGF expression in cortical neurons by increased signaling through the P38/MAPK pathway.[110] The premature infant is sensitive to neurologic injury partly due to the exposure of their immature vascular network to extrauterine physiologic abnormalities in oxygen tension, biochemical, and environmental factors.

Intraventricular hemorrhage occurs in about 20% of infants born before 32 weeks' gestation and is a major cause of neurodevelopmental morbidity and mortality in premature infants.[111,112] IL-6 may be an important early biomarker for IVH. In one study, serum IL-6 levels were elevated in infants with IVH and were associated with increased risk of neonatal morbidity at less than 28 days after birth.[113] If high levels of IL-8, an important neutrophil and monocyte chemokine that is produced by macrophages, smooth muscle cells, and the endothelium, persisted for >1 day, the risk of IVH and white matter injury was higher.[114]

Hypoxic-ischemic encephalopathy (HIE) is a encephalopathy resulting from perinatal asphyxia that leads to neuronal death from activation of inflammatory cells and overexpression of apoptosis-related proteins.[115] In HIE, elevated inflammatory cytokine levels such as IL-6, TNF, and IL-8 recruit leukocytes to the site of injury and damage endothelial cell integrity.[116,117] In infants with HIE, early microvascular injury may have a critical impact on neuronal damage.[118]

Endothelial Cells in Retinopathy of Prematurity

Premature infants continue to develop the retinal vasculature after birth and are susceptible to altered vascular development such as in retinopathy of prematurity (ROP). These abnormalities in angiogenesis can be recapitulated in murine models such as those of oxygen-induced retinopathy (OIR).[119–121] ROP involves altered endothelial

cell proliferation and survival,[122–124] and consequent abnormal retinal vascularization. Increasing data suggest that ROP involves dysregulation of VEGF expression.[125,126] The vascular development in ROP shows two distinct phases: an initial phase of vaso-obliteration that is triggered by hypoxia and a subsequent period of abnormal neovascularization triggered by retinal hypoxia to meet the demands of the metabolically hyperactive retinal cells and neurons.[127] These abnormalities can be seen in the mouse model of OIR, where mouse pups exposed first to hyperoxia develop vaso-obliteration of retinal vessels and then show abnormal neovascularization.[128,129]

In vivo assessments in a mouse oxygen-induced retinopathy model have revealed several physiologic and functional phenotypes in the developing retinal as a result of aberrant angiogenesis. Alterations in arterial and venous oxygen tension (PO_2) result in increased arterio-venous PO_2 gradients, which indicate increased oxygen extraction and possible underlying ischemia.[130] Whole-mount staining of retinas shows central vaso-obliteration in neonatal OIR mice with recovery to full vascularization by P21.[120,128,129] However, longitudinal live retinal imaging using fluorescein angiography revealed capillary avascularity, arterial tortuosity, and venous dilation in neonatal OIR mice compared to fully vascularized, normal caliber arteries and veins in room-air-raised mice, and consequent prolonged loss of capillary density with the paucity of neovascular buds on capillaries of adult OIR mice in spite of full peripheral vascularization. Spectral-domain optical coherence tomography revealed thinner retinas in neonatal mice with OIR,[131–134] more pronounced in the hypovascular retinal areas,[132] and restricted to the inner retina.[133] Electroretinograms correlate retinal vascular abnormalities to inner retinal dysfunction in OIR mice.[134,135] Comparative retinal histology following *in vivo* imaging showed prolonged overexpression of VEGF, microglial activation, abnormal malaligned neuronal synapses, and apoptosis in OIR mice.[136] A subpopulation of resident macrophages (M2) has been shown to be an important phenotype during angiogenesis.[137,138] Exogenous administration of pro-angiogenic isoform of $VEGFA_{165a}$ in a mouse model of OIR promoted earlier revascularization,[126] likely by targeting endothelial cell proliferation via increased angiogenic signaling through VEGF receptors.

Several proteins and support cells are intricately linked to endothelial cell function. Endothelial cells have surface protein receptors for integrins that play a role in angiogenesis and inflammation.[139] VEGF induces expression of the collagen receptors, $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins.[140] The recruitment of pericytes has been demonstrated to be important in vascular maturation, for stabilization of the vasculature and remodeling of the early endothelial plexus into a more mature vascular network.[141,142] Disruption of endothelial-pericyte connections leads to exaggerated regression of vasculature and abnormal remodeling.[141] Angiopoietins play a role in the pathogenesis of ROP. Ang-2 was inhibited by hyperoxia and increased during relative hypoxia in a rat model of OIR.[143] Biomarkers have been investigated for ROP monitoring and disease severity. IL-6 levels in the umbilical cord were noted to be elevated in preterm infants with severe ROP, while high cord levels plasma C5a were associated with ROP that required laser therapy.[144]

Inflammatory cytokines have been associated with ROP in both the peri- and postnatal periods. Studies of amniotic fluid samples from 175 premature infants born between 23–

32 weeks showed that higher IL-6 and IL-8 levels were associated with a higher risk of advanced ROP.[145] Similarly, Pieh et al. showed that premature infants with high plasma levels of the soluble VEGF receptor 2 (sVEGFR-2) and its soluble membrane-bound tyrosine kinase receptor (sTie) are associated with an increased risk of ROP.[146]

Endothelial Cells in Bronchopulmonary Dysplasia

Bronchopulmonary dysplasia (BPD), also known as chronic lung disease of prematurity, is related to increased supplemental oxygen use during the early neonatal period[147,148] and occurs in about 40% of infants born below 29 weeks gestation.[149,150] A coordinated development of the pulmonary vasculature is required for normal lung development growth. Preterm birth may disrupt the lung vascular growth during the saccular and alveolar stages of pulmonary development, and aberrant development of the pulmonary vascular bed may lead to impaired alveolar development.[148,150,151] Postmortem lung examination of infants with BPD showed low levels of VEGF mRNA and reduced VEGF immunostaining, as well as a reduction in angiogenic receptors Flt-1 and Tie 2 in the infants with BPD compared to those without BPD.[152] Inhibiting VEGF during development decreases alveolarization and pulmonary arterial density.[153,154] Higher levels of ICAM-1, Ang-2, and IL-1β, and reduced levels of Ang-1 and MCP-1 are correlated with BPD severity.[155]

Endothelial Cells in Pulmonary Hypertension

Endothelial dysfunction is centrally implicated in pulmonary hypertension. Pulmonary hypertension is a multifactorial and complex condition, associated with the aberrant endothelial cell proliferation with concurrent neoangiogenesis and the alteration in the secretion of vasoactive mediators, such as prostacyclin, NO, serotonin, ET-1, and thromboxane. The lung endothelium is heterogenous and different from systemic endothelium in both function and structure. The pulmonary endothelium's function includes maintaining barrier integrity, homeostasis, vascular tone, leukocyte trafficking, and production of necessary growth factors.[156] The normal endothelium is typically in a stable, "quiescent" state. When the endothelium is disturbed and "activated" by stress, infection, disease, or injury, endothelial cells tend to express specific proteins and markers, such as ICAM-1, VEGF, and E-selectin, which causes exaggerated proliferation, coagulability, and vasoconstriction.[156,157] In pulmonary hypertension, some of the triggers of endothelial activation are inflammation, shear stress, reactive oxygen species, genetic mutations, and defect in angiogenesis.[156]

Endothelial Cells in Necrotizing Enterocolitis

Necrotizing enterocolitis (NEC) is an inflammatory bowel disease seen in premature infants that is associated with high morbidity and mortality. Maldevelopment of the microvasculature of the intestinal mucosal and abnormally altered intestinal blood flow are implicated in the pathogenesis of NEC. There is low resistance of the intestinal vasculature across the intestines of the newborn infant, mediated by increase in the production of nitric oxide by the endothelium. Neonatal swine models showed abnormal vasoconstriction responses to severe hypoxemia, resulting in intestinal ischemia.[158] Hypoxia in the preterm neonate can inhibit NO production and result in intestinal injury and NEC.[159] There is also evidence of VEGF dysregulation; premature infants exposed to hyperoxia may

show decreased VEGF expression and VEGF/VEGFR-regulated pro-angiogenic signaling pathways and diminished development of the intestinal microvasculature. These limitations in the splanchnic vasculature may not be insufficient for the relatively limited metabolic needs in the first few days after birth but may become inadequate with increasing feeding volumes in the later neonatal period. In experimental NEC, VEGFR2 protein and VEGFR2 activity have been shown to be low preceding the onset of intestinal injury.[160,161] Similarly, inhibition of VEGFR2 led to decreased endothelial cell proliferation and intestinal microvascular network development.[161] Administration of dimethyloxalylglycine (DMOG), a propyl hydroxylase enzyme inhibitor, increased the expression of VEGF-A in the intestines of neonatal pups, but the splanchnic effects of DMOG were abolished by inhibiting VEGFR2 signaling.[162] Further investigations are needed to investigate the strategies to modulate angiogenic signaling through the VEGF-VEGFR2 pathway, which may possibly protect against NEC.

Endothelial Cells in Neonatal Acute Kidney Injury

Early changes in capillary blood flow and endothelial cell injury leading to inflammation, ischemia, and pro-coagulation may play a crucial role in the pathogenesis of early and chronic ischemic AKI. In rat models, ischemic kidneys were unable to autoregulate blood flow and exhibited vasoconstriction when renal perfusion pressure decreased.[163] The organization of the cytoskeletal network of endothelial cells and small arterioles is altered during renal ischemia-reperfusion injury, which disrupts endothelial cell tight junctions as indicated by the disintegration of VE-cadherin in renal microvasculature.[164,165] The loss of the integrity of barrier function could have been the result of matrix metalloproteinase-2 or -9 activation.[166] There is also some evidence to show impaired endothelial-dependent vasodilator activity in AKI. L-arginine and eNOS cofactor tetrahydrobiopterin may attenuate acute ischemia-reperfusion renal injury by preserving medullary perfusion.[167–169]

Endothelial Cells as Therapeutic Targets

Therapeutic advances to regulate angiogenesis have been challenging and limited in success employing pro- and anti-angiogenic factors. This could be due to the complex biology of angiogenic factors, their multiple receptors, and versatile functions. Preclinical studies of pro-angiogenic cell therapies or microRNAs targeting show promise of alternate therapeutic strategies.[170]

Bevacizumab (Avastin) is a promising non-selective anti-VEGF drug that was first used to treat metastatic cancers[171] but was subsequently approved for the treatment of ROP and other ocular conditions.[171–173] Selective pro-angiogenic VEGF isoforms are being explored preclinically, such as administration of VEGFA165a microparticles for the treatment of ROP.[126] Ranibizumab, a humanized Fab fragment that can block all VEGF isoforms, reverses VEGF-stimulated delocalized tight junctions, proliferation and migration of cells, and delocalization of tight junction proteins in retinal endothelial cells, may also be useful in some stages of ROP.[68] Targeting endothelial-to mesenchyme transitions may also be useful in specific stages of vascular disease. Relaxin, a calcimimetic agent, Cinacalcet, and Losartan are shown to inhibit endothelial-mesenchymal transitions.[174–176]

There may be some utility in monitoring biomarkers indicative of damage to the endothelium during neonatal sepsis, such as endothelial growth factors or components of tight junctions (TJs) that shed into circulation upon endothelial damage and quantifying plasma and urine levels of soluble components of endothelial wall and glycocalyx and degraded glycocalyx.[177] Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) is able to differentiate sepsis from non-sepsis cases, with an area under curve (AUC) of 0.97 to diagnose proven or suspected neonatal sepsis, compared to 0.96 of IL-6, and 0.8 of Endocan.[178] The ratio of Ang-1 is shown to correlate with bacteremia.[179] Higher Ang-2 levels correlate with clinical sepsis.[180] Endothelial cell dysfunction has also been implicated in NEC, and several therapies are being explored to modulate the ensuing inflammatory necrosis. Enteral administration of TGF-β_2 was protective in mice with experimental NEC-like injury.[181] PAF has been implicated in NEC pathogenesis and shows promise as a biomarker.[182] Resveratrol (*trans*-3,4′,5-trihydroxystilbene) is a naturally occurring polyphenol found in red wine, berries, and peanuts and has been shown to improve endothelial NO production and endothelial redox balance, as well as inhibit the activation of the endothelium following pro-inflammatory and metabolic stress.[183] Protocols have been developed that enable the differentiation of h-iPSCs very efficiently into competent h-iECs, thereby enabling the development of perfused vascular networks *in vivo*.[184] Despite the early promises of tissue engineering involving endothelial cells, applications to clinical practice are limited. Understanding the cellular and molecular mechanisms related to physiologic and pathologic angiogenesis, both in pediatric and adult tissues, will enhance advances in tissue engineering.[185]

Conclusion

Endothelial cells are critical regulators of vascular homeostasis through intricate interactions with vascular smooth muscle cells, circulating cells, and surrounding support cells, and their connections to blood and tissue components make them vulnerable to minute alterations in the composition of blood, mechanical stress of blood flow, injury, or inflammation. Endothelium based on the microenvironment can transform from pro-inflammatory to anti-inflammatory properties, as well as vasodilation or vasoconstriction, and pro- and anti-thrombotic properties. Future investigations focused on understanding endothelial cell heterogeneity may provide insights into vascular-bed-specific therapies in neonates.

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Impact

- We reviewed the scope of endothelial cell heterogeneity, along with the endothelial cell structure and function as seen in the fetus and neonate.

- Endothelial cells are a diverse subtype of cells and play vital roles in innate immunity, angiogenesis, tissue homeostasis, repair of tissues, tissue inflammation, and cellular apoptosis in numerous inflammatory and infectious diseases.

- Evolutionary mechanisms regulating endothelial cell heterogeneity vary *in vivo* and *ex vivo*.

- Endothelial cells are important therapeutic mediators in the vasculature of numerous neonatal disorders.
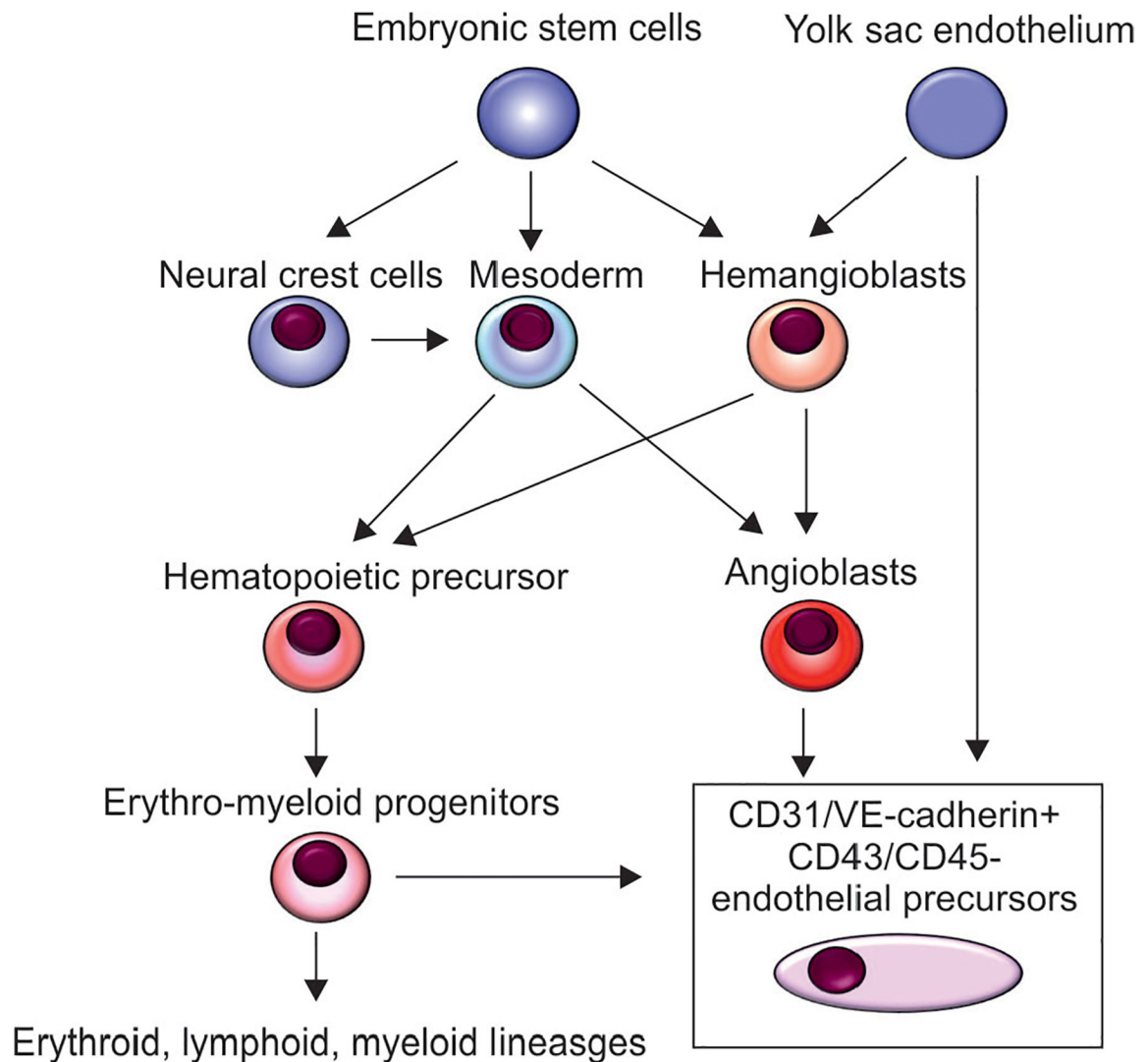
Fig. 1:

Origin of endothelial cells. Overall schematic of the common origin of endothelial
progenitor cells and the erythroid, lymphoid, myeloid precursors. Hematopoietic and
endothelial progenitor cells are derived from a common precursor, the hemangioblast.
Embryonic stem cells give rise to neural crest cells, mesoderm, and hemangioblasts.
Hemangioblasts are derived from the yolk sac endothelium. Neural crest cells differentiate
into mesenchymal stem cells, which tissue-resident precursors through chondro-, osteo- and
adipogenesis. Endothelial precursors can arise from the yolk sac, myeloid precursors, and
hemangioblasts
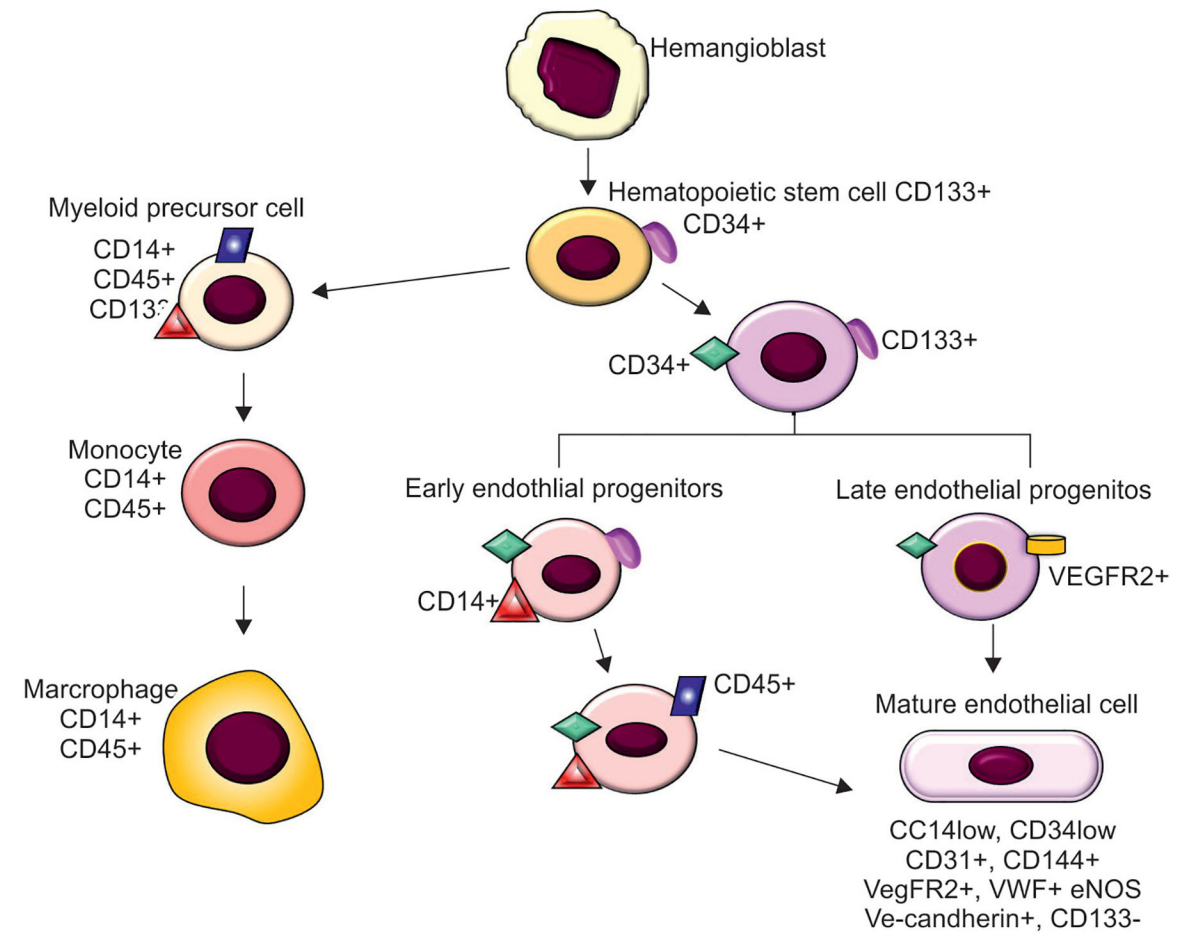
Fig. 2:

Differentiation of endothelial progenitor cells. Hemangioblasts differentiate into hematopoietic stem cells and endothelial progenitor cells. Hematopoietic stem cells and endothelial progenitor cells express three markers cluster of differentiation (CD) 34, CD 45, CD133, and vascular endothelial growth factor receptor-2 (VEGFR2). CD133 is a marker for immature hematopoietic stem cell, while CD34 is a classic hematopoietic stem cell marker. Hematopoietic stem cells give rise to myeloid cell lineage, which express CD14 and CD45, and are CD133 negative, which ultimately give rise to monocytes and macrophages. As endothelial progenitor cells differentiate, they lose CD133 and begin to express CD31, CD144, vascular endothelial cadherin, VEGFR2, endothelial nitric oxide synthase (eNOS), and von Willebrand factor (vWF). Endothelial progenitor cells are positive for both hematopoietic stem cell marker CD34 or CD133 and an endothelial marker, such as VEGFR2. Endothelial progenitor cells do not have exclusive surface markers, rather share similar markers with mature endothelial cells
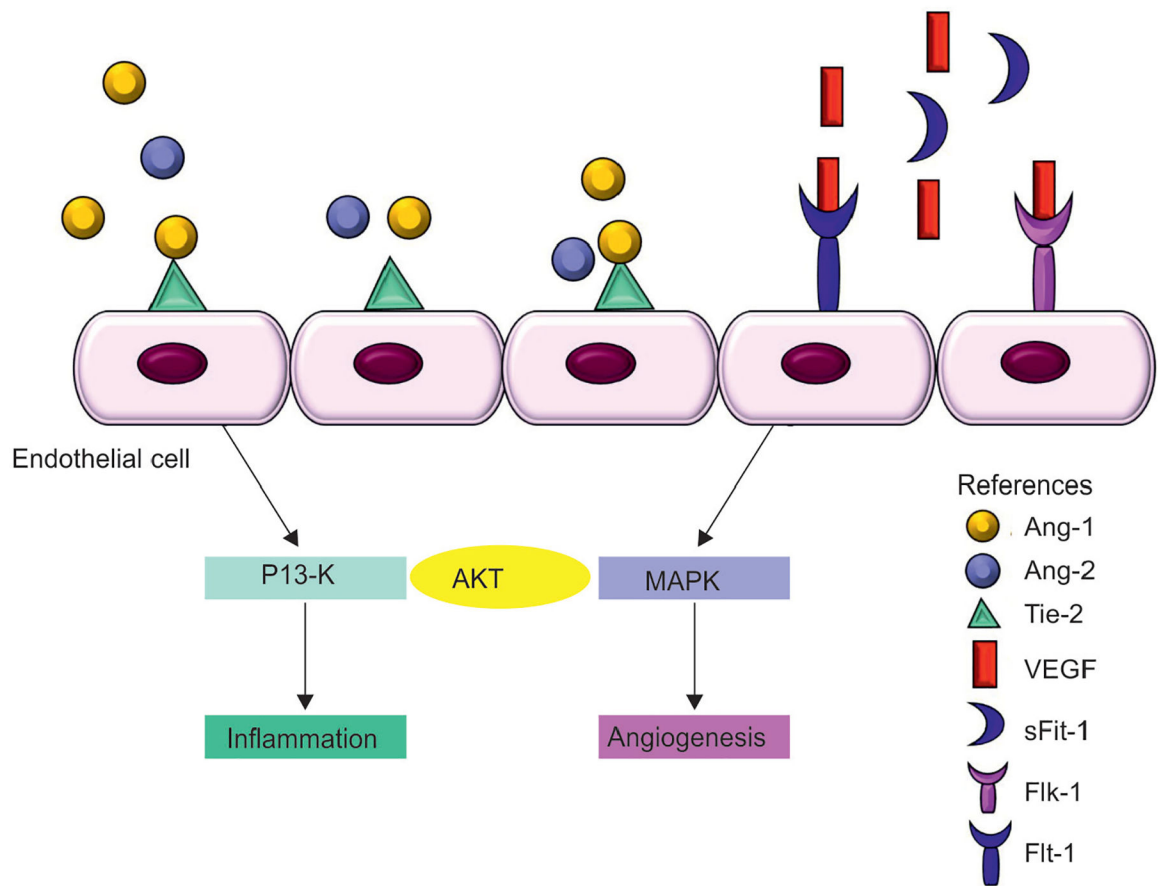
Fig. 3:
Endothelial markers in inflammation and angiogenesis. VEGF works together with angiopoietins during inflammation and angiogenesis, and both have receptors on endothelial cells. Ang-1 and -2 bind to their receptors Tie 2. Ang-1–Tie 2 signaling contributes to maintaining a quiescent endothelial cell phenotype. Ang-1 is pro-angiogenic and required for vascular remodeling, while Ang-2 counteracts their effects as anti-angiogenic. VEGF has two transmembrane receptors, Flt1 or VEGFR1 and Flk-1 or VEGFR2. VEGFR1 has two forms generated by alternative splicing, a membrane-bound Flt1 and a soluble Flt1 receptor, VEGF signals through VEGFR2 to promote angiogenesis. VEGFR1 (Flt-1) serves to limit the actions of (VEGFR2) Flk-1. Ang-2 binds to Tie 2 to activate P13-K/Akt signaling. VEGF-VEGFR2 activates MAPK/AKT signaling pathways

Table 1:

Specific human and murine endothelial cell markers

Type of marker	Name of marker	Species expressed	Cells expressed
Constitutive markers expressed in different endothelium	CD31/PECAM-1[154]	Human, murine	Endothelial cells, B and T lymphocytes, platelets, monocytes, neutrophils
	Bandeirea simplicifolia lectin binding[155]	Murine	Endothelial cells
	Vascular endothelial cadherin[39,156]	Human, murine	Endothelial cells, trophoblasts, macrophages
	CD34[20]	Human, murine	Endothelial cells, hemopoietic precursors
	Thrombomodulin[157]	Human, murine	Endothelial cells, smooth muscle cells
Monoclonal antibodies used to identify specific endothelial cells	BMA-120[158]	Human	ECs, mesothelium, glomerular epithelium
	EN4[158]	Human	Endothelial cells, leukocytes, platelets
	EN 7/44[159]	Human	Endothelial cells in tumors and inflammatory tissues
Endothelial cell markers induced by inflammatory cytokines	CD54/ICAM-1[22,160]	Human, murine	ECs, leukocytes, epithelium, fibroblasts
	CD62E/E-selectin[21]	Human, murine	Endothelial cells, postcapillary venules
Endothelial cell markers induced by angiogenesis	KDR/Flk-1 (VEGFR-2)[120,132,161]	Human, murine	Endothelial cells
	Flt-1 (VEGFR-1)[120,126,162]	Human, murine	Endothelial cells
	Tie-1[57,163]	Human, murine	Endothelial cells
	Tie-2/Tek[57,163]	Human, murine	Endothelial cells

CD, cluster differentiation; PECAM-1, platelet endothelial intercellular adhesion molecule; BMA, biotinylated monoclonal antibody; EN, endothelium antibody; E-selectin, endothelial cells selectin; KDR/Flk-1, kinase insert domain receptor/fetal liver kinase 1; Flt-1, Fms-related receptor tyrosine kinase 1; VEGFR, vascular endothelial growth factor receptor; Tie and Tek, receptor tyrosine kinase genes

Table 2:

Endothelial biomarkers in neonatal diseases

Neonatal disease	Biomarker	Functional properties	Functional use
IVH	IL-6[86]	Pro-inflammatory	Increased serum levels in IVH
	IL-8[87]	Pro-inflammatory	Increased serum levels in IVH and white matter injury
ROP	VEGF-A	Pro-angiogenic	Increased in ROP
	sVEGFR-2[120]	Pro-angiogenic	Elevated in premature infants with ROP
	sTie2[120]	Pro-angiogenic	Elevated in premature infants with ROP
	IL-6[118,119]	Pro-inflammatory	Increased amniotic fluid levels
	IL-8[119]	Pro-inflammatory	Increased amniotic fluid levels
NEC	PAF[70,154,165]	Pro-inflammatory	Elevated in blood early in NEC
	TGF-β[153,166]	Pro-inflammatory	Increased blood levels in NEC
Sepsis	Ang-1[81]	Anti-angiogenic	Decreased in sepsis
	Ang-2[81]	Pro-angiogenic	Elevated in sepsis
BPD	Ang-1[128]	Anti-inflammatory	Reduced serum levels in BPD
	Ang-2[128]	Pro-inflammatory	Increased levels in BPD
	ICAM-1[128]	Pro-inflammatory	Increased serum levels correlate with BPD severity
	IL-1β[128]	Pro-inflammatory	Increased serum levels in infants with both BPD and pulmonary hypertension
	MCP-1[128]	Pro-inflammatory	Lower serum levels of MCP-1
	VEGF[126]	Pro-angiogenic	Decreased levels in BPD
	Tie 2[126]	Pro-angiogenic	Decreased levels in BPD

IL, interleukin; VEGF, vascular endothelial factor; VEGFR, vascular endothelial factor receptor; Tie, receptor tyrosine kinase gene; TGF-β, transforming growth factor-beta; PAF, platelet-activating factor; Ang, angiopoietin; ICAM, intercellular adhesion molecule; MCP, monocyte chemoattractant protein