New paradigms in inflammatory signaling in vascular endothelial cells

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Inflammation is a set of interrelated processes in response to injuries caused by a variety of biological, chemical, and physical stimuli (19). Vascular endothelial cells (ECs) form an interface between blood flow and the vessel wall and execute a number of important functions in the maintenance of body’s homeostasis (2). The endothelium not only provides a nonadhesive and highly selective physical barrier to control the vascular permeability, it also secretes a large number of vasoactive substances to regulate the vascular tone and the remodeling of vessel wall (106). Most importantly, ECs are indispensable components of inflammation as the key regulator and major target of the inflammatory process. Clinically, vascular ECs are involved in nearly every of the cardinal signs of inflammation. Vasodilation increases blood flow and causes the redness (rubor) and an elevated focal heat (calor). Increased endothelial permeability results in a leakage of plasma proteins and fluid into the tissue, which is manifested as swelling (tumor). The mediators released from ECs and leukocytes, such as bradykinin and prostaglandins, contribute to the sensitivity to pain (dolor). Pathologically, inflammation is defined as the local infiltration and activation of leukocytes. By orchestrated expression of a series of chemokines and cell adhesion molecules, the endothelium plays a pivotal role in regulating the place, extent, and duration of the inflammatory process to ensure appropriate defense and ensuing repair against infection and injury (59). On the other hand, ECs by themselves are targets of inflammatory response. Proinflammatory activation of ECs have been well recognized as a key pathophysiological step in many diseases including infections, autoimmunity, and cancer and metabolic disorders such as hypertension, coronary heart disease, obesity, and diabetes. In particular, recent studies have made significant progresses in the understanding of the complex molecular pathways that mediate the pro- and anti-inflammatory signaling in ECs (121). Thus this article will briefly summarize these new mechanisms with a special emphasis in the context of cardiovascular diseases.

Endothelial Activation and Inflammation

ECs are constantly exposed to various biological, chemical, and mechanical milieus and maintain a quiescent state with antithrombotic, anti-inflammatory, and antiproliferative properties (18). During inflammatory responses, ECs are phenotypically converted into an “activated” or perturbed state that is characterized by increased permeability, induced leukocyte adhesion, and a prothrombotic feature. This phenotypic conversion is referred as EC activation. EC activation is commonly classified into type I and II responses (82). Type I activation loosen the EC junctions to increase the permeability and export Weibel-Palade bodies (exocytosis) to release the
stored vonWillebrand factor (vWF) and P-selectin, initiating the endothelial interaction with leukocyte and platelets. It is a rapid but transient response independent of de novo gene expression. Type II activation provides a more sustained inflammatory response and invokes the gene expression of a variety of proinflammatory cytokines and adhesion molecules.

**Circulating Factors and Endothelial Inflammation**

Diverse stimuli in circulation activate ECs and provoke proinflammatory responses. Among these, infectious agents, such as lipopolysaccharide (LPS), an endotoxin of Gram-negative bacteria, and tumor necrosis factor-α (TNF-α), a prototypic proinflammatory cytokine, are most extensively studied agents in ECs (12). Thus the basic model for the proinflammatory response in terms of the endothelial/leukocyte interaction and the molecular cascades has been established in regard to these two stimuli and extended to other scenarios later on (55, 59, 81, 97).

**Adhesion molecules: the mediators of EC-leukocyte interactions.** Resting ECs are considered not adhesive to circulating leukocytes. The inflammatory response depends on the migration of leukocytes across ECs. Upon perturbation by proinflammatory stimuli, ECs express E-selectin [also known as endothelial-leukocyte adhesion molecule-1 (ELAM)] (58) and P-selectin (64) to induce the “tethering” and “rolling” of leukocytes onto the inflamed endothelium via the weak binding between selectins and their low affinity ligands Sialyl-LewisX and P-selectin glycoprotein ligand 1 (PSGL-1) (13, 65). ECs (and vascular residing macrophages) also secrete chemokines such as monocytic chemotactic protein-1 (MCP-1 or CCL2), which acts via the receptor C-C chemokine receptor type 2 (CCR2) and CCR4 on monocytes and T lymphocytes, and interleukin-8 (IL-8), which binds to the IL-8 receptor α (CXCR1 or IL8RA) and β (CXCR2 or IL8RB) on neutrophils (14, 15). Rolling leukocytes are further activated by the chemokines displayed on endothelial surface, resulting in spreading and clustering of surface integrins, such as lymphocyte function-associated antigen-1 (LFA-1) and very late antigen-4 (VLA-4) (8, 16). Induced surface expression of intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) on ECs interact with the integrins counterreceptors on leukocytes and, hence, mediate the firm adhesion (arrest) and transmigration of the leukocytes into the subendothelial spaces of vessel wall or extravasation into the injured tissue to initiate the inflammation (20, 27, 96). Progress have been made to further define steps involving the leukocyte-adhesion cascade, including rolling, activation and capture (or tethering), slow rolling, adhesion strengthening and spreading, intravascular crawling, and paracellular and transcellular transmigration (55). The identified molecules participating in these processes include endothelial cell-selective adhesion molecule (ESAM) (110), junctional adhesion molecule (JAM) (76), macrophage antigen 1 (MAC1) (27), and mucosal vascular addressin cell-adhesion molecule 1 (MAdCAM1) (5, 55, 68).

**Nuclear Factor-κB: a Major Proinflammatory Transcription Factor**

Induced expression of the proinflammatory cytokines and adhesion molecules is a critical step and hallmark of endothelial proinflammatory activation. Nuclear factor (NF)-κB is a master regulator of proinflammatory responses in ECs as well as in other types of cells (87). NF-κB can be activated by various agonists such as oxidized low-density lipoprotein (LDL) (11, 52), lyso phosphatidic acid (LPA) (78), very low-density lipoprotein (VLDL) (23), angiotensin II (ANG II) (85), glucose (71), viral infection (39), and disturbed flow (70). As a transcription factor, NF-κB activates the transcription of the proinflammatory genes including TNF-α, interleukin-1 (IL-1), interleukin-8 (IL-8), E-selectin, VCAM-1, and ICAM-1 by binding to the cognate cis-elements within the regulatory regions of these target genes (22, 69, 80, 98). In mammals, NF-κB has five members: NF-κB1 (p50 and the precursor p105), NF-κB2 (p52 and the precursor p100), Rel A (p65), Rel B, and c-Rel. In the NH2 terminus, they all have a Rel homology domain (RHD), which is responsible for nuclear translocation, dimerization, DNA binding, and interaction with the inhibitor of NF-κB (IκB) (25, 50, 72). In most cases, NF-κB acts as a heterodimer between p65 and p50/p52 to stimulate gene expression.

**IκB kinase signalosome.** In unstimulated ECs, NF-κB is inhibited by IκB, which forms complexes with NF-κB via the ankyrin repeat domains, masking the nuclear localization signal (NLS) and sequestering NF-κB in cytoplasm (45, 107). The IκBs are comprised of typical IκBα, IκBβ, and IκBe, the precursor of p52 and p50 (p100 and p105) (7), and the atypical IκB proteins such as IκBɛ and Bcl-3. Proinflammatory stimuli cause the phosphorylation and the ubiquitination of IκB proteins, leading to the degradation of IκB proteins in the 26S proteasomes and the release of NF-κB for nuclear translocation (10). The phosphorylation of IκB is catalyzed by the upstream kinase IκB kinase (IKK). The IKK complex-mediated site-specific phosphorylation of IκBs represents the most critical regulatory machinery in the proinflammatory signaling. IKK complex is composed of catalytic subunits IKKα (IKK1) (24), IKKβ (IKK2) (111), and a regulatory subunit IκKγ [also known as NF-κB essential modifier (NEMO)] (88). Among the three major members forming this macromolecular “signalosome,” IKKβ is predominantly responsible for TNF-α, IL-1, and LPS-stimulated phosphorylation of IκBα, which is considered as the canonical pathway for NF-κB activation (117). Alternatively, NF-κB can be activated by LPS, CD40, lymphotoxin, and latent membrane protein 1 (LMP1) via a noncanonical pathway in which IKKα phosphorylates p100 (the precursor for NF-κB2) and leads to its processing and release of p52/RelB heterodimer (37). Notably, IKK-independent atypical pathways of NF-κB activation by hypoxia and hydrogen peroxide have also been described, which may be related to the actions of casein kinase-II (CK2), tyrosine-kinase-dependent pathways, or generation of reactive oxygen species (ROS) (75).

**Proinflammatory Signaling from Specific Receptors**

**TNF-α receptor signaling.** The IKK/NF-κB pathway functions as a master regulator and a signaling hub for various proinflammatory stimuli to which ECs are exposed. The upstream signaling pathways leading to the activation of the IKK/NF-κB and other pathways in response to proinflammatory cytokines such as TNF-α has been intensively investigated (17). Therefore, the TNFR pathway has been considered to be the prototype of proinflammatory signaling.
The binding of TNF-α with its receptor (TNFR1) causes the recruitment of TNFR-associated via death domain protein (TRADD) (42), which in turn recruits the receptor-interacting serine/threonine-protein kinase 1 (RIPK1, also called as RIP) (41), and TNFR-associated factor 2 (TRAF2), an E3 ubiquitin ligase and adaptor protein (95, 97). TRAF2 also binds the cellular inhibitor of apoptosis proteins (cIAPs), which have E3 ubiquitin ligase activity and link the inflammatory and survival pathways (62). These molecules, TRADD, RIP, TRAF2, and cIAPs, form the core components of the TNFR1 signalosome to mediate the diverse pathways of TNF-α signaling (Fig. 1) (17). TRAF2 catalyzes the ubiquitination of RIP and enables the recruitment of the NEMO/IKK complex leading to the phosphorylation of IKK2, the ensuing activation of the canonical pathway of NF-κB, and inflammation; cIAPs as E3 ubiquitin ligases not only regulate the activation of NF-κB downstream of TNFR1 but also mediate the activation of NF-κB resulted from the innate immunity signalosomes. (109). Apart from the roles in regulating inflammation, the signaling from the membrane cytokine receptors to NF-κB also plays an important role in regulating endothelial apoptosis, a critical cellular process intrinsically interacting with inflammation. However, the cell death-related pathways are beyond the scope of this review and, thus, will not be discussed here.

Innate immunity-associated receptors. Emerging evidence has revealed that the activation of innate immunity is associated with cardiovascular diseases. The endothelium is among the first line of the body’s defense system to encounter and combat the perturbation of invading microbes and endogenous substances resulted from tissue damages. As a protective response to such injuries, ECs first recognize such potentially dangerous milieu by repressing a number of pattern-recognition receptors (PRRs) including Toll-like receptors (TLRs), nucleotide-binding oligomerization-domain (NOD)-like receptors (NLRs), RNA helicases retinoic acid inducible gene-I (RGI-I)-like receptors (RLRs), cytosolic DNA sensors (CDS), and C-type lectin receptors (CLRs) (63, 94). The specific receptor/signalosome interactions elicit inflammatory response to eliminate the insults to restore the tissue homeostasis.

PRRs recognize the molecular patterns shared by various microbes that distinguished from the endogenous molecules, which are collectively named as pathogen-associated molecular patterns (PAMPs) (1, 105), or the endogenous molecules produced from the injured/stressed cells, which are known as danger-associated molecular patterns (DAMPs). PAMPs include various bacterial components such as LPS, peptidoglycan, flagellin, and viral double-stranded RNAs. DAMPs include intracellular or extracellular proteins or their fragments such as heat shock proteins (HSPs), high-mobility group box 1 protein (HMGB1), and hyaluronan fragments. In addition, ATP, uric acid, cholesterol crystals and host DNAs can also act as nonprotein DAMPs.

TLRs are belong to the “interleukin-1 receptor/Toll-like receptor superfamily” because they have Toll-IL-1 receptor (TIR) domain (66). TLRs are evolutionarily conserved. Ten TLRs (from TLR1 to TLR10) have been identified both in human and in mice whereas TLR11, 12, and 13 are only expressed in mice. All TLRs are type I transmembrane proteins with an ectodomain consist of leucine-rich repeats (LRRs). TLRs can be classified into two broad groups by their cellular localization. TLR1, 2, 4, 5, 6, and 11 are cell membrane TLRs. TLR3, 7, 8, 9, 10, and 13 are localized to intracellular vesicles including endoplasmic reticulum, endosome, and lysosome (34). Cell-surface TLRs recognize conserved PAMPs that are accessible on the cell surface. TLR4 is the receptor for LPS and may also be activated by viral proteins, HSP60, oxidized LDL, and fibrinogen (83). Endogenous ligands of TLR2 include HMGB1, which is a transcription factor and a secreted proinflammatory cytokine, hyaluronic acid fragments, and biglycan (47, 51, 79, 91). TLR5 recognizes bacterial flagellin (36). The intracellular nucleic acid-sensing TLRs mainly recognize microbial nucleic acids, such as double-stranded RNA (dsRNA)
pathways leading to the proinflammatory response, whereas TLR3 activates a TIR-domain-containing adapter-inducing interferon-β (TRIF)-dependent pathway leading to interferon-β (IFNβ) activation (113). Upon activation by PAMPs or DAMPs, TLRs dimerize and associate with the adaptor protein MyD88, which recruits IL-1 receptor-associated kinases (IRAKs) to TLR (31). IRAKs are activated by phosphorylation and then associate with an E3 ubiquitin ligase TRAF6 (74). Then, the IRAK-1/TRAF6 complex dissociates from the TLRs and associates with TGF-β-activated kinase 1 (TAK1) and TAK1-binding proteins, TAB1 and TAB2. TRAF6 in turn polyubiquitinates and facilitates the binding of TAK1 with IKKβ, leading to the activation of NF-κB (46) (Fig. 2).

NLRs are another family of PRRs that mainly sense intracellular microbial invaders and danger molecules produced under stress (33). Structurally, NLRs are characterized by three distinct domains: the ligand-sensing leucine-rich repeats (LRRs), the self-oligomerizing NACHT (NOD) domain, and the effector domain [NH2-terminal pyrin domain (PYD), caspase recruitment domain (CARD), or inhibitor of apoptosis domain (BIR)]. According to phylogenic distribution, NLRs can be divided into three subfamilies: NACH (NOD), LRR, and PYD domain-containing proteins (NLRPs), ICE-protease activating factor (IPAF)/neuronal apoptosis inhibitory protein (NAIP), and NODs. NLRPs have 14 members ranging from NLRP1 to NLRP14 and all contain an NH2-terminal pyrin domain (PYD) (104). IPAF and NAIP contain a caspase recruitment domain (CARD) and BIR domain, respectively. NOD subfamily members NOD1, NOD2, NOD3, and NOD4 contain the CARD domain. NOD5 lacks the NH2-terminal domain. NODs recognize peptidoglycans, sugar chains cross-linked with peptide chains from invading bacteria such as *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Streptococcus*...
pneumoniae, and mycobacterium tuberculosis. NLRPs sense PAMPS such as microbial toxins and whole microbes (fungi and viruses) and danger molecules such as ATP, glucose, cholesterol, and environmental substances including silica, asbestos, and UV irradiation. IPAF senses flagellin from salmonella typhimurium, pseudomonas aeruginosa, and listeria monocytogenes.

Recognition of these PAMPS and DAMPs triggers the oligomerizations of the NODs and the assembly of NOD signalosomes to which CARD-containing serine-threonin kinase (RIP2) is recruited through CARD-CARD interaction (44). Oligomerization of NLRPs triggers the formation of inflammasome, a high-molecular weight protein complex that activates proinflammatory caspases and cytokines IL-1β and IL-18. The term “inflammasome” was coined 10 yr ago by Martinon by identifying a caspase-activating complex that comprises caspase 1, caspase 5, apoptosis-associated speck-like protein containing a caspase recruitment domain (PYCARD/ASC), and NLRP1 (92). To date, several types of inflammasomes have been described, including NLRP1, NLRP3, NLR family CARD domain-containing protein 4 (NLRC4)/IPAF inflammasomes, and a non-NLRP subset absent in melanoma 2 (AIM2) inflammasome (40). The exact components of an inflammasome depend on the type of PAMPS or DAMPs that triggers the assembly. Among the proteins forming the inflammasomes, NLRPs serve as the scaffolding molecule. The pyrin domain of NLRs binds to an adaptor protein ASC via PYD-PYD interaction. ASC contains PYD and CARD by which to PAMPs and DAMPs. Nucleotide-binding oligomerization-domain (NOD)-like receptors (NLRs) serve as the scaffolding molecule with their pyrin domain (PYD) binding to ASCs, which recruit inactive procaspase-1. The aggregation of the procaspase-1 causes its autocleavage and activation of enzymatic activity. Subsequently, caspase-1 in its active form proteolytically processes the pro-IL-1β at Asp116 into IL-1β and cleaves pro-IL-18 into IL-18, resulting in the secretion of the proinflammatory cytokines.

![Fig. 3. Endothelial inflammasomes. Inflammasomes are assembled in response to PAMPS and DAMPs. Nucleotide-binding oligomerization-domain (NOD)-like receptors (NLRs) serve as the scaffolding molecule with their pyrin domain (PYD) binding to ASCs, which recruit inactive procaspase-1. The aggregation of the procaspase-1 causes its autocleavage and resulting in the secretion of the proinflammatory cytokines.](image)

MicroRNAs and Posttranscriptional Regulation of Endothelial Inflammation

While transcription factors exemplified with NF-κB exert important functions in the regulation of de novo synthesis of mRNAs for the inflammation-related molecules in ECs, inflammatory signaling pathways are also tightly regulated beyond the transcriptional control. Recently, microRNAs (miRs) have been discovered as new signaling molecules that regulate gene expression at posttranscriptional level and are implicated in a variety of important cellular processes and diseases (28). Being a family of highly conserved noncoding RNAs, miRs repress gene expression via degradation or translational inhibition of their target mRNAs. In particular, emerging evidence has demonstrated that miRs are a class of novel inflammatory regulators by modifying the target genes at different levels of the proinflammatory signaling cascades. NF-κB is regulated by the miR-based mechanism. miR-10a was found to be down-regulated at the athero-prone area within the vasculature (29) and can be induced in ECs exposed to laminar flow (86). miR-10a suppresses NF-κB activation by targeting two upstream molecules involved in the activation of the IκK/IκB complex, TAK1 and β-transducin-repeat containing gene (β-TRC), and preventing the phosphorylation of IκBα. The nuclear translocation of p65/NF-κB in ECs can also be inhibited by miR-181b-mediated targeting of importin-α3, a protein that binds and import p65/p50 to the nucleus (102). Anti- or proinflammatory effects of miRs are also imposed through

![Diagram](image)
other transcription factors. As such, miR-221/222 cluster suppress the endothelial expression of Ets-1, a critical regulator of vascular inflammation (116, 122). miR-21 is one of the miRs highly expressed in ECs and the first member of the oncomiR family. The validated targets of miR-21 include ras homolog family member B (Rho B) (48, 89), phosphatase and tensin homolog (PTEN) (67), peroxisome proliferator-activated receptor-α (PPARα) in ECs (120) and Sprouty 1 (103) and 2 (32), programmed cell death protein 4 (PDCD4) (6), B-cell lymphoma 2 (Bcl-2), transforming growth factor-β receptor II (TGFRβRII) (53), and myocyte-specific enhancer factor 2C (MEF2C) (114) and etc. in other types of cells. miRNAs also regulate endothelial response by suppressing the genes encoding for the proinflammatory agonists or their membrane receptors. miRNA-125a/b-5p inhibits endothelin-1 (ET-1) expression (56) whereas miR-155 was found to target angiotensin II type I receptor (AT1R) and attenuate the endothelial activation in response to angiotensin II (122). In addition, many miRs are involved in endothelial inflammation by directly targeting the genes responsible for leukocyte recruitment. Among these are miR-126, targeting VCAM-1 (35), and miR-31 and miR-17–3p, targeting E-selectin and ICAM-1, respectively (101). Recent evidence has demonstrated that miRNA is released from ECs to function as signaling molecules. miR-126 is enriched in apoptotic bodies released from ECs and convey paracrine signals to perform an anti-atherosclerotic effect (115). Monocytic cells secreted miR-15a can target c-myb in ECs (118, 120) whereas miR-155 targets angiotensin II type 1 receptor (AT1R) and attenuate the endothelial activation in response to angiotensin II (122). In addition, many miRs are involved in endothelial inflammation by directly targeting the genes responsible for leukocyte recruitment. Among these are

In conclusion, inflammatory responses in ECs are tightly controlled by specific yet interactive signaling networks in response to various stimuli. The nature of proinflammatory milieu is diverse, ranging from invading microorganisms and their toxins to those resulted from metabolic disturbances. Beside ECs, other vascular cells such as SMCs and adventitial fibroblasts also serve as the targets and/or regulators of vascular inflammation and are implicated in the pathogenesis of atherosclerosis, hypertension, fibrosis, and diabetes and its vascular complications. In particular, meta-inflammation, the chronic low-grade inflammation caused by excessive nutrients, has been recognized to be implicated in the pathogenesis of cardiovascular diseases. Future studies are warranted to dissect the interplays between the classical proinflammatory signaling and the intracellular energy-sensing pathways. In addition, the signaling networks mediating the cross-talk among vascular endothelium with extracellular matrix, SMCs, adventitia, as well as the vascularized organs remain to be elucidated.

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DISCLOSURES

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