Review Article

Endothelium - an organ system

Suganthi V

Department of Physiology, Vinayaka Missions Kirupananda Variyar Medical College, Salem

Abstract

Endothelium forms the inner lining of blood vessels and lymphatic ducts. It lines all the vessels and hence it is distributed throughout the body. It exhibits structural and functional heterogeneity and has multiple functions. It regulates angiogenesis and maintains a delicate balance between coagulation and fibrinolysis. It is not just a simple barrier between intra and extravascular compartments but it regulates exchange of solutes, water, regulates permeability, synthesizes, converts and metabolises many hormones/mediators. Endothelial cells release vasoconstrictors and vasodilators to regulate vascular tone and expresses adhesion molecules for the extravasation of leucocytes and lymphocytes in health and inflammation. The endothelium present in all the blood vessels exhibits wide variety of functions and therefore endothelial dysfunction results in widespread disease of the body. This review gives a gross view of the various functions of endothelium and a basic idea about its dysfunction.

Keywords: adhesion molecules, angiogenesis, endothelial cells, fibrinolysis, hemostasis , leucocyte trafficking

Corresponding Author

Dr. Suganthi V, Associate Professor, Department of Physiology, Vinayaka Missions Kirupananda Variyar Medical College, Chinna Seeragapadi, Salem 636308
Telephone: +91 9443908855, Email: vsuganthiarasan@ gmail.com

Introduction

Endothelium lines the inner aspect of blood vessels and regulates the vascular permeability. It is considered to be the largest organ of the body covering a surface area of 1 to $7m^2$.[1,2] It is made up of 6 x 10^{13} cells and weighs approximately 1 kg. [1,2] The endothelial cells (EC) show heterogeneity in their structure in different tissues. It is either continuous as in the brain and retina or fenestrated as in the villus and glomerulus or discontinuous as in the liver sinusoids.[2] The heterogeneity is not only expressed in structure and arrangement of the cells but also in protein expression - Von Williebrand factor (vWF) which is expressed only in cells of certain vessels.[2] These variations in ECs are due to their exposure to environmental factors, growth factors, cytokines etc.[2] These cells not only show structural heterogeneity they also have different functions in tissues.[1,2]

Endothelial cells line the vessels of all organs and regulate flow of nutrients, metabolic products and blood cells across the vessel wall. They also help in maintaining the fluidity of the blood by balancing the anticoagulant and procoagulant properties and thereby maintain a smooth flow through the vessels.[3] EC regulates

tone of the vessel wall, blood pressure and blood flow to various organs by secreting vasodilators and vasoconstrictors. They take part in inflammation and immune reponses by trafficking blood cells across capillary and lymphatic vessel walls.[3] EC are involved in allergic reactions and are responsible for corticosteroid resistant allergies. They are responsible for angiogenesis - development of new vessels, wound repair and in cancerous tissues.[3] Endothelium dysfunction is responsible for many diseases like atherosclerosis, hypertension, diabetes mellitus etc.[3]

The endothelial cells are heterogenic in their structure and function and their role in various normal and abnormal functions in health and disease are diverse. In this review, after first discussing the structure of endothelial cells and the types of endothelium, we will be discussing the contribution of endothelial cells for various functions. The discussion of the functions of endothelium will be under the following headings:

	Functions of endothelium
1.	Role in angiogenesis and repair of vessels and tissues
2.	Role in regulation of vasomotor tone in blood vessels
3.	Role in regulation of vascular
4.	permeability
5.	Role in haemostasis
6.	Role in fibrinolysis
7.	Role in leucocyte trafficking
8.	Role in immunity
9.	Role in allergic reactions
	Role in metabolic activities and other functions

The review ends with a brief note on endothelial dysfunction, its role in the pathogenesis of various diseases and pharmacological and non-pharmacological interventions that can keep it in check.

Structure of endothelial cells

Anatomically endothelium is defined as the innermost lining of blood and lymphatic vessels[2]. They develop from the mesoderm by differentiation of hemangioblasts/angioblasts.[2] Shape of the cells varies in different vasculature. In capillaries they are flat and in high endothelial venules (HEVs) they are plump and cuboidal. Their thickness varies from 0.1 µm to 1 µm.[2] The cells are aligned along the direction of flow of blood in all the vessels. This feature helps them to bring about modifications based on shear stress.[2] The Endothelial cells contain many vesicular structures – Weibel-Palade bodies, vesiculo-vacular orgenelles (VVO), caveoli etc.[2] There are a few protein markers which are expressed in all the ECs, most common ones are – Platelet endothelial cell adhesion molecules (PECAM-1) and vascular endothelial cadherins (VE- cadherins).[4]

Types of endothelium

Endothelium is either continuous or discontinuous.[2] Continuous ones are either fenestrated or non-fenestrated. Non-fenestrated endothelium is present in the arteries, veins and capillaries of brain, skin, heart and lung.[2,5] Fenestrated continuous endothelium is present in the vessels where filtration happens as in the glomeruli, choroid plexus, intestinal villi and in endocrine and exocrine glands.[2,6] The fenestrae are transendothelial pores (≈70nm). Discontinuous endothelium is present in the sinusoids of the Liver.[2,6] They have large fenestrations (100-200nm) without the diaphragm and gaps are present within the cells. The basement membrane is poorly developed.[5, 6]There is a basement membrane underlying the endothelial cells and a glycocalyx layer along their luminal surfaces.[5] The thickness of glycocalyx and the basement membrane differs in different vascular beds. The pericytes cover the endothelial cells.[7]

Role in angiogenesis

Formation of new vessels in the developing embryo without any prevascular structure is vasculogenesis and formation or sprouting of new vessels from existing vessels is angiogenesis.[8] Studies from the embryos have shown that new arteries and veins form initially as a small vessel made up of only endothelial cell lining and pericytes.[8] Later, they acquire connective tissue and muscle layer by signals from endothelial cells to the surrounding tissues. Signals like shear stress sensed by endothelial cells regulate the diameter of the vessel and formation of various layers of the vessel. Growth factors also stimulate growth of capillaries.[8]

Endothelial cells form new capillaries by dividing and extending from an existing capillary to form a sprout which grows to form a hollow tube.[8] This tube continues to grow to reach another capillary sprout. The sprouts now establish as a new vessel and circulation is established through it.[8] This happens in the presence of growth factors like vascular endothelial growth factor (VEGF), Fibroblast growth factors (FGF), angiopectins 1 and 2. [8,9]

Angioblasts and the endothelial cells need to have cell to cell contact and adhesion with like cells to form new capillaries. This happens in the presence of adhesion molecules like PECAM and vascular endothelial cadherin (VE-cadherin).[1]VE- cadherin of one cell is recognised by the VE- cadherin on the other cell and brings them together for formation of new vessels.[1]

The endothelial cells have the property to divide and proliferate and thereby repair the endothelial damage in the vessels.[8] They repair injured tissue by extending into the tissues and forming new capillaries. Growth of blood vessels into the damaged tissues is due to signals from damaged tissues, and VEGF released by injured tissue is the main signal.[8] Also Hypoxia Inducible factors (HIF -1) in the damaged tissues stimulate capillary growth.[8,9] Once the capillary has developed and circulation established, the hypoxia gets corrected and the HIF -1 stimulation is switched off and the angiogenesis stops. This on-off switch restricts the growth of blood vessels beyond the tissue needs.[8]

Role in regulation of vascular tone

Endothelium secretes vasodilators like Nitric oxide (NO), Endothelium derived hyperpolarizing factor (EDHF) and Prostacyclin and vasoconstrictors like Endothelins, Prostaglandin H2 and Thromboxane A2 and thereby regulates vasomotor tone.[3]

Nitric Oxide: Nitric oxide (NO) is synthesized from oxidation of L –arginine by NO synthase, the synthesis being enhanced by thrombin, bradykinin, muscarinic agonists, substance P and shear stress.[10] NO activates guanylyl cyclase and forms cGMP. cGMP diffuses into the vascular smooth muscle cells and causes relaxation and induces vasodilation.[11] Robert Furchgott, Ferid Murad, and Luis Ignarro were awarded the Nobel Prize in Physiology and Medicine in 1998 for the discovery of NO as the Endothelium-derived relaxing factor (EDRF). NO also activates prostacyclin through cGMP and vasodilation is potentiated. Exercise induced vasodilation is by action of NO on shear stress stimulation. [12] NO also Inhibits platelet activation, secretion, aggregation and favours platelet disaggregation and it prevents vascular adhesion and extrusion of leucocytes, repairs vascular injury and limits the smooth muscle migration and proliferation.[13]

Endothelium-derived hyperpolarizing factor (EDHF): EC produces EDHF, which promotes vascular smooth muscle relaxation. Muscarinic agonists stimulate EC to release EDHF, causing a transient hyperpolarization of the cell membrane.[3] EDHF exerts its vascular effects by activating ATP-sensitive potassium channels or smooth muscle sodium-potassium ATPase, or both or by a hyperpolarizing current to smooth muscles.[14]

Prostacyclin and other vasodilators of EC origin: Epoxyeicosatrienoic acids (EET) derived from arachidonic acid metabolism and prostacyclin (PGI_2) and PGE_2 are also relaxing factors.[3, 15]

Endothelin: Endothelin is the most potent vasoconstrictor synthesized by the ECs.[3] The secretion of endothelin is stimulated by hypoxia, ischemia, shear stress, growth factors, catecholamines, thrombin etc.[1] Catecholamines and endothelins potentiate the actions of each other. Synthesis and secretion of NO and endothelin reciprocally inhibit each other.[3, 11]
Endothelin acts on receptors ET-A and ET-B in vascular smooth muscles and ET-B in endothelial cells.[16] The receptors are bound to Gq protein on muscle membrane which causes synthesis of IP_3 and release of Ca^{++} and vasoconstriction. [16] On binding with ET-B on ECs, it stimulates production of NO and thereby causes vasodilation. In endothelial dysfunction where atherosclerosis is potentiated, unopposed action of endothelin promotes smooth muscle proliferation and vasoconstriction.[16]

Prostaglandins: Prostaglandin H2 formed by the arachidonic acid metabolism pathway is the precursor of all prostanoids including thromboxane A2. [11] Prostaglandin H2 and thromboxane A2 bind to their receptors and initiate vascular smooth muscle contraction. In physiological conditions their actions are overridden by endothelium-derived vasorelaxants.[11]

The balance between the vasodilators and vasoconstrictors regulates vascular tone. The key consequence in endothelial dysfunction is inhibition of release of vasodilators. Age, hypertension, atherosclerosis and hyperlipidemia may provoke endothelial dysfunction.[17]

Role in vascular permeability

The endothelial lining separates the tissues from the circulating blood. It provides nutrients to tissues and maintains plasma oncotic pressure.[18] Endothelium is permeable to molecules ranging from 0.1 nm to 11.5 nm in diameter.[18]

Movement of substances across vessel wall is through transcellular (larger particles) and para or intercellular pathways (smaller particles).[6,18]
Transcellular pathway: The Weibel-Palade bodies, vesicles and caveoli in EC aid in the transport of substances across the luminal to basolateral surface.[18]
Intercellular pathways: Transport is aided by intercellular junctions - Tight junctions (TJ) and Adherens junctions (AJ) [18,6] TJ are placed along the luminal surface of ECs and they prevent movement of substances between neighboring cells. They are well organised in arteries than veins and formed by proteins like occludins, claudins and junction adhesion molecules (JAMs). [18] Occludins are more in number in the arteries and are highly expressed in brain. AJs are located below the TJs. [18] AJs are formed by VE-cadherin. VE-cadherins are responsible for endothelial cell to cell contacts necessary for angiogenesis and barrier function. [18,8]There are also gap junctions between adjacent ECs formed by connexons.[18]

Solutes and ions move across the EC through gaps in between, paracellular route or transcellularly. Blood cells are also capable of moving across the barrier by any of the above routes. Vesicular transfer of plasma proteins like albumin across the membrane is a highly regulated process. [18] Disruption of the highly regulated barrier makes the endothelial lining leaky and may lead to detrimental results as in pulmonary edema. [18.19]

The barrier: The barrier function of the endothelial monolayer is regulated by cell-cell and cell-extracellular matrix adhesion.[19] Structures making the barrier are the

glycocalyx, an extracellular covering on the apical side of endothelium, the adhesive property provided by the intercellular endothelial junctions (described above) and the integrin receptors.[19] They maintain the albumin-impermeable nature of the vessel wall under basal conditions. Glycocalyx acts as a sieve, allowing transendothelial transport of low-molecular weight solutes, it inhibits adhesion of red blood cells and neutrophils and acts as a mechanotransducer.[19,20]

Regulation of the barrier function: Barrier disruption is due to compromised interendothelial junctions, resulting in the formation of gaps between cells and barrier reinforcement arises from stabilization of junctional complexes. [19] Barrier disruptive substances are thrombin, platelet activating factor (PAF), tumor necrosis factor-α (TNF-α), histamine and bradykinin.[19]

The barrier function is regulated by small GTPases such as RhoA, Rac1, and Cdc42. They integrate signals between the cell membrane and the cytoskeleton (actin filaments). Signaling via Rac1 and Cdc42 increase stability and RhoA activation increases permeability.[19,] Intracellular Ca^{2+} concentration regulate endothelial permeability via activation of myosin light chain kinase (MYLK).[20] Vascular endothelial growth factor (VEGF) also known as the vascular permeability factor (VPF) is 50,000 times more potent than histamine. [21]

Hemodynamic forces in the vessels also influence endothelial permeability. Laminar flow stabilizes the junctions and perturbed flow disintegrates actin cytoskeleton organization and leads to disassembly.[22] Occludin and VE-cadherin convert mechanical forces to intracellular signaling and regulate permeability.[23,24] cAMP, Fibroblast growth factor (FGF), VE-cadherin and sphingosine-1-phosphate (S1P) are the barrier stabilizing agents.[6,]

Role of endothelial cells in haemostasis

The main function of EC is to maintain blood in a fluid state and induces a normal blood flow. In times of injury, they help in clot formation and repairs the injured vessel. In health, endothelial luminal surface prevents adhesion and activation of platelets and leucocytes; therefore it is anticoagulant and non-thrombogenic.[25] But the macromolecules in the basal lamina below the endothelium, on exposure stimulate platelet activation, aggregation and clot formation. The endothelial cells, therefore, regulate the equilibrium between thrombosis, hemostasis and thrombo-resistance.[25]

There are anticoagulant and procoagulant factors present in the EC. On the anticoagulant side it expresses tissue factor pathway inhibitor (TFPI), heparin, thrombomodulin, endothelial protein C receptor (EPCR), tissue-type plasminogen activator (t-PA), ecto-ADPase, prostacyclin, and nitric oxide.[26] On the procoagulant side, EC synthesize tissue factor, plasminogen activator inhibitor (PAI)-1, von Willebrand factor (vWF), and protease activated receptors.[26]

Hemostasis involves vasoconstriction, temporary platelet plug formation and coagulation.

Role in temporary platelet plug formation: In health, thrombocytes do not get attached to EC surfaces due to the presence of NO, prostacyclin and ecto-ADPase.[26] Following injury to vascular wall the receptors on platelets and EC are activated. Factors/Receptors favouring platelet adhesion are:

- Glycoprotein (GP) Ib-IX-V complex: Under the influence of high shear stress or injury the platlets bind to the ECs via platelet glycoprotein (GP) Ib-IX-V complex with the help of vWF.[1]
- vWF present in the Weibel-Palade bodies in the endothelial cells are

released and initiates platelet activation. At the site of injury vWF helps in binding of platelet and the tissues and activates Factor VIII.[25]

- GPIIb/IIIa, also known as integrin $\alpha_{IIb}\beta_3$ is an integrin complex found on platelets. It is a receptor for fibrinogen and vWF and aids platelet activation. Platelet activation induces a conformational change in GPIIb/IIIa and binds with fibrinogen and favours aggregation.[26]
- Platelet activating factor (PAF) released by EC stimulates platelet adherence and activation.[25]

Activated platelets degranulate and release ADP, ATP, Thromboxane A2, serotonin, vWF and it further enhances platelet aggregation, activation and vasoconstriction, all together resulting in formation of temporary hemostatic plug. The neighboring normal endothelium limits the plug formation by releasing prostacyclin and NO.[25,,26, 27]

Role in coagulation: Coagulation involves formation of fibrin from fibrinogen which forms a mesh on the temporary platelet plug and forms a permanent clot. Thrombin formation is the major step in clotting. Thrombin is a serine protease which activates many clotting factors and enzymes. It also stimulates the procoagulant pathways within the ECs and therefore its activation is highly regulated by:

- Activation of antithrombin III and thereby inhibition of thrombin activation.[28]
- Tissue factor pathway inhibitor (TFPI) which binds with tissue-factor/VIIa/Xa complex expressed by the EC.[29]
- Expression of thrombomodulin (TM) by the EC prevents activation of thrombin.[1]
- Activation of Protein C inactivates Va and VIIIa and inhibits thrombin dependent activation of platelets, factors V, XIII, fibrinogen and prothrombin activator.[29]
- Proteins S synthesized from EC enhance the action of protein C.[1]

Initiation of coagulation by EC: The important step in initiation of coagulation process is expression of tissue factor (TF)[1]. In the normal vessels, TF is present in the matrix beneath the endothelium and in the cells surrounding the vessel and subcutaneous tissues.[8] On exposure, TF along with factor VIIa activates factor IX (FIX) and factor X (FX). FXa further enhances formation of FXa and promotes synthesis of thrombin.[25] Thrombin cleaves fibrinogen to fibrin and fibrin polymerize to form the clot along with the platelets and aggregated cells. Thrombin also activates other factors and stabilizes the clot by activating factor XIII (FXIII).[25]

Endothelium initiates clotting by the presence of the different factors. Tissue Factor (TF) is expressed by EC when stimulated by activated platelets, endotoxins, cytokines, fibrin, thrombin, shear stress or hypoxia.[25,28] EC express thrombin receptors, also termed protease-activated receptor-1 (PAR1). Binding of thrombin to EC expresses prothrombotic and antithrombotic molecules. It expresses several receptors for fibrin and fibrin degradation products.[18,25] Binding of fibrin promotes endothelial cell adhesion, spreading, proliferation, and migration, leukocyte adhesion and inhibition of PGI_2 synthesis. EC in the regions of disturbed blood flow in arteries show increased levels of Nuclear factor –KB (NF-KB) and in conditions like sepsis and coronary artery disease, the NF-KB moves on to the nucleus and induces expression of procoagulants.[28]

All these factors are not expressed all the time. Under normal conditions, endothelial cells in different sites of the vasculature bring about a balance between different anticoagulants and procoagulants for local hemostasis.[28]

Other major organs involved in the coagulation process and generation of clotting factors are the liver and the bone marrow. Liver produces serine proteases, cofactors (factors V and VIII, and protein S), fibrinogen, and antithrombin III. The bone marrow produces and releases monocytes and platelets each day.[18] Monocytes express TF and platelets provide the surface for assembly of clotting factors. The products from the liver and bone marrow interact with the local endothelium derived factors and they all function together for hemostatic balance in every vascular bed which differs from one area to the other.[18,29]

Role of endothelium in fibrinolysis

Endothelial surface is profibrinolytic and helps to maintain blood in a fluid state. The balance between the anti and pro-coagulants shifts towards the latter in vessel wall injury and clot is formed. Folllowing this, fibrinolytic system dissolves the clot and restores a patent blood vessel.[3]

The fibrinolytic system includes an inactive proenzyme in plasma, plasminogen, which is activated to plasmin by tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). Plasmin degrades the fibrin component of the clot into soluble degradation products.[30]

t-PA is released by endothelial cells constitutively and upon stimulation, by shear stress, thrombin or bradykinin.[31] Its action is inhibited by plasma inhibitor of t-PA and plasminogen activator inhibitor type-I (PAI-1), secreted by EC.[31] t-PA is produced only in the micro vessels following stimulation and its role in regulation of blood flow in large vessels is questionable. It is found in humans following exercise and venous compression.[31]

u-PA is synthesized and released only by activated endothelial cells involved in wound healing and angiogenesis and levels of u-PA increases with cell migration and tissue remodelling.[30,31] EC express t-PA and u-PA receptors. One of the EC binding proteins is found to be annexin II. The binding of t-PA to EC stimulates fibrinolytic activity and cell proliferation.[32] u-PA receptors are found in the migrating EC taking part in angiogenesis rather than in quiescent cells.[33,34]

Plasminogen activator inhibitors (PAI): There are PAIs produced by EC and they are of 3 types – PAI 1, PAI-2 & PAI-3. PAI-1 is found to be associated with the extracellular matrix and is stimulated by thrombin, endotoxin, various cytokines and oxidized low density lipoprotein. PAI-2 is found normally in plasma only during pregnancy.[35] PAI-3 (also known as protein C inhibitor) has a much lower affinity for u-PA and t-PA. Binding of thrombin to thrombomodulin accelerates its capacity to activate a protein known as thrombin-activatable fibrinolysis inhibitor (TAFI). This results in the loss of plasminogen/plasmin and a t-PA binding site on fibrin and fibrinolysis is inhibited.[36]

A balance exists between profibrinolytic and antifibrinolytic pathways in the unperturbed endothelium and thereby helps to maintain blood fluidity, but EC seem to express more antifibrinolytic than profibrinolytic activity.[36]

Role of endothelial cells in leucocyte trafficking and inflammation

Endothelium allows passage of WBC and thrombocytes in health and disease conditions through trans-endothelial or inter-endothelial spaces.[18] EC take part in immunity - both innate and acquired.[3] The non-adherent leucocytes circulating in the blood become adhesive to EC in conditions of infection or inflammation through adhesion molecules. The adhesion molecules are part of the leucocytes as well as the EC. On adhesion, the cells enter the tissues to combat the infection/inflammation.[27]

In health, lymphocytes move in and out of lymphatic ducts and blood vessels for immune surveillance. Extravasation of lymphocytes normally happens through postcapillary venules at high endothelial venules (HEVs).[37] Leukocytes migrate through postcapillary venules and small veins, at the site of inflammation.[18] The extravasation happens by binding of active receptor on the leucocyte to its ligand on the ECs. These receptor-ligand pairs belong to a large, adhesion molecule-surface receptor family.[37]

The following are the steps involved in the transport of leucocytes: Leucocyte rolling on endothelial cells, triggering by rapid activation of integrins via G-proteins, tight adhesion to EC and diapedesis.[26,37]

Leucocyte rolling: The process starts with tethering of leucocytes to the vessel wall. Rolling happens by interaction between selectins and glycated ligands on EC and this slows down the movement of leucocytes and on slowing down they recognize the endothelial signals for adhesion.[18,37]Selectin – The adhesion molecule includes 3 types - L-selectin (Leucocyte), P-selectin (Platelet) and E-selectin (Endothelium).[37] L-selectin is expressed in the leucocytes and lymphocytes and the other two are present in the ECs. L- Selectin and binding sites for P and E-selectins are present in the microvilli of leucocytes.[37] Selectins are induced in the ECs and leukocytes by cytokines. The ligands on the ECs for L-selectin include glycosylation dependent cell adhesion molecule 1 (GlyCAM-1), CD34, podocalyxin and Addressin (mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1)).[37]Other factors responsible for the rolling phase are- In lymphocytes, VLA-4 (Very Late Antigen-4 ([Integrin $\alpha 4\beta 1$]) and Vascular cell adhesion molecule – 1 (VCAM-1), an endothelial integrin ligand that belongs to the immunoglobulin superfamily.[38]

The rolling of leucocytes on the endothelial surface establishes a close contact between the two cells and leads to firm adhesion and arrest of WBC.

Activation of integrins: The next step is stimulated by chemokines. Chemokines produced by the EC, on bacterial stimulation or by inflammatory mediators, are substances like the monocytic chemotactic proteins (MCP).[39] The chemokines bind by means of a heparin binding domain with proteoglycans on the luminal surface of EC and are presented to the leukocytes.[39]The effects of chemokines on WBCs are mediated by G-protein-coupled receptors.[39] This results in conformational change in the integrins, a group of adhesion molecules on the leukocyte, followed by increased integrin activity and adhesion of the leucocyte to EC receptors (Members of immunoglobulin superfamily). This brings about stable arrest of the leucocyte on the endothelium.[37.39] Chemokine attraction leads to - Arrest and adhesion of leucocytes to EC and movement of leucocytes to target tissues.[18]

Tight adhesion: Tight adhesion of leukocytes to ECs is due to adhesion of integrins with their ligands of Ig superfamily on the ECs.[37] Integrins on leucocytes are heterodimers and there are 17 α and 8 β chains. The integrins, $\alpha 4\beta 1$ (VLA-4), $\alpha L\beta 2$ (LFA-Lymphocyte function-associated antigen), macrophage differentiation antigen-1 (Mac-1) and $\alpha 4\beta 7$ have major role in leukocyte-endothelial cell interactions.[37.39] The ligands for these integrins are members of the Ig superfamily located on the EC: VECAM-1, intercellular adhesion molecule (ICAM-1 & 2), PECAM-1 and JAMs. ICAM-2 binds to LFA-1, and VCAM-1 binds to VLA-4. ICAM-1 and VCAM-1 are upregulated in inflamed endothelium by the cytokines - IL-1 and TNF-α.[37] Mechano-stimulation also regulates the expression of adhesion molecules on endothelial cells.[40]

Transmigration of Leucocytes: Migration of leucocytes happens across the endothelial cells through adherens junction (AJ).[18] Cadherins, in the EC - VE-cadherin, make up the AJ. VEGF and histamine increase the vascular permeability by acting on VE-cadherin.[18,41] Increased permeability is followed by migration of leucocytes. Other molecules like PECAM-1 and JAMs also help in migration.[37] JAMs are concentrated in the apical region of intercellular junctions and are readily engaged by a Leucocyte that has adhered to the surface of an EC. Along with PECAM-1, JAMs help in guiding the movement of leukocytes through the inter-endothelial-cell junctions.[41]

In quiesence, inter-endothelial cell junction is closed, and the integrity is by binding of VE-cadherin, PECAM-1 and JAM molecules from adjacent cells. VE-cadherin is linked to the cytoskeleton (Actin) via β-catenin, and is required for its adhesive activity. [41] As the adhesion of leucocytes proceed, it triggers the dissociation of the VE-cadherin-β-catenin complex by degradation of the β-catenin and internalization of the VE-cadherin. [41]

On leucocyte migration, the homophilic interactions of molecules between adjacent cells are disrupted and results in loosening of the junction. [41] Engagement of the endothelial molecules provides a scaffold to 'walk' the leucocytes through the junction. This is the mechanism of leukocyte transmigration by the intercellular or paracellular pathway.[41]

Tight junctions play a major role in transendothelial migration of leukocytes which takes place at the tricellular corners of TJ.[37] Transcellular migration happens through intracellular adhesion molecule (ICAM-1) and VECAM-1 which are highly expresssed at the transmigratory cups across which migration happens.[37]

Role of endothelial cells in immunity

ECs play 2 major roles in cell mediated immunity (CMI) :

(i) Antigen presentation to memory T cells
(ii) Recruitment of inflammatory cells.[3]

Macrophages and EC act as antigen presenting cells (APC). EC present antigens only to memory cells whereas macrophages present antigens to both naïve and memory cells. EC express both MHC I and II and therefore can stimulate CD8 and CD4 cells. The EC also provide co-stimulation to T cells via CD 58.[1] Memory T cells on activation stimulates various endothelial functions in relation to inflammatory cell recruitment. It is done by contact stimulation or by cytokines. Endothelial cells respond by forming vasodilators to increase delivery of leucocytes, tethering, release of chemokines and transendothelial migration of leukocytes and leakage of plasma proteins.[1,42]

Initially neutrophils are recruited through the endothelium and later are gradually replaced by other cells and the chemokines also change from those of which are for neutrophils to that for other leucocytes.[1] The infiltrate through the endothelium changes from a neutrophil rich one to a T-cell–rich and monocyte-rich delayed hypersensitivity (DTH) reactions. This cytokine rich infiltrate eliminates the pathogen and induces change in the EC and promotes leukocyte extravasation, angiogenesis and tissue remodelling.[1]

CD8 cells are involved in graft rejection. In some organ transplants, vascular endothelial cells are the antigen-bearing cells and are targets for lymphocyte attack. This leads to intimal arteritis or endothelialitis and EC become the cause of transplant rejection. The reactions precipitate fibrinoid vessel wall necrosis, thrombus formation and severely compromises organ perfusion.[42]

Role of endothelial cells in allergic inflammation:

Endothelial cells have been identified to have a role in the development of allergic reactions based on production of substances like periostin and thymus activation-regulated chemokine (TARC).[43] These substances are biomarkers for the pathogenesis & progression of allergic reactions. ECs are potential targets for IL-33 which is a key regulator of allergic inflammation. IL-33 stimultes various cell types involved in allergic reactions like the T helper type 2 cells (TH2 cells), eosinophils, basophils, dendritic cells and mast cells.[43] Most of the responses of the EC towards the allergens are not controlled by corticosteroids. The refractoriness of EC mediated responses towards steroid treatment is due to angiogenesis in airways, leucocyte adhesion, production of perisostin and TARC and the actions mediated by IL-33. Therefore in steroid-resistant allergies endothelial cell targeted therapies may be considered.[43]

Role in metabolic activities

Endothelins are also involved in the metabolism or activation of various substances like hormones, free radicals, amines, nucleotides, metabolites of arachidonic acid pathway etc.[18] ECs express various enzymes like angiotensin converting enzyme (ACE), Catechol-e-methyl transferase, Monoamine oxidase, neutral endopeptidase which metabolizes various compounds like Norepinephrine, Angiotensin II, Serotonin, Bradykinin etc.[18]

Other functions of endothelial cells

Demyelination due to white matter ischemia results in loss of brain function. Studies have shown that transpalntation of brain microvascular endothelial cells (MVEC) in infarcted areas induced remyelination and improvement of functions. MVEC were also able to reduce the apoptosis of oligodendrocyte precursor cells (OPC) in the infarct.[44] The MVEC produce substances of pro-survival activity (FGF-2, PDGF and VEGF) of OPC and thereby help in recovery of white matter following infarct.[44]

Endothelial dysfunction:

Having discussed the varied functions of the endothelial cells, endothelial dysfunction is now briefly addressed. Disease of the endothelium leads to endothelial cell dysfunction. Endothelial dysfunction results in expression of proadhesive, procoagulant and antigen-presenting properties and decrease in vasodilatation induced by NO.[45] Quiescent endothelium is anticoagulant, antiadhesive and of vasodilator phenotype.[45] Shift towards dysfunctional properties is due to inflammatory mediators – Endotoxin, TNF -α, IL-1 and free radicals and this happens in hypertension, coronary artery disease, peripheral vascular disease, diabetes, chronic renal failure and viral infections.[45]

Free radicals injure the endothelium and disrupt the balance of NO resulting in increased permeability of endothelium to toxins and inflammatory cells.[3] In most instances, the human body has an adequate supply of antioxidants to neutralize the free radicals; but if the body is depleted of these antioxidants or if there are too many coexistent factors, a change in the balance of NO may result in widespread disease of endothelium.[3]

Factors that increase free radical generation in the body are smoking, high calorie intake, sleeplessness, obesity, infections and exposure to pollutants. In endothelial dysfunction any one or more of the above functions of ECs are deranged. Till date, assessment of endothelial

function is not possible; but the response of endothelium to vasodilators can give an approximate idea about the functioning of endothelium.[3,46]

Endothelial dysfunction plays a major role in the pathogenesis of various diseases like atherosclerosis, angiogenesis in cancer, infections, stroke and vascular pathologies as in peripheral vascular disease. Diseases like diabetes, hypertension, atherosclerosis, sedentary lifestyle and smoking can induce endothelial dysfunction.[47]

In view of our knowledge about the functioning of the endothelium, many treatment strategies can be targeted at endothelium to bring it back to the quiescent state. Many drugs like the ACE-inhibitors, statins and drugs which increase insulin sensitivity, L-Arginine, HMG-CoA reductase inhibitors improve endothelium-dependent coronary vasomotion and are used to improve endothelial function.[3, 48]

There are non-pharmacological interventions which can prevent endothelial dysfunction and improve its function - cessation of smoking, control of hypertension, dietary modifications like low cholesterol diet, supplement of dietary L-Arginine, consumption of fish, lifestyle modifications like increase in physical activity and anti-oxidant therapy.[3]

Conclusion:

Endothelial cells lining the inner walls of the vasculature are heterogenic, based on their structure, location and functions. Structurally they are continuous, fenestrated or discontinuous based on their location in various organs and their functions. Also intracellular structures like the Weibel-Palade bodies and caveoli are not present in all the EC. Functionally there are distinct functions for EC in certain vessels as in leucocyte trafficking happening in postcapillary venules, arteriolar endothelium regulating vasomotor tone, etc. Endothelial cells are not just vascular wall barriers but are dynamic heterogenic organs in the vasculature present all over the body with many physiological functions.

They regulate vasomotor tone by secreting vasodilators and vasoconstrictors, maintain the fluidity of blood by their physical nature and anti-coagulant property and regulate hemostasis. They regulate the permeability of the vessel for water, solutes and blood cells in health and inflammatory conditions by modulating the adhesion molecules in the inter-endothelial junctions. It regulates cell mediated immunity by acting as APCs and recruiting inflammatory cells. EC take part in allergic reactions by secreting periostin & thymus and activation-regulated chemokine which are involved in pathogenesis of allergic reactions and are targets for IL-33. The endothelial cell responses are also responsible for corticosteroid-resistant allergies. In the brain, implantation of microvascular endothelial cells (MVEC) in infarcted areas can induce re-myelination and recovery of white matter. They also have metabolic functions and take part in angiogenesis.

Endothelial dysfunction can occur in many metabolic diseases and the dysfunction may induce many widespread disorders in the body. The dysfunction can be kept in check by pharmacological and non-pharmacological interventions.

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Conflicts of interest: Nil

References

1. Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, et al. Endothelial Cells in Physiology and in the Pathophysiology of Vascular Disorders. Blood. 1998;91:3527-3561.

2. Aird WC. Endothelial Cell Heterogeneity, Cold Spring Harb Perspect Med. 2012 Jan; 2(1):a006429.
3. Cooke JP. The endothelium: a new target for therapy. Vascular Medicine. 2000;5:49-53.
4. Garlanda C, Dejana E. Heterogeneity of endothelial cells. Specific markers. Arterioscler Thromb Vasc Biol. 1997;17: 1193-1202.
5. Van den Berg BM, Vink H, Spaan JA. The endothelial glycocalyx protects against myocardial edema. Circ Res. 2003;92:592-594.
6. Aird WC. Phenotypic Heterogeneity of the Endothelium Structure, Function, and Mechanisms. Circ Res. 2007; 100: 158-173.
7. Simionescu M, Simionescu N, Palade GE. Morphometric data on the endothelium of blood capillaries. J Cell Biol. 1974;60:128-152.
8. Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th ed. New York: Garland Science;2002
9. Clapp C, Thebault S, Jeziorski MC, De La Escalera GM. Peptide Hormone Regulation of Angiogenesis. Physiol Rev. 2009 Oct; 89(4):1177-1215.
10. Topper JN, Cai J, Falb D, Gimbrone MA Jr. Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli: Cyclooxygenase-3, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. Proc Natl Acad Sci USA. 1996;93:10417.
11. Stankevičius E, Kėvelaitis E, Vainorius E, Simonsen U. Role of nitric oxide and other endothelium-derived factors. Medicina (Kaunas). 2003;39(4):333-341.
12. Loscalzo J, Vita JA. Ischemia, hyperemia, exercise, and NO: Complex physiology and complex molecular adaptations. Circulation. 1994;90(5):2556-59
13. Kubes P, Suzuki M, Granger DN. Nitric oxide: An endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA. 1991;88(11):4651-4655
14. Garland CJ, Plane F, Kemp BK, Cocks TM. Endothelium-dependent hyperpolarization: A role in the control of vascular tone. Trends Pharmacol Sci. 1995;16(1):23-30
15. Campbell WB, Gebremedhin D, Pratt PF, Harder DR. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. Circ Res. 1996;78:415-23.
16. Lopez JA, Armstrong ML, Piegors DJ, Heistad DD. Vascular responses to endothelin-1 in atherosclerotic primates. Arteriosclerosis. 1990;10:1113-1118
17. Mombouli JV, Vanhoutte PM. Endothelial dysfunction: from physiology to therapy. J Mol Cell Cardiol. 1999;31:61-74.
18. Feletou M. The Endothelium: Part 1: Multiple Functions of the Endothelial Cells-Focus on Endothelium-Derived Vasoactive Mediators. San Rafael (CA): Morgan & Claypool Life Sciences;2011
19. Mehta D, Ravindran K, Kuebler WM. Novel regulators of endothelial barrier function. Am J Physiol Lung Cell Mol Physiol. 2014 Dec 15;307(12):L924–L935.
20. Cioffi DL, Pandey S, Alvarez DF, Cioffi EA. Terminal sialic acids are an important determinant of pulmonary endothelial barrier integrity. Am J Physiol Lung Cell Mol Physiol. 2012;302:L1067–L1077.
21. Roberts WG, Palade GE. Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor, J Cell Sci. 1995;108:2369-2379.
22. Ting LH, Jahn JR, Jung JI,Shuman BR, Feghhi S, Han SJ, et al. Flow mechanotransduction regulates traction forces, intercellular forces, and adherens junctions. Am J Physiol Heart Circ Physiol. 2012;302:H2220–H2229.
23. Chistiakov DA, Orekhov AN, Bobryshev YV. Endothelial Barrier and Its Abnormalities in Cardiovascular Disease. Front Physiol. 2015;6:365.
24. Sukriti S, Tauseef M, Yazbeck P, Mehta D. Mechanisms regulating endothelial permeability. Pulm Circ. 2014 Dec;4(4):535–551
25. Wu K, Thiagarajan P. Role of endothelium in thrombosis and hemostasis. Annu Rev Med. 47(1):315-31
26. Fullard JF. The role of the platelet glycoprotein IIb/IIIa in thrombosis and haemostasis. Curr Pharm Des. 2004;10(14): 1567-76.
27. Aird WC. Phenotypic Heterogeneity of the Endothelium Structure, Function, and

Mechanisms. Circ Res. 2007; 100(2):158-173.
28. Jesty J, Lorenz A, Rodriguez J, Wun TC. Initiation of the tissue factor pathway of coagulation in the presence of heparin: Control by antithrombin III and tissue factor pathway inhibitor. Blood. 1996;87:2301-7
29. Aird W. Spatial and temporal dynamics of the endothelium. J Thromb Hemost. 3:1392-1406.
30. Levin EG, Osborn K. The expression of endothelial cell tissue plasminogen activator in vivo: A function defined by vessel size and anatomic location. J Cell Sci. 1997;11:139.
31. Bacharach E, Itin A, Keshet E. In vivo patterns of expression of urokinase and its inhibitor PAI-1 suggest a concerted role in regulating physiologic angiogenesis. Proc Natl Acad Sci USA. 1992;89:10686-10690
32. Hajjar KA, Hamel NM. Identification and characterization of human endothelial cell membrane binding sites for tissue plasminogen activator and urokinase. J Biol Chem. 1990;265(5):2908-2916
33. Pepper MS, Sappino AP, Stocklin R, Montesano R, Orci L, Vassalli JD. Upregulation of urokinase receptor expression on migrating endothelial cells. J Cell Biol. 1993;122(3):673.
34. Loskutoff DJ, Sawdey M, Mimuro J. Type 1 plasminogen activator inhibitor. In: Coller BS, ed. Progress in Hemostasis and Thrombosis. Philadelphia, PA: Saunders; 1989.p 87.
35. Scarpati EM, Sadler JE. Regulation of endothelial cells coagulant properties. J Biol Chem. 1989;264:20705-13
36. Bajzar L, Morser J, Nesheim M. TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin-thrombomodulin complex. J Biol Chem. 1996;271:16603-8
37. Johnson-Léger C, Aurrand-Lions M and Imhof BA. The parting of the endothelium: miracle, or simply a junctional affair? Journal of Cell Science. 2000;113:921-933.
38. Mantovani A, Bussolino F, Introna M. Cytokine regulation of endothelial cell function: from molecular level to the bedside. Immunol Today. 1997;18(5):231-240.
39. Stewart M, Thiel M, Hogg N. Leukocyte integrins. Curr Opin Cell Biol. 1995;7:690-696.
40. Alon R, Kassner PD, Carr MW, Finger EB, Hemler ME, Springer TA. The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. J Cell Biol. 1995;128:1243-1253.
41. Ayalon O, Sabanai H, Lampugnani MG, Dejana E, Geiger B. Spatial and temporal relationships between cadherins and PECAM-1 in cell-cell junctions of human endothelial cells. J Cell Biol. 1994;126: 247-258.
42. Biedermann BC. Vascular Endothelium: Checkpoint for Inflammation and Immunity. American Physiological Society. 16(2):84-88.
43. Shoda T, Futamura K, Orihara K, Emi-Sugie M, Saito H, Matsumoto K, Matsuda A. Recent advances in understanding the roles of vascular endothelial cells in allergic inflammation. Allergol Int. 2016 Jan; 65(1):21-29.
44. Kurachi M, Mikuni M, Ishizaki Y. Extracellular Vesicles from Vascular Endothelial Cells Promote Survival, Proliferation and Motility of Oligodendrocyte Precursor Cells. PLOS One.2016.DOI:10.1371/journal.pone.0159158
45. Aird WC. Endothelium in health and disease. Pharmacological reports. 2008; 60:139-143.
46. Rajendran P, Rengarajan T, Thangavel J, Nishigaki Y, Sakthisekaran D, Sethi G, et al., The Vascular Endothelium and Human Diseases. Int J Biol Sci. 2013;9(10):1057-1069.
47. Mancini GBJ, Henry GC, Macaya C, O'Neil BJ, Pucillo AL, Carere RG, et al. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease: the TREND (Trial on Reversing ENdothelial Dysfunction) Study. Circulation. 1996;94(3):258–65.