Chapter 4
Endothelial Cells

Caterina Sturtzel

4.1 Introduction

The commonly accepted roles of endothelial cells (EC) in homeostasis of body physiology are to safeguard transport logistics, control vascular permeability, and regulate vascular tone. In the immunology of cardiovascular homeostasis and pathology, EC can be regarded from two perspectives: on the one hand, they are a constitutive and integral part of the cardiovascular system and therefore intrinsically causing disease if dysfunctional, and on the other hand, they actively mediate immune responses at places of injury or infection [1].

Cobblestone shape is a main histological characteristic of EC, but they constitute more than static mechano-protective plates. EC that line the inner vessel wall are not at all inert bystander cells but central and active parts of two major systems in the body—the immune and the vascular system. As a consequence, damage, (hyper) activation, and dysfunction of EC are frequently part of the etiology of cardiovascular diseases. These two systems often act in concert, for example, during wound healing, but there are also conditions where EC engage in distinctive roles in each of them, such as during development and tissue regeneration.

The vascular system is composed of EC lining the inside of vessels and of smooth muscle cells or pericytes supporting the vessel structure. Strongly adapted to the various tissues, the lymphatic and the blood vessel system pervade the entire body. Lymphatic vessels are blind-ended tubes equipped with valves to fulfill efficiently their major task of collecting and draining interstitial fluid leaking out of blood vessels. Thereby, they are also a transport route for nutrients or mobile cells, such as white blood cells, again interlinking function with the immune system [2]. Blood vessels

C. Sturtzel, PhD
Innovative Cancer Models, Children's Cancer Research Institute,
St. Anna Kinderkrebsforschung e.V, Vienna, Austria
e-mail: caterina.sturtzel@ccri.at

S. Sattler, T. Kennedy-Lydon (eds.), *The Immunology of Cardiovascular Homeostasis and Pathology*, Advances in Experimental Medicine and Biology 1003,
DOI 10.1007/978-3-319-57613-8_4

exist as capillaries, veins, or arteries, covered by supporting cells, and their major tasks are the transport of oxygen as well as nutrients and immune cells to the various tissues of the body [3]. Therefore, genesis of the lymphatic system in the developing embryo is triggered by rising fluid pressure, whereas blood vessel vasculogenesis and angiogenesis are driven by hypoxia [4, 5]. During embryonic development, the first vascular plexus is established by coalescing hemangioblasts from which eventually vessels are built by sprouting of EC, the process defined as angiogenesis [3, 6].

EC isolated from different tissues will exhibit organ-specific adaptations and adjustments in shape and function. For example, EC of the central nervous system form the blood-brain barrier, uterine EC express estrogen receptors, EC of the high endothelial venules allow for transcellular routes for leukocyte extravasation during homing, EC of the endocardium fold up and adapt to the constant heartbeat, and EC committed for either arterial or venous vessels show different sprouting capacities [7–10].

As active part of the immune system, EC not only function as a transport device for mobile immune cells and form a mechanical barrier against intruders, but they also (1) have essential paracrine function by secreting chemokines, interleukins, interferons, and growth factors; (2) organize recruitment of immune cells and regulate leukocyte extravasation at places of inflammation by inducible expression of adhesion molecules like E-selectin, P-selectin, ICAM, or VCAM [11]; as well as (3) maintain appropriate hemostasis or coagulation.

The classical four signs of inflammation described by Celsus (30 BC–38 AD), dolor (pain), calor (heat), rubor (reddishness), and tumor (swelling), already illustrated the significant role of EC during inflammation, since these reactions are all mediated by EC through local changes of vessel barrier function. Although EC are not immune cells in the classical sense, as they cannot kill, phagocytose, and produce antibodies or similar, they essentially coordinate the immune response.

4.2 Classical Functions of Endothelial Cells and Their Immunological Relevance

4.2.1 Hemostasis

To maintain barrier function and to prevent intrusion of pathogens and their quick systemic spread, junctions need to be kept tight and repaired quickly after vessels rupture. During tissue trauma vessels are often damaged, and, in cooperation with platelets, the endothelium initiates processes aiming to stop bleeding and close holes. Coagulation results in the formation of solid blood clots to plug the opening in the vessel. Thereby, hemostasis, the cessation of bleeding, is reached (Fig. 4.1). However, as crucial as this activity of the endothelium and the platelets is in the correct situation, as dangerous can it be when activated aberrantly, for example, during disseminated coagulation, which carries a high risk for fatal outcomes [12]. When clots are formed uncontrollably, they can occlude vessels, especially narrowing capillaries, and reach the state of thrombosis. Therefore, the endothelium in its basal state is anticoagulant.

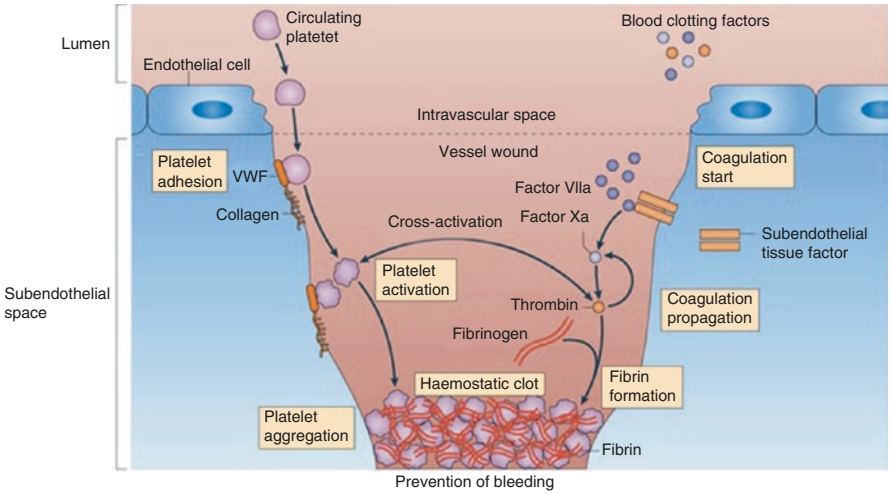

Fig. 4.1 Hemostasis involves two principle components: platelets and the coagulation system with its major product, fibrin. Both systems act in concert to generate a hemostatic clot that seals the wound. The efficient recruitment of platelets involves specific surface receptors, such as the platelet glycoprotein Ib (GPIb)-GPV-GPIX complex, GPVI, and several integrins (not shown). These platelet receptors recognize distinct ligands that are normally concealed by the endothelial barrier and become exposed only after vessel damage. These ligands include von Willebrand factor (VWF) and collagens, as well as fibrinogen, vitronectin, and fibronectin (not shown). Platelet recruitment does not induce hemostasis unless fibrin is also formed by the coagulation system. Coagulation requires the sequential activation of blood-based serine proteases and their cofactors (collectively known as blood clotting factors). The process is initiated by tissue factor, which is expressed by subendothelial cells and is therefore hidden in the intact vessel wall. In response to injury, however, tissue factor is exposed to blood and can interact with blood-based factor VIIa to trigger the coagulation cascade, which culminates in the formation of fibrin (Modified from [12])

To control and balance the homeostasis of hemostasis, the activation of coagulation is tightly regulated and depends on a full cascade of progressive protease activities on proenzymes [13]. Activation of the coagulation cascade culminates in the formation of fibrin from fibrinogen. Polymerized and cross-linked fibrinogen becomes a sticky substance providing a mesh on activated platelets, which supports formation of a clot. These thrombi can be degraded again by proteases, especially plasmin, when healing progresses [12–14].

During hemostasis, the endothelium provides a crucial base whereupon activities are organized and regulated. Procoagulant molecules, including von Willebrand factor (vWF) and tissue factor (TF), are expressed in subendothelial areas on collagen and fibroblasts and become exposed to blood upon injury of the vessel wall. There, platelet aggregation is induced by receptor binding of vWF, and TF binds circulating coagulation factor VII, which initiates the proteolytic coagulation cascade. The proteolysis of coagulation factor VII (fVII) generates fVIIa, which further supports cleavage of fX into fXa and forces thrombin generation from prothrombin. Other circulating factors including factor V, VIII, and IX contribute as cofactors to enhanced thrombin formation. Ultimately, thrombin releases fibrin from fibrinogen, which further strengthens clot formation of the platelets.

Weibel-Palade bodies (WPB) are preformed endothelial-specific first-aid kits, perfectly equipped to rapidly and highly efficiently respond to an insult to the vasculature, without losing time of activating the translational machinery. These rod-shaped subcellular organelles are filled with a whole battery of bioactive compounds such as their major component vWF to recruit platelets to close the wound, by concentrating them to the vessel wall by binding through their glycoprotein 1balpha [15].

They further contain P-selectin to recruit leukocytes to guard the wound, IL-8 to boost inflammation, endothelin-1 for vasoconstriction to close off the affected area, angiopoietin-2 to destabilize endothelial junctions and its barrier function for flexibility during tissue repair, and tPA to prevent excessive fibrin formation [16–18]. Relative allocation of the content of WPB is situation dependent, with an increased amount of IL-8 during inflammatory situations, mutual exclusive presence of P-selectin and angiopoietin-2 due to different transport sources, and varying amounts of tPA [17, 19]. Activation and exocytosis of WPB can be triggered by several stimuli including thrombin, vascular endothelial growth factor (VEGF), or epinephrine either through Ca^{2+}/calmodulin-dependent pathways or in response to cAMP-raising agonists distinctly influencing cytoskeletal function [20].

Tissue factor expression on EC increases upon pro-inflammatory stimulation with, e.g., TNF-alpha, oxidized phospholipids, pro-angiogenic factors, or shear stress [21–23]. During inflammation, clot formation can contribute to pathogen containment and regeneration of the vessel barrier function. However, this scenario also substantially contributes to atherosclerotic plaque formation [12].

Several pathways control aberrant initiation of the sequentially amplifying coagulation cascade. To prevent coagulation, it is necessary to interfere at the very top of the cascade. Tissue factor pathway inhibitor (TFPI) is a serine protease inhibitor that precludes complexing of TF to fVIIa and thus dampens coagulation. To form a clot now, fVIII and fX need to override this blockage. There are two main splice isoforms in humans; TFPIalpha is secreted by EC and present in the plasma but also stored in platelets. TFPIbeta is EC specific and anchored by GPI into the plasma membrane [14, 24]. Regulation of the coagulation cascade can thereby be spaciously differently controlled in the fluid phase and at the vessel wall.

Disruption of this intricate balance can cause severe disorders, such as the congenital bleeding disease hemophilia A or B, when the function of fVIII or fX is impaired, respectively. Still, acute conditions such as thrombosis or disseminated intravascular coagulation (DIC) are the result of uncontrolled activation. There is a high risk for developing this pathologic state during systemic inflammation when fibrin is over-consumed and clot formation is not effective [25].

To reconstitute unperturbed flow, removal of the thrombus is necessary once healing has progressed sufficiently. Fibrinolysis of clots is crucial to prevent thrombosis and is accomplished by plasmin. Plasmin is cleaved off its liver-secreted zymogen plasminogen by the serine proteases (serpins) tissue plasminogen activator (tPA), mainly produced by EC, or urokinase (uPA), secreted by many different cell types. The process of fibrin degradation needs to be as tightly controlled as its generation and includes several safety check points [26]. tPA requires fibrin as cofactor,

and thus only cleaves clot associated plasminogen, and is irreversibly and rapidly inhibited by its specific plasminogen activator inhibitor (PAI-1) when circulating. Whereas tPA acts mostly in the bloodstream, uPA works mostly extravascularly. Furthermore, nonfibrin-bound plasmin is bound by alpha2-antiplasmin rendering it inactive through blocking its fibrin-binding site. Taking advantage of these specificities may allow for targeted therapy during clinical thrombotic events [27].

Acute occlusion of main vessels by thrombi is detrimental for the affected ischemic tissue, for example, during a myocardial infarct; however, rapid sealing of injured vessels is necessary to keep up vital nutrient support and protection from intruding pathogens. Therefore, depending on the specific situation, maintaining the appropriate balance between pro- and anticoagulative action of the endothelium is crucial for effective immunity of the human body.

4.2.2 Vascular Tone

Under homeostasis, the vascular tone is regulated in balance of vasodilative and vasoconstrictive signals to adapt blood pressure and flow to current activity requirements. EC control vascular tone by sending paracrine signals to smooth muscle cells surrounding the vessels, which can constrict vessels by contraction or dilate them by relaxation.

The most potent vasoconstrictor is endothelin (ET), a 21aa peptide existing in three isoforms mainly synthesized by EC [28]. Serum levels of ET-1, the predominant form of endothelin, are elevated by pro-inflammatory, EC-activating signaling, with transcription factor binding sites for AP1, NF-kB, GATA2, SMAD, or HIF1alpha detected in the endothelin gene [29]. However, ET-1 itself induces expression of pro-inflammatory signals. Constriction of a vessel in an inflamed area achieves a containment effect for pathogens and decelerates passing leukocytes for transmigration. ET-1 signaling increases expression of adhesion molecules such as VCAM (vascular cell adhesion molecule) on endothelial cells and supports the clustering of neutrophils, which contributes to the massive neutrophil infiltration observed in ischemic myocardium [28].

The main counter-player against vasodilation is nitric oxide (NO), a gasotransmitter, produced by NO synthases (NOS) in a stepwise redox reaction from L-arginine. There are three isoforms of NO synthase, nNOS (neuronal), eNOS (endothelial), and iNOS (cytokine-inducible). Under healthy conditions, eNOS in the endothelium provides NO to keep the vascular tone to adjusted levels under altering blood pressure and blood flow conditions [30, 31]. Endothelial dysfunction is a state of impaired NO bioavailability and linked to development of atherosclerosis and cardiovascular disease [32]. Flow-mediated dilation (FMD) is typically measured to determine endothelial dysfunction in patients, where a reduction can be used as prognostic marker for heart failure and presents concomitantly with vascular remodeling of arterial vessels [33]. Capacity overload of the system or depletion of L-arginine or the essential cofactor tetrahydrobiopterin leads to eNOS uncoupling, a switch in the NO generation process that also produces reactive oxygen species (ROS). This oxidative stress

in the long term causes subtle remodeling of the vasculature to a chronic pro-inflammatory state and contributes to atherosclerotic plaque formation [34].

In contrast, iNOS, as reflected by the name, is steeply induced by cytokines under inflammatory conditions, especially to generate ROS to combat pathogens. NO itself has radical potential and exerts microbicidal function. iNOS is also expressed in EC, but the main source are leukocytes, especially macrophages [35]. Under sterile inflammatory conditions, iNOS activation constitutes a significant threat to the cardiovascular system and may lead to the detrimental development of septic shock.

4.3 Endothelial Cells as Part of the Immune Response

4.3.1 Expression of Innate Immune Receptors

Toll-Like Receptors EC express several innate immune receptors including the toll-like receptor (TLR) family recognizing pathogen-associated molecular patterns (PAMPs) [36, 37]. Expression of all TLRs is detectable in EC. TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, and TLR9 are found in all different kinds of tissue-specific EC. In resting EC, TLR7, TLR8, and TLR10 are absent, but they are inducible under inflammatory activation [38]. Upon agonist binding, dimerization, and activation of TLRs, NF-kappaB and MAPK signaling are initiated via MyD88 and/or TRIF. This leads to a pro-inflammatory cell response, which in EC means structural changes of adhesion molecules in order to increase vascular permeability, production of inflammatory cytokines, presentation of adhesion molecules to recruit leukocytes, and the switch to a procoagulant state [36]. Specifically, direct activation of TLR1/2, TLR3, and TLR4, by their respective agonists Pam3CSK4, Poly(I:C), or LPS, elicits a strong pro-inflammatory response by stimulating the production of cytokines such as IL-6, IL-8, TNF-alpha, and IL-1 beta, altering adhesion molecule expression, including E-selectin, P-selectin, ICAM, and VCAM, elevating vascular permeability through reduced junction protein claudin-5 as well as secretion of procoagulant factors like tissue factor, PAI-1, uPa, and vWF [38–40].

Notably, there are crucial functional differences between TLR responses in monocytes and EC. Some are mediated via different routes of NF-kB and MAPK signaling, as ERK5 seems to regulate endothelial TLR2-dependent transcriptional response including its own upregulation, whereas MEK1 controls monocytic activation [41]. TLR2 in endothelial cells is expressed only at low level at baseline but is strongly upregulated by initial contact with its ligand. In contrast, leukocytes show a constitutively high TLR2 expression. This seems to be a functional adaptation to prevent overshooting endothelial activation and in consequence harmful vascular hyperpermeability, thrombosis, or septic shock [39].

Vascular TLR2 and TLR4 do not only sense pathogens but also tissue damage, for example, by the presence of extracellular histones, and respond with elevated tissue factor production, which may challenge the balance between beneficial local microthrombus formation and the risk for sepsis [42]. In contrast to other reports, this study also describes the TLR2 and TLR4 to be displayed on the endothelial

surface as on leukocytes. However, in EC TLR2 and TLR4 are often found intracellularly [43]. This discrepancy might be correlated with the activation state of the EC.

Hyperglycemia is another pro-inflammatory stimulus for the activation of EC. It is also mediated via TLR2 and TLR4/MyD88/NF-kB/AP1, leading to shedding of the repellent glycocalyx of the endothelium. This enables improved leukocyte adhesion and increased ROS production. In a chronic state, this contributes to the vascular complications of diabetes patients [44].

Circulating endothelial progenitor cells also present certain levels of TLRs. They are responsive to agonist stimulation, which, however, only induces cytokine production, but not differentiation, excluding TLR activation as a trigger for endothelial progenitor cell differentiation at sites of injury [45].

In addition to TLR, various other types of pattern recognition receptors exist in EC. NOD1 and NOD2, containing a nucleotide-binding oligomerization domain (NOD) and caspase recruitment domain (CARD), sense degraded bacterial components released from endosomes in the endothelial cytosol and activate NF-kB signaling [46]. The RNA helicase retinoic acid-inducible gene-I (RIG-I) recognizes particular viral ssRNA structures [47].

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) of EC mediates uptake of the glycation end-product oxidized LDL, which is generated under oxidative stress during inflammatory events. Its activation leads to exacerbation of the inflammatory response, chronic inflammation, endothelial dysfunction, and atherosclerosis [48]. LOX-1 is a member of a lectin-like receptor family, encoded in the natural killer (NK) gene complex together with dectin-1 and CLEC-1 [49]. C-type lectin-like receptor 1 (CLEC-1), which is also expressed in EC, is not known to be involved in leukopheresis, as other C-type lectins described later, but represents an intracellular pattern recognition receptor. It is upregulated by immune regulation TGF-beta and is involved in control of immune response to transplants [50, 51].

4.3.2 Endothelial Cells in Leukopheresis

One of the most crucial functions of EC during an inflammatory response is to organize and coordinate controlled transition of leukocytes through the vessel wall for recruitment into damaged/infected tissue. It is a major challenge for EC to keep up the balance between tightly sealing vessel walls to prevent leakage of transported fluids on the one hand and to facilitate extravasation of mobile immune cells on the other hand. However, during inflammation, increased vascular permeability and transendothelial migration (TEM), reflecting innate and adaptive immunity, are required at different time points.

Release of molecules such as antibodies or complement components to injured tissue should constrain infections. As a second line of defense, leukocytes join in. For this purpose, EC express adhesion molecules on their surface with various operative specializations to direct the leukocyte extravasation cascade. In general, TEM can be pictured as leukocytes migrating through the vessel wall in sequential steps of (1) tethering, (2) rolling, (3) firm adhesion, (4) crawling, and (5) eventually diapedesis (Fig. 4.2) [52]. L-, P-, and E-selectin constitute one main group of adhesion

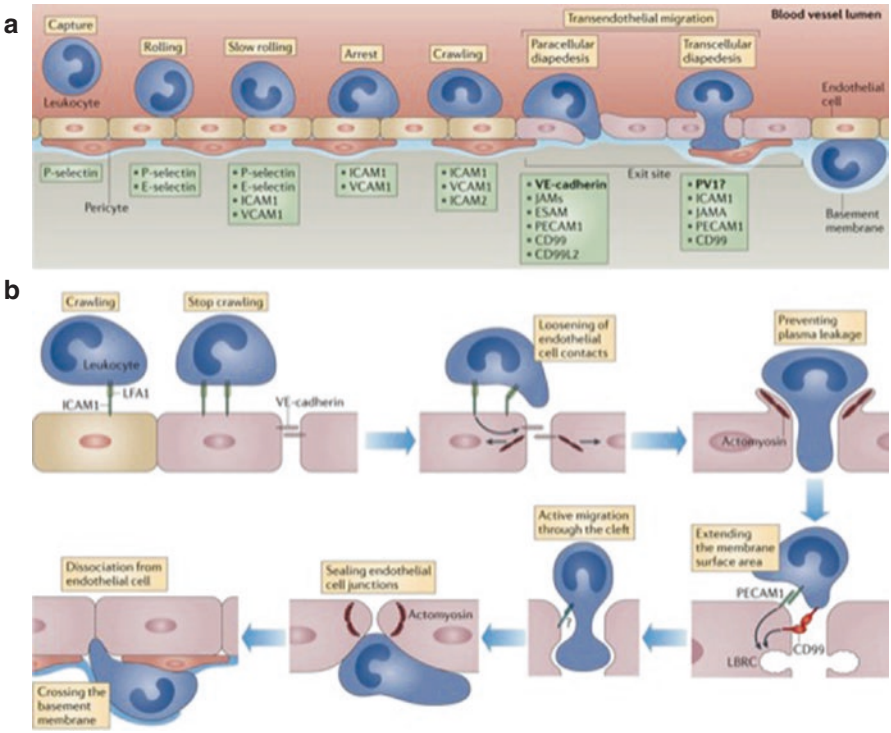

Fig. 4.2 (**a**) A range of cell adhesion receptors on endothelial cells (as shown at the *bottom* of the *panel*) mediates the capture, rolling, arrest, and crawling of leukocytes on the luminal endothelial cell surface. This is the prelude for the actual transmigration through the endothelial barrier—the diapedesis process. Leukocyte diapedesis is usually paracellular but can also occur in a minority of cases through a transcellular route. Whereas transmigration through the endothelial barrier takes about 2–3 min, leukocytes take up to 15–20 min to overcome the basement membrane, leading to the transient accumulation of leukocytes between endothelial cells and the basement membrane. Some of the adhesion receptors that participate in paracellular diapedesis are also relevant for transcellular diapedesis. Vascular endothelial cadherin (VE-cadherin) is exclusively involved in the paracellular route, functioning as a barrier to transmigration. The only candidate for a cell surface protein that might be exclusively involved in transcellular diapedesis is plasmalemma vesicle protein 1 (PV1), which is an essential component of fenestral and stomatal diaphragms. Owing to space limitations, the list of endothelial cell adhesion receptors shown here is not exhaustive but represents the most well studied. (**b**) The diapedesis process requires many functions mediated by leukocytes and endothelial cells: stopping intraluminal crawling at suitable exit sites, loosening of endothelial cell contacts, preventing plasma leakage, extending the membrane surface area at endothelial cell junctions through mobilization of the lateral border recycling compartment (LBRC), active leukocyte migration through the junctional cleft, and sealing of the junction after diapedesis. Finally, leukocytes dissociate from endothelial cells followed by transmigration through the basement membrane. *CD99L2* CD99 antigen-like protein 2, *ESAM* endothelial cell-selective adhesion molecule, *ICAM* intercellular adhesion molecule, *JAM* junctional adhesion molecule, *LFA-1* lymphocyte function-associated antigen 1, *PECAM1* platelet endothelial cell adhesion molecule 1, *VCAM1* vascular cell adhesion molecule 1 (Modified from [52])

molecules. They are C-type (Ca^{2+}-dependent) lectins binding to glycoconjugated ligands. These three closely related selectins recruit leukocytes out of blood flow toward an inflamed area by establishing first specific but dynamic contacts with EC [53]. This initial step of leukocyte tethering to EC is preceded by local EC glycocalyx shedding executed by heparinase, matrix metalloproteinases (MMPs), and ROS, as this charged cell surface coating prevents adhesion molecule and carbohydrate ligand presentation under normal conditions as a barrier for unintended extravasation [54].

E-selectin has to be synthesized de novo and is accordingly steeply induced by pro-inflammatory signals such as TNF-alpha or IL-1, whereas P-selectin is stored in Weibel-Palade bodies, which fuse upon stimulation with the cell membrane. L-selectin in contrast is constitutively expressed on leukocytes, and its main purpose is to guide them toward high endothelial venule structures during regular homing processes, where expression of the ligands glycosylated cell adhesion molecule-1 (GlyCAM1) and P-selectin glycoprotein ligand-1 (PSGL-1) captures them. However, similar structures are also present in atherosclerotic-prone vascular walls [55]. PSGL-1 is a low-affinity ligand for all selectins, thereby decelerating leukocytes close to extravasation sites, but firm adhesion has still to be established [56, 57].

PSGL-1 is resident in lipid rafts on the tips of microvilli, and simultaneous binding of several PSGL-1 molecules leads to formation of secondary structures to further supporting adhesion, whereby transition of tethering to rolling is smoothly proceeding [53].

CD44 is another versatile glycoprotein binding to L- or E-selectin when fitted with the appropriate posttranslational modification of certain carbohydrate residues. Furthermore, CD44 protein harbors docking sites for the interaction with components of the extracellular matrix, contributing to fixation of leukocytes in damaged areas. CD44 can regulate rolling velocity by cooperation with PSGL-1, thereby especially recruiting T cells [53].

E-selectin ligand-1 (ESL-1) can further support binding of PSGL-1 to P-selectin when ESL-1 is recognizing E-selectin in parallel [53].

All these molecules in their different combination and density can fine-tune the tethering/rolling process and indeed regulate which subsets of leukocytes are eventually recruited. Chemokines were found to decisively influence the type of captured immune cells due to distinct spatiotemporal regulation of expression of selectins and their ligands. The chemokine CCL2 elicits a rapid surface presence of L-selectin, PGSL-1, and CD44, crucial for neutrophil recruitment, and subsequently induces E-selectin expression to attach monocytes. This is in accordance with the observed phenomenon of a first wave of infiltrating neutrophils and a second wave of imigrating monocytes at sites of cardiovascular pathologies [58]. This study also depicts that E-selectin is coexpressed with CD31/PECAM at cell-cell endothelial junctions. Such junctions have to be opened in places where leukocytes should squeeze through, and E-selectin seems to pull them directly toward the lateral cell-cell borders.

The processes of arrest and crawling are mediated by stronger anchoring through interaction of adhesion molecules and their various integrin ligands, intercellular adhesion molecule (ICAM-1) and lymphocyte function-associated antigen-1 (LFA-1), vascular cell adhesion molecule-1 (VCAM-1) and VLA-4 (very late antigen-4), and platelet endothelial cell adhesion molecule (PECAM), respectively [59]. Binding of these ligands to integrins, transmembrane receptors for cell-cell and cell-ECM interactions, leads to conformational changes, inducing downstream intracellular signaling to alter cytoskeleton structures [60]. Thereby fixation and crawling of leukocytes even against of flow direction are supported. Inside-out signaling induces transition from ICAM-1 binding to LFA-1 for an anchoring arrest toward crawling mediating binding of macrophage antigen 1 (Mac-1).

ICAM-1/-2 and VCAM-1 are inducible by various inflammatory stimuli, such as TNF-alpha, IL-1beta, oxLDL, or C-reactive protein. This is regulated by signaling via the transcription factors NF-kB and AP1 for which several binding sites were discovered, for example, in the VCAM promoter [61, 62]. Detection of ICAM-1 or VCAM-1 on EC and on endothelial microparticles was shown to constitute a prognostic marker for cardiovascular diseases with a severe outcome [63]. Highly expressed ICAM-1 is an indication for activated EC as well as increased infiltration, and thereby attracted leukocytes destabilize atherosclerotic plaques [64]. Further, ICAM-1 is involved in the formation of unstable plaques through mineralocorticoid aldosterone, which normally regulates blood volume by shifting electrolyte concentrations. Mineralocorticoid receptor-responsive elements were found in the ICAM-1 promoter, and increased leukocyte infiltration destabilizes atherosclerotic plaques [65]. In addition, EC-derived ICAM-1 induced by IL-1b and IL-6 in circulation is responsible for attracting T-cell and monocyte infiltration into the left ventricle subsequent to heart failure [66].

PECAM-1 is an endothelial cell adhesion molecule that can be found at the lateral borders of an EC monolayer but is also expressed on leukocytes. In a complex with VEGFR2 and VE-cadherin, it functions as shear stress sensor and thereby activates signaling to further induce ICAM-1 expression [67].

The described adhesion molecules transiently attach leukocytes to endothelial cells, whereas connections between EC are tight and adherent junctions. The involved protein complexes regulate the barrier function of the endothelium under noninflamed conditions. To cross this sealed cell wall for leukocytes during inflammation, two routes are conceivable: either trans- or paracellular. Although data are yet controversial due to limitations of live microscopy of this complex cellular process, there is consent that transcellular passage of leukocytes through EC is happening, with an approximate proportion of 10% trans- and 90% paracellular TEM also depending on blood vessel type and thickness as well as potentially the type of initial TEM stimulus [52].

For the paracellular route, adherent junctions have to loosen and open up in a harmonized way as to not completely disband the vessel but just to decrease barrier function. In EC adherent junctions are constituted by the single membrane protein VE-cadherin (cadherin 5) which forms homodimers with molecules on neighboring cells [68]. To anchor these junctions, VE-cadherins associate with beta- and

alpha-catenin, and these further interconnect to the cytoskeleton. Experiments using constitutively stabilized VE-cadherin-catenin proteins revealed that leukocyte transmigration is strongly prevented then [69]. It is suggested that phosphorylation of VE-cadherin by tyrosine kinases and dissociation of vascular endothelial protein tyrosine phosphatase (VE-PTP) are prerequisite for opening the junctions through conformational changes and by internalization. There are tyrosine residues specific for induction of vascular leakage, while others are responsible for destabilizing junctions as preparation of diapedesis [70–72]. Leukocytes are required to unwrap junctions before passing through. When ICAM-1 molecules ligate and cluster, phosphorylation of VE-cadherin is induced, which also involves proteins of the Rho family [73]. This system is also responsible for the actin stress fiber rearrangements that pull apart the adherent junctions. Dissociation of VE-PTP can be triggered by binding of leukocytes but also by VEGF. It is also necessary to loosen contacts during vessel growth [74].

A whole system of counteracting proteins is required for successful TEM: the molecules PECAM, CD155, and CD99 on the membrane system termed lateral border recycling compartment (LBRC). Homophilic interactions between PECAM and CD99 expressed on leukocytes and EC direct the leukocyte toward a junction and then through it, respectively [75, 76]. The LBRC is moved with the help of kinesins. Other junctional proteins such as junctional adhesion molecule (JAM-)A and C also function as counter-receptors for passaging leukocytes. These molecules seem to surround the leukocytes in circular structures, forming an orientation guide to prevent hesitant or even backward migration. During ischemic reperfusion conditions, JAM-C expression is reduced at EC junctions, and disrupted polarized TEM of neutrophils but not of monocytes has been observed [77].

All these different proteins involved in TEM reflect the critical point of opening up a normally tightly sealed tissue structure to enable transition of whole cells without perturbing the entire vessel system [11, 78]. Furthermore the endothelium preserves a crucial selective capacity by regulating the recruited leukocyte type by differential expression of chemokines and adhesion molecules [79]. Knowing the different molecules involved in recruitment of different leukocytes to atherosclerotic plaques or damaged tissue after an ischemic event might offer more targeted therapeutic strategies.

4.3.3 Angiogenesis

Angiogenesis, defined as the formation of new blood vessels from preexisting vessels, is not only required during development and growth but is necessarily linked to tissue repair and restoration of oxygen and metabolite supply as well as barrier function in wounded, ischemic, or inflamed areas. During angiogenesis, EC are activated, and single lines of cells start to migrate out of a preexisting vessel toward a gradient of vascular endothelial growth factor (VEGF), a growth factor produced under hypoxic conditions [80]. However, a lot of factors including chemokines are

known to have pro-angiogenic capacity, reflecting the interlinked need for new vessels during an immune response. For example, IL-8 is highly angiogenic, and its expression is associated with poor prognosis in different cancers but induces EC proliferation in ischemic myocardium [81, 82].

It is commonly accepted that new vessels are formed as outgrowing sprouts of tip/stalk cells. The leading tip cell is highly migratory and protrudes many filopodia, which express VEGF receptors to sense the VEGF gradient. So-called stalk cells follow behind the tip cell. These are highly proliferative and will eventually form a new, lumenized vessel. Delta-like 4 (Dll4), a Notch receptor ligand, expressed on tip cells, induces Notch signaling in the neighboring cell, upon which surface expression of KDR/VEGF receptor 2 is downregulated implementing the stalk cell phenotype [83, 84]. Several factors might select for a cell to become a tip cell, including random local overexpression of KDR, metabolic advantages of some cells in terms of elevated glycolysis conferring higher motility, or cell arrangements orientating cells into certain directions to facilitate migration [85, 86]. When a sprout encounters another, they will anastomose and blood can flow through a newly formed vessel [87, 88]. This process is often bridged by macrophages via conferring additional sources of Notch receptors especially at branching points [89]. Monocytes can also be an additional resource of VEGF, which is necessary for the process of arteriogenesis, the rapid maturation of collateral capillaries into arteries after an occlusion event of the arterial circulation [90, 91]. This involves NF-kB signaling that can stabilize hypoxia-inducible factor 1alpha under non-hypoxic conditions.

Stimulation and support of angiogenesis by macrophages is also a potential indirect mechanism induced by the immune-suppressive cytokine IL-19. IL-19, a member of the IL-10 family, is expressed by EC of inflamed coronary tissue. It boosts bFGF-dependent angiogenesis in the absence of hypoxia by effects exerted on vascular smooth muscle cells [92, 93].

Originally, VEGF was described as vascular permeabilization factor referring to one of its obvious physiological effects [94]. To allow vessel growth, junctional adhesion has to be destabilized, similar to inflammation-induced permeability, to allow for restructuring of the EC layer. VEGF usually stands for VEGF-A, the classical pro-angiogenic factor, representing a whole structurally related family. VEGF crucially sustains EC survival and promotes blood vessel angiogenesis. VEGF proteins are the ligands for the receptor tyrosine kinase family of VEGF receptors. There are three receptors, VEGFR1, VEGFR2, and VEGFR3, also known as Flt1, Flk1, and Flt4, respectively. VEGFR1 binds VEGF-A with higher affinity than VEGFR2, but VEGFR1 is hardly mitogenic and the intrinsic signaling kinase activity is very low. VEGFR1 and its soluble splice form sFlt1 are therefore regarded as decoy receptors, titrating out abundant VEGF at times of massive angiogenesis or to maintain avascularity of corneas [95].

VEFGR2 possesses high intrinsic tyrosine kinase activity upon ligand binding. Proliferation of EC is stimulated by VEGF primarily via phospholipase gamma and protein kinase C leading to RAF/ERK/MAPK pathway signaling. The pro-survival effect of VEGF-A-VEGFR2 signaling is mediated through PI3K/PIP3/PKB/AKT-dependent phosphorylation and blocking of proapoptotic caspases [96].

Inflammatory signals induce a qualitatively, partly overlapping transcription profile in EC compared to angiogenic stimuli, but quantitative differences reflect the fine-tuning potential of the system. For example, VCAM-1 is steeply induced by IL-1 as well as by VEGF, however, with a factor 100 difference in magnitude. This reflects the significantly greater need for leukocyte recruitment into inflamed/damaged tissue, than during sterile angiogenesis, where only a small number is needed for surveillance. However, some endothelial genes are specifically activated only under pro-angiogenic conditions, specifically required for the sprouting process itself, while numerous others are activated under pro-inflammatory conditions only, since hypoxic conditions are not always present when inflammation has to be triggered [97, 98].

As mentioned above, many cytokines also exert pro-angiogenic, EC-activating effects. Another prominent example, IL-33, especially exemplifies how entangled the different functions of EC are. IL-33, member of the IL-1 superfamily, is released during inflammatory tissue damage or trauma by necrotic or affected cells. IL-33 activates endothelial migration in a potentially pro-angiogenic way and increases vascular permeability by elevated eNOS-dependent NO levels [99]. At the same time, IL-33 upregulates TF expression in EC and downregulates TFPI, pushing them to a procoagulant state [100]. IL-33 and TF can be co-detected in atherosclerotic plaques, and levels of IL-33 correlate to disease activity in CAD [101].

All processes of EC activation, inflammation, hemostasis, and angiogenesis have one principle in common: the end is already part of the program. Therefore, VEGF inducible transcription factors control targets, which will neutralize the pro-angiogenic activation of EC if no restimulation occurs, as part of a negative-feedback mechanism when normoxia is restored. VEGF-dependent MEF2C induced A2M, which functions as a global serine protease inhibitor [102]. This likely prevents excessive extracellular matrix (ECM) and tissue degradation or aberrant angiogenesis [103]. Also the inflammatory response has to be brought back to a resting level to prevent excessive inflammatory damage. Strong pro-inflammatory stimuli such as IL-1 or TNF-alpha induce, during a second wave, inhibitors of the NF-kB, NFAT, and MAPK pathway to resolve activation [104].

4.3.4 EC in Cardiovascular Disease

In contrast to smooth muscle cells, EC can tolerate hypoxia very well, in culture even for weeks through different expression of HIF-1 and HIF-2, but, for example, in a myocardial infarct after occlusion of a vessel after 20–40 min, the clinically long observed wavefront phenomenon of necrotic areas sets in [105, 106]. However, they are very sensitive to reperfusion injury when blood flow is restored. Necrotic cardiomyocytes and hypoxia activate EC, rendering them targets for infiltrating leukocytes. Notably, however, EC seem not to tolerate hypothermia very well. Cardiac arrest patients treated with hypothermia to improve neurological prognosis show aggravated endothelial dysfunction and elevated levels of sVCAM-1 [107].

ROS are commonly considered detrimental to cells, and EC are also very vulnerable for their destructive potential. However, there are conflicting reports regarding this aspect, as antioxidant treatment by diet or drugs was not effective in EC-dependent CVD risk factor prevention [108]. Elucidating this phenomenon on molecular level revealed that coronary EC become proapoptotic upon long-term ROS burden in the mitochondrial cell compartment but need short-term ROS stimuli for survival [109].

Further, EC are very sensitive to flow and adapt to different flow conditions. Flow-mediated dilation and low-flow-mediated vasoconstriction are regulated by healthy responsive EC to balance homeostasis, but loss of these functions is indicative for endothelial dysfunction and correlates with an adverse prognosis for CVD [110].

Pulsatile laminar flow is atheroprotective, especially at vessel branches, as it induces eNOS and consequently elevates the level of NO and suppresses endothelin-1 [111]. Shear stress is decisive for the balanced expression of these two counterplayers in the vascular system, but the potential of high wall shear stress to remodel the endothelium is also considered as a driver of formation of non-stable atherosclerotic plaques. However, imaging possibilities are not yet meaningful enough for correlating shear stress, morphologic changes of plaque, and adverse events [112, 113].

As extensively reviewed by Heusch et al., after myocardial infarction, the area of risk is the critical region that determines the outcome of adverse events [114]. Reperfusion injury manifests as increased vascular permeability along with edema formation, which is not only caused by lost barrier function and glycocalyx loss upon inflammation but also by electrolyte concentration shifts due to loss of energy-dependent ion pumps. Edema impairs further microvascular perfusion by compression. Nitric oxide can attenuate deprivation of the barrier function; however, vasomotion in infarcted areas is often shifted to hyperconstriction through endothelin, leading to vascular remodeling. Microembolisms resulting from atherothrombotic debris or after percutaneous coronary intervention, or cell aggregates assembled through the increased expression of adhesion molecules, can further reduce circulation. If the swelling of the vessels is too severe, these might rupture, and the resulting hemorrhage leads to severe myocardial necrosis. The area of risk can be reduced by cardioprotective interventions like pre- or postischemic conditioning but only if some collateral flow is preserved to transfer protective factors [114]. Remote ischemic preconditioning can be cardioprotective in animal models where several cycles of few minutes ischemia seem to be an effective schedule [115]. Detailed definition of the involved molecular mediators sent and received by EC poses a new therapeutic target to treat ischemic maladies.

4.4 Conclusion

EC are culprits and victims during myocardial infarction at the same time. Although EC tolerate hypoxia better than other cardiac resident cell types, their pro-angiogenic activation causes loss of barrier function and edema formation. Furthermore their

pro-inflammatory activation increases expression of adhesion molecules and causes leukocyte influx. Excessive immune cell infiltration can be detrimental to the already damaged tissue. Loss of vasodilative NO synthesis by EC further aggravates vessel occlusion in the heart. In addition, endothelial activation shifts them toward a hazardous prothrombotic state.

In summary, disruption of the classical endothelial functions of vessel formation, hemostasis, and regulation of vascular tone underlies most cardiovascular pathologies, and their central role in immune response organization exacerbates damage. Therefore it is necessary to also therapeutically target EC during cardiovascular diseases.

References

1. Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. Nat Rev Immunol. 2007;7:803–15.
2. Alitalo K. The lymphatic vasculature in disease. Nat Med. 2011;17:1371–80.
3. Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. Cell. 2011;146:873–87.
4. Planas-Paz L, Strilic B, Goedecke A, Breier G, Fassler R, Lammert E. Mechanoinduction of lymph vessel expansion. EMBO J. 2012;31:788–804.
5. Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer. 2003;3:721–32.
6. Risau W. Differentiation of endothelium. FASEB J. 1995;9:926–33.
7. Domigan CK, Iruela-Arispe ML. Recent advances in vascular development. Curr Opin Hematol. 2012;19:176–83.
8. Adams RH, Alitalo K. Molecular regulation of angiogenesis and lymphangiogenesis. Nat Rev Mol Cell Biol. 2007;8:464–78.
9. Mickoleit M, Schmid B, Weber M, Fahrbach FO, Hombach S, Reischauer S, Huisken J. High-resolution reconstruction of the beating zebrafish heart. Nat Methods. 2014;11:919–22.
10. Haack T, Abdelilah-Seyfried S. The force within: endocardial development, mechanotransduction and signalling during cardiac morphogenesis. Development. 2016;143:373–86.
11. Vestweber D. Relevance of endothelial junctions in leukocyte extravasation and vascular permeability. Ann N Y Acad Sci. 2012;1257:184–92.
12. Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. Nat Rev Immunol. 2013;13:34–45.
13. Kazmi RS, Boyce S, Lwaleed BA. Homeostasis of hemostasis: the role of endothelium. Semin Thromb Hemost. 2015;41:549–55.
14. Yau JW, Teoh H, Verma S. Endothelial cell control of thrombosis. BMC Cardiovasc Disord. 2015;15:130.
15. Andre P, Denis CV, Ware J, Saffaripour S, Hynes RO, Ruggeri ZM, Wagner DD. Platelets adhere to and translocate on von Willebrand factor presented by endothelium in stimulated veins. Blood. 2000;96:3322–8.
16. Weibel ER. Fifty years of Weibel-Palade bodies: the discovery and early history of an enigmatic organelle of endothelial cells. J Thromb Haemost. 2012;10:979–84.
17. Rondaij MG, Bierings R, Kragt A, van Mourik JA, Voorberg J. Dynamics and plasticity of Weibel-Palade bodies in endothelial cells. Arterioscler Thromb Vasc Biol. 2006;26:1002–7.
18. Denis CV, Andre P, Saffaripour S, Wagner DD. Defect in regulated secretion of P-selectin affects leukocyte recruitment in von Willebrand factor-deficient mice. Proc. Natl. Acad. Sci. U.S.A. 2001;98:4072–7.
19. Utgaard JO, Jahnsen FL, Bakka A, Brandtzaeg P, Haraldsen G. Rapid secretion of prestored interleukin 8 from Weibel-Palade bodies of microvascular endothelial cells. J Exp Med. 1998;188:1751–6.

20. Vischer UM, Wollheim CB. Purine nucleotides induce regulated secretion of von Willebrand factor: involvement of cytosolic Ca^{2+} and cyclic adenosine monophosphate-dependent signaling in endothelial exocytosis. Blood. 1998;91:118–27.
21. Mechtcheriakova D, Schabbauer G, Lucerna M, Clauss M, De Martin R, Binder BR, Hofer E. Specificity, diversity, and convergence in VEGF and TNF-alpha signaling events leading to tissue factor up-regulation via EGR-1 in endothelial cells. FASEB J. 2001;15:230–42.
22. Bochkov VN, Mechtcheriakova D, Lucerna M, Huber J, Malli R, Graier WF, Hofer E, Binder BR, Leitinger N. Oxidized phospholipids stimulate tissue factor expression in human endothelial cells via activation of ERK/EGR-1 and Ca(++)/NFAT. Blood. 2002;99:199–206.
23. Houston P, Dickson MC, Ludbrook V, White B, Schwachtgen JL, McVey JH, Mackman N, Reese JM, Gorman DG, Campbell C, Braddock M. Fluid shear stress induction of the tissue factor promoter in vitro and in vivo is mediated by Egr-1. Arterioscler Thromb Vasc Biol. 1999;19:281–9.
24. Mast AE. Tissue factor pathway inhibitor: multiple anticoagulant activities for a single protein. Arterioscler Thromb Vasc Biol. 2016;36:9–14.
25. Levi M. Current understanding of disseminated intravascular coagulation. Br J Haematol. 2004;124:567–76.
26. Chapin JC, Hajjar KA. Fibrinolysis and the control of blood coagulation. Blood Rev. 2015;29:17–24.
27. Lippi G, Mattiuzzi C, Favaloro EJ. Novel and emerging therapies: thrombus-targeted fibrinolysis. Semin Thromb Hemost. 2013;39:48–58.
28. Davenport AP, Hyndman KA, Dhaun N, Southan C, Kohan DE, Pollock JS, Pollock DM, Webb DJ, Maguire JJ. Endothelin. Pharmacol Rev. 2016;68:357–418.
29. Stow LR, Jacobs ME, Wingo CS, Cain BD. Endothelin-1 gene regulation. FASEB J. 2011;25:16–28.
30. Siragusa M, Fleming I. The eNOS signalosome and its link to endothelial dysfunction. Arch Eur J Physiol. 2016;468:1125–37.
31. Shesely EG, Maeda N, Kim HS, Desai KM, Krege JH, Laubach VE, Sherman PA, Sessa WC, Smithies O. Elevated blood pressures in mice lacking endothelial nitric oxide synthase. Proc Natl Acad Sci USA. 1996;93:13176–81.
32. Kuhlencordt PJ, Gyurko R, Han F, Scherrer-Crosbie M, Aretz TH, Hajjar R, Picard MH, Huang PL. Accelerated atherosclerosis, aortic aneurysm formation, and ischemic heart disease in apolipoprotein E/endothelial nitric oxide synthase double-knockout mice. Circulation. 2001;104:448–54.
33. Kishimoto S, Kajikawa M, Maruhashi T, Iwamoto Y, Matsumoto T, Iwamoto A, Oda N, Matsui S, Hidaka T, Kihara Y, Chayama K, Goto C, Aibara Y, Nakashima A, Noma K, Higashi Y. Endothelial dysfunction and abnormal vascular structure are simultaneously present in patients with heart failure with preserved ejection fraction. Int J Cardiol. 2017;231:181–7.
34. McNeill E, Channon KM. The role of tetrahydrobiopterin in inflammation and cardiovascular disease. Thromb Haemost. 2012;108:832–9.
35. Uehara EU, Shida Bde S, de Brito CA. Role of nitric oxide in immune responses against viruses: beyond microbicidal activity. Inflamm Res. 2015;64:845–52.
36. Khakpour S, Wilhelmsen K, Hellman J. Vascular endothelial cell toll-like receptor pathways in sepsis. Innate Immun. 2015;21:827–46.
37. Opitz B, Eitel J, Meixenberger K, Suttorp N. Role of toll-like receptors, NOD-like receptors and RIG-I-like receptors in endothelial cells and systemic infections. Thromb Haemost. 2009;102:1103–9.
38. Fitzner N, Clauberg S, Essmann F, Liebmann J, Kolb-Bachofen V. Human skin endothelial cells can express all 10 TLR genes and respond to respective ligands. Clin Vaccine Immunol. 2008;15:138–46.
39. Shin HS, Xu F, Bagchi A, Herrup E, Prakash A, Valentine C, Kulkarni H, Wilhelmsen K, Warren S, Hellman J. Bacterial lipoprotein TLR2 agonists broadly modulate endothelial function and coagulation pathways in vitro and in vivo. J Immunol. 2011;186:1119–30.

40. Pegu A, Qin S, Fallert Junecko BA, Nisato RE, Pepper MS, Reinhart TA. Human lymphatic endothelial cells express multiple functional TLRs. J Immunol. 2008;180:3399–405.
41. Wilhelmsen K, Mesa KR, Lucero J, Xu F, Hellman J. ERK5 protein promotes, whereas MEK1 protein differentially regulates, the toll-like receptor 2 protein-dependent activation of human endothelial cells and monocytes. J Biol Chem. 2012;287:26478–94.
42. Yang X, Li L, Liu J, Lv B, Chen F. Extracellular histones induce tissue factor expression in vascular endothelial cells via TLR and activation of NF-kappaB and AP-1. Thromb Res. 2016;137:211–8.
43. Dunzendorfer S, Lee HK, Soldau K, Tobias PS. Toll-like receptor 4 functions intracellularly in human coronary artery endothelial cells: roles of LBP and sCD14 in mediating LPS responses. FASEB J. 2004;18:1117–9.
44. Pahwa R, Nallasamy P, Jialal I. Toll-like receptors 2 and 4 mediate hyperglycemia induced macrovascular aortic endothelial cell inflammation and perturbation of the endothelial glycocalyx. J Diabetes Complicat. 2016;30:563–72.
45. Mazzucchelli I, Lisini D, Garofoli F, Dragoni S, Angelini M, Pozzi M, Bonetti E, Tzialla C, Kramer BW, Spinillo A, Maccario R, Rosti V, Moccia F, Borghesi A, Stronati M. Expression and function of toll-like receptors in human circulating endothelial colony forming cells. Immunol Lett. 2015;168:98–104.
46. Opitz B, Puschel A, Beermann W, Hocke AC, Forster S, Schmeck B, van Laak V, Chakraborty T, Suttorp N, Hippenstiel S. *Listeria monocytogenes* activated p38 MAPK and induced IL-8 secretion in a nucleotide-binding oligomerization domain 1-dependent manner in endothelial cells. J Immunol. 2006;176:484–90.
47. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol. 2004;5:730–7.
48. Lubrano V, Balzan S. Roles of LOX-1 in microvascular dysfunction. Microvasc Res. 2016;105:132–40.
49. Sattler S, Ghadially H, Reiche D, Karas I, Hofer E. Evolutionary development and expression pattern of the myeloid lectin-like receptor gene family encoded within the NK gene complex. Scand J Immunol. 2010;72:309–18.
50. Sattler S, Reiche D, Sturtzel C, Karas I, Richter S, Kalb ML, Gregor W, Hofer E. The human C-type lectin-like receptor CLEC-1 is upregulated by TGF-beta and primarily localized in the endoplasmic membrane compartment. Scand J Immunol. 2012;75:282–92.
51. Thebault P, Lhermite N, Tilly G, Le Texier L, Quillard T, Heslan M, Anegon I, Soulillou JP, Brouard S, Charreau B, Cuturi MC, Chiffoleau E. The C-type lectin-like receptor CLEC-1, expressed by myeloid cells and endothelial cells, is up-regulated by immunoregulatory mediators and moderates T cell activation. J Immunol. 2009;183:3099–108.
52. Vestweber D. How leukocytes cross the vascular endothelium. Nat Rev Immunol. 2015;15:692–704.
53. Zarbock A, Ley K, McEver RP, Hidalgo A. Leukocyte ligands for endothelial selectins: specialized glycoconjugates that mediate rolling and signaling under flow. Blood. 2011;118:6743–51.
54. Becker BF, Jacob M, Leipert S, Salmon AH, Chappell D. Degradation of the endothelial glycocalyx in clinical settings: searching for the sheddases. Br J Clin Pharmacol. 2015;80:389–402.
55. Galkina E, Kadl A, Sanders J, Varughese D, Sarembock IJ, Ley K. Lymphocyte recruitment into the aortic wall before and during development of atherosclerosis is partially L-selectin dependent. J Exp Med. 2006;203:1273–82.
56. Vandendries ER, Furie BC, Furie B. Role of P-selectin and PSGL-1 in coagulation and thrombosis. Thromb Haemost. 2004;92:459–66.
57. Ramachandran V, Yago T, Epperson TK, Kobzdej MM, Nollert MU, Cummings RD, Zhu C, McEver RP. Dimerization of a selectin and its ligand stabilizes cell rolling and enhances tether strength in shear flow. Proc Natl Acad Sci U.S.A. 2001;98:10166–71.

58. Zuchtriegel G, Uhl B, Hessenauer ME, Kurz AR, Rehberg M, Lauber K, Krombach F, Reichel CA. Spatiotemporal expression dynamics of selectins govern the sequential extravasation of neutrophils and monocytes in the acute inflammatory response. Arterioscler Thromb Vasc Biol. 2015;35:899–910.
59. Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. Arterioscler Thromb Vasc Biol. 2007;27:2292–301.
60. van Wetering S, van den Berk N, van Buul JD, Mul FP, Lommerse I, Mous R, ten Klooster JP, Zwaginga JJ, Hordijk PL. VCAM-1-mediated Rac signaling controls endothelial cell-cell contacts and leukocyte transmigration. Am J Physiol Cell Physiol. 2003;285:C343–52.
61. Cybulsky MI, Fries JW, Williams AJ, Sultan P, Eddy R, Byers M, Shows T, Gimbrone MA Jr, Collins T. Gene structure, chromosomal location, and basis for alternative mRNA splicing of the human VCAM1 gene. Proc Natl Acad Sci U.S.A. 1991;88:7859–63.
62. Kawanami D, Maemura K, Takeda N, Harada T, Nojiri T, Saito T, Manabe I, Imai Y, Nagai R. C-reactive protein induces VCAM-1 gene expression through NF-kappaB activation in vascular endothelial cells. Atherosclerosis. 2006;185:39–46.
63. Radecke CE, Warrick AE, Singh GD, Rogers JH, Simon SI, Armstrong EJ. Coronary artery endothelial cells and microparticles increase expression of VCAM-1 in myocardial infarction. Thromb Haemost. 2015;113:605–16.
64. Hoke M, Winter MP, Wagner O, Exner M, Schillinger M, Arnold Z, Mlekusch W, Maurer G, Koppensteiner R, Minar E, Goliasch G. The impact of selectins on mortality in stable carotid atherosclerosis. Thromb Haemost. 2015;114:632–8.
65. Marzolla V, Armani A, Mammi C, Moss ME, Pagliarini V, Pontecorvo L, Antelmi A, Fabbri A, Rosano G, Jaffe IZ, Caprio M. Essential role of ICAM-1 in aldosterone-induced atherosclerosis. Int J Cardiol. 2017;232:233–42.
66. Salvador AM, Nevers T, Velazquez F, Aronovitz M, Wang B, Abadia Molina A, Jaffe IZ, Karas RH, Blanton RM, Alcaide P. Intercellular adhesion molecule 1 regulates left ventricular leukocyte infiltration, cardiac remodeling, and function in pressure overload-induced heart failure. J Am Heart Assoc. 2016;5:e003126.
67. Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, Cao G, DeLisser H, Schwartz MA. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. Nature. 2005;437:426–31.
68. Corada M, Mariotti M, Thurston G, Smith K, Kunkel R, Brockhaus M, Lampugnani MG, Martin-Padura I, Stoppacciaro A, Ruco L, McDonald DM, Ward PA, Dejana E. Vascular endothelial-cadherin is an important determinant of microvascular integrity in vivo. Proc Natl Acad Sci U.S.A. 1999;96:9815–20.
69. Schulte D, Kuppers V, Dartsch N, Broermann A, Li H, Zarbock A, Kamenyeva O, Kiefer F, Khandoga A, Massberg S, Vestweber D. Stabilizing the VE-cadherin-catenin complex blocks leukocyte extravasation and vascular permeability. EMBO J. 2011;30:4157–70.
70. Wessel F, Winderlich M, Holm M, Frye M, Rivera-Galdos R, Vockel M, Linnepe R, Ipe U, Stadtmann A, Zarbock A, Nottebaum AF, Vestweber D. Leukocyte extravasation and vascular permeability are each controlled in vivo by different tyrosine residues of VE-cadherin. Nat Immunol. 2014;15:223–30.
71. Orsenigo F, Giampietro C, Ferrari A, Corada M, Galaup A, Sigismund S, Ristagno G, Maddaluno L, Koh GY, Franco D, Kurtcuoglu V, Poulikakos D, Baluk P, McDonald D, Grazia Lampugnani M, Dejana E. Phosphorylation of VE-cadherin is modulated by haemodynamic forces and contributes to the regulation of vascular permeability in vivo. Nat Commun. 2012;3:1208.
72. Adam AP, Sharenko AL, Pumiglia K, Vincent PA. Src-induced tyrosine phosphorylation of VE-cadherin is not sufficient to decrease barrier function of endothelial monolayers. J Biol Chem. 2010;285:7045–55.
73. Saito H, Minamiya Y, Saito S, Ogawa J. Endothelial rho and rho kinase regulate neutrophil migration via endothelial myosin light chain phosphorylation. J Leukoc Biol. 2002;72:829–36.

74. Broermann A, Winderlich M, Block H, Frye M, Rossaint J, Zarbock A, Cagna G, Linnepe R, Schulte D, Nottebaum AF, Vestweber D. Dissociation of VE-PTP from VE-cadherin is required for leukocyte extravasation and for VEGF-induced vascular permeability in vivo. J Exp Med. 2011;208:2393–401.
75. Schenkel AR, Mamdouh Z, Chen X, Liebman RM, Muller WA. CD99 plays a major role in the migration of monocytes through endothelial junctions. Nat Immunol. 2002;3:143–50.
76. Schenkel AR, Chew TW, Muller WA. Platelet endothelial cell adhesion molecule deficiency or blockade significantly reduces leukocyte emigration in a majority of mouse strains. J Immunol. 2004;173:6403–8.
77. Woodfin A, Voisin MB, Beyrau M, Colom B, Caille D, Diapouli FM, Nash GB, Chavakis T, Albelda SM, Rainger GE, Meda P, Imhof BA, Nourshargh S. The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo. Nat Immunol. 2011;12:761–9.
78. Chistiakov DA, Orekhov AN, Bobryshev YV. Endothelial barrier and its abnormalities in cardiovascular disease. Front Physiol. 2015;6:365.
79. Schnoor M, Alcaide P, Voisin MB, van Buul JD. Crossing the vascular wall: common and unique mechanisms exploited by different leukocyte subsets during extravasation. Mediat Inflamm. 2015;2015:946509.
80. Gerhardt H. VEGF and endothelial guidance in angiogenic sprouting. Organogenesis. 2008;4:241–6.
81. Inoue K, Slaton JW, Eve BY, Kim SJ, Perrotte P, Balbay MD, Yano S, Bar-Eli M, Radinsky R, Pettaway CA, Dinney CP. Interleukin 8 expression regulates tumorigenicity and metastases in androgen-independent prostate cancer. Clin Cancer Res. 2000;6:2104–19.
82. Xie Q, Sun Z, Chen M, Zhong Q, Yang T, Yi J. IL-8 up-regulates proliferative angiogenesis in ischemic myocardium in rabbits through phosphorylation of Akt/GSK-3beta(ser9) dependent pathways. Int J Clin Exp Med. 2015;8:12498–508.
83. Phng LK, Gerhardt H. Angiogenesis: a team effort coordinated by notch. Dev Cell. 2009;16:196–208.
84. Hellstrom M, Phng LK, Hofmann JJ, Wallgard E, Coultas L, Lindblom P, Alva J, Nilsson AK, Karlsson L, Gaiano N, Yoon K, Rossant J, Iruela-Arispe ML, Kalen M, Gerhardt H, Betsholtz C. Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. Nature. 2007;445:776–80.
85. Fraisl P, Mazzone M, Schmidt T, Carmeliet P. Regulation of angiogenesis by oxygen and metabolism. Dev Cell. 2009;16:167–79.
86. Jakobsson L, Franco CA, Bentley K, Collins RT, Ponsioen B, Aspalter IM, Rosewell I, Busse M, Thurston G, Medvinsky A, Schulte-Merker S, Gerhardt H. Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. Nat Cell Biol. 2010;12:943–53.
87. Wacker A, Gerhardt H. Endothelial development taking shape. Curr Opin Cell Biol. 2011;23:676–85.
88. Blanco R, Gerhardt H. VEGF and notch in tip and stalk cell selection. Cold Spring Harb Perspect Med. 2013;3:a006569.
89. Outtz HH, Tattersall IW, Kofler NM, Steinbach N, Kitajewski J. Notch1 controls macrophage recruitment and notch signaling is activated at sites of endothelial cell anastomosis during retinal angiogenesis in mice. Blood. 2011;118:3436–9.
90. Grunewald M, Avraham I, Dor Y, Bachar-Lustig E, Itin A, Jung S, Chimenti S, Landsman L, Abramovitch R, Keshet E. VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. Cell. 2006;124:175–89.
91. Tirziu D, Jaba IM, Yu P, Larrivee B, Coon BG, Cristofaro B, Zhuang ZW, Lanahan AA, Schwartz MA, Eichmann A, Simons M. Endothelial nuclear factor-kappaB-dependent regulation of arteriogenesis and branching. Circulation. 2012;126:2589–600.
92. Kako F, Gabunia K, Ray M, Kelemen SE, England RN, Kako B, Scalia RG, Autieri MV. Interleukin-19 induces angiogenesis in the absence of hypoxia by direct and indirect immune mechanisms. Am J Physiol Cell Physiol. 2016;310:C931–41.

93. Jain S, Gabunia K, Kelemen SE, Panetti TS, Autieri MV. The anti-inflammatory cytokine interleukin 19 is expressed by and angiogenic for human endothelial cells. Arterioscler Thromb Vasc Biol. 2011;31:167–75.
94. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol. 1995;146:1029–39.
95. Rahimi N. VEGFR-1 and VEGFR-2: two non-identical twins with a unique physiognomy. Front Biosci. 2006;11:818–29.
96. Koch S, Tugues S, Li X, Gualandi L, Claesson-Welsh L. Signal transduction by vascular endothelial growth factor receptors. Biochem J. 2011;437:169–83.
97. Schweighofer B, Testori J, Sturtzel C, Sattler S, Mayer H, Wagner O, Bilban M, Hofer E. The VEGF-induced transcriptional response comprises gene clusters at the crossroad of angiogenesis and inflammation. Thromb Haemost. 2009;102:544–54.
98. Testori J, Schweighofer B, Helfrich I, Sturtzel C, Lipnik K, Gesierich S, Nasarre P, Hofer-Warbinek R, Bilban M, Augustin HG, Hofer E. The VEGF-regulated transcription factor HLX controls the expression of guidance cues and negatively regulates sprouting of endothelial cells. Blood. 2011;117:2735–44.
99. Choi YS, Choi HJ, Min JK, Pyun BJ, Maeng YS, Park H, Kim J, Kim YM, Kwon YG. Interleukin-33 induces angiogenesis and vascular permeability through ST2/TRAF6-mediated endothelial nitric oxide production. Blood. 2009;114:3117–26.
100. Stojkovic S, Kaun C, Basilio J, Rauscher S, Hell L, Krychtiuk KA, Bonstingl C, de Martin R, Groger M, Ay C, Holnthoner W, Eppel W, Neumayer C, Huk I, Huber K, Demyanets S, Wojta J. Tissue factor is induced by interleukin-33 in human endothelial cells: a new link between coagulation and inflammation. Sci Rep. 2016;6:25171.
101. Demyanets S, Konya V, Kastl SP, Kaun C, Rauscher S, Niessner A, Pentz R, Pfaffenberger S, Rychli K, Lemberger CE, de Martin R, Heinemann A, Huk I, Groger M, Maurer G, Huber K, Wojta J. Interleukin-33 induces expression of adhesion molecules and inflammatory activation in human endothelial cells and in human atherosclerotic plaques. Arterioscler Thromb Vasc Biol. 2011;31:2080–9.
102. Sturtzel C, Testori J, Schweighofer B, Bilban M, Hofer E. The transcription factor MEF2C negatively controls angiogenic sprouting of endothelial cells depending on oxygen. PLoS One. 2014;9:e101521.
103. Bhattacharjee G, Asplin IR, Wu SM, Gawdi G, Pizzo SV. The conformation-dependent interaction of alpha 2-macroglobulin with vascular endothelial growth factor. A novel mechanism of alpha 2-macroglobulin/growth factor binding. J Biol Chem. 2000;275:26806–11.
104. Winsauer G, de Martin R. Resolution of inflammation: intracellular feedback loops in the endothelium. Thromb Haemost. 2007;97:364–9.
105. Nauta TD, van den Broek M, Gibbs S, van der Pouw-Kraan TC, Oudejans CB, van Hinsbergh VW, Koolwijk P. Identification of HIF-2alpha-regulated genes that play a role in human microvascular endothelial sprouting during prolonged hypoxia in vitro. Angiogenesis. 2016;20(1):39–54.
106. Reimer KA, Lowe JE, Rasmussen MM, Jennings RB. The wavefront phenomenon of ischemic cell death. 1. Myocardial infarct size vs duration of coronary occlusion in dogs. Circulation. 1977;56:786–94.
107. Brugaletta S, Scalone G, Dantas AP, Ortega-Paz L, Garabito M, Roque M, Martin V, Masotti M, Freixa X, Sabate M. Endothelial function impairment in STEMI patients with out-of-hospital cardiac arrest under therapeutic hypothermia treatment. Int J Cardiol. 2017;232:70–5.
108. Willcox BJ, Curb JD, Rodriguez BL. Antioxidants in cardiovascular health and disease: key lessons from epidemiologic studies. Am J Cardiol. 2008;101:75D–86D.
109. Shafique E, Torina A, Reichert K, Colantuono B, Nur N, Zeeshan K, Ravichandran V, Liu Y, Feng J, Zeeshan K, Benjamin LE, Irani K, Harrington EO, Sellke FW, Abid MR. Mitochondrial redox plays a critical role in the paradoxical effects of NAPDH oxidase-derived ROS on coronary endothelium. Cardiovasc Res. 2017;113(2):234–46.

110. Gori T, von Henning U, Muxel S, Schaefer S, Fasola F, Vosseler M, Schnorbus B, Binder H, Parker JD, Munzel T. Both flow-mediated dilation and constriction are associated with changes in blood flow and shear stress: two complementary perspectives on endothelial function. Clin Hemorheol Microcirc. 2016;64(3):255–66.
111. Young A, Wu W, Sun W, Benjamin Larman H, Wang N, Li YS, Shyy JY, Chien S, Garcia-Cardena G. Flow activation of AMP-activated protein kinase in vascular endothelium leads to Kruppel-like factor 2 expression. Arterioscler Thromb Vasc Biol. 2009;29:1902–8.
112. Eshtehardi P, Brown AJ, Bhargava A, Costopoulos C, Hung OY, Corban MT, Hosseini H, Gogas BD, Giddens DP, Samady H. High wall shear stress and high-risk plaque: an emerging concept. Int J Cardiovasc Imaging. 2017; doi:10.1007/s10554-016-1055-1.
113. Dolan JM, Kolega J, Meng H. High wall shear stress and spatial gradients in vascular pathology: a review. Ann Biomed Eng. 2013;41:1411–27.
114. Heusch G. The coronary circulation as a target of cardioprotection. Circ Res. 2016;118:1643–58.
115. Johnsen J, Pryds K, Salman R, Lofgren B, Kristiansen SB, Botker HE. The remote ischemic preconditioning algorithm: effect of number of cycles, cycle duration and effector organ mass on efficacy of protection. Basic Res Cardiol. 2016;111:10.