BRIEF REVIEWS

Immune Regulation by Microvascular Endothelial Cells: Directing Innate and Adaptive Immunity, Coagulation, and Inflammation[1]

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An effective immune response depends not only on the proper activation, regulation, and function of immune cells, but also on their distribution and retention in diverse tissue microenvironments where they encounter a number of stimuli and other cell types. These activities are mediated by endothelial cells, which form specialized microcirculatory networks used by immune cells under both physiological and pathological circumstances. Endothelial cells represent a highly heterogeneous population of cells with the ability to interact with and modulate the function of immune cells. This review is focused on the role of microvascular endothelial cells in innate and adaptive immunity, inflammation, coagulation, angiogenesis, and the therapeutic implications of targeting endothelial cells in selected autoimmune and chronic inflammatory disorders. The Journal of Immunology, 2007, 178: 6017–6022.

The endothelium is a highly specialized cellular system that is composed of $1-6 \times 10^{13}$ endothelial cells (ECs)[3] lining a total surface area of $4000-7000 \ m^2$ (1, 2) and plays a key role in physiological processes such as blood supply, nutrient delivery, metabolic homeostasis, and immune cell trafficking, as well as pathological processes such as inflammation (3–5). Inflammation can be seen as a vascular response (6), where ECs become activated, display increased leakiness, enhanced leukocyte adhesiveness, and procoagulant activity, and form new vessels (7). Thus, an immune response resulting in inflammation depends strictly on a permissive microvasculature, which normally exerts the opposite function of preventing the indiscriminate influx of immune cells into a tissue. Compared with large blood vessels, the microvascular bed constitutes the bulk of the overall endothelial surface, covering an area ~50 times greater than that of all large vessels combined (8).

Major qualitative differences exist between macro- and microvascular ECs, the latter being able to generate a range of mediators, to display distinct adhesion molecule patterns, to activate unique sets of genes, and to form capillaries (9–11).

Much of the information on the contribution of ECs to immunity and inflammation derives from HUVECs (12), but these cells do not reflect the highly specialized nature of microvascular ECs, and the study of ECs from distinct body compartments has confirmed their heterogeneity (9, 10, 13–15). This is best exemplified by the differential expression of homing ligands involved in immune cell trafficking. Mad-CAM-1 is expressed by Peyer's patches high endothelial venules to recruit $\alpha_4\beta_7$ homing receptor-positive naive lymphocytes (16). Likewise, ECs from brain, liver, and other organs express distinct surface markers, protein transporters, and intracellular enzymes (15, 17, 18). The mechanisms responsible for EC heterogeneity are unclear, but tissue-specific and transcription factors likely contribute to the induction or maintenance of specialized EC features (13, 17). In addition to its function in leukocyte trafficking, distribution, and homing, recent evidence indicates that microvascular ECs play a far more direct role in immunity. This review will show that the multifaceted properties of the organ-specific microvasculature convert the perceived passive role of ECs to an active one that controls innate and adaptive immunity, coagulation, and inflammation.

Innate immunity

The primary function of innate immunity is to recognize pathogen-associated molecular patterns (PAMPs) through "pattern recognition receptors." Among these, TLRs are surface molecules that trigger signals resulting in proinflammatory gene expression, leukocyte chemotaxis, phagocytosis, cytotoxicity, and activation of adaptive immune responses (19). Several reports have demonstrated TLRs on ECs (20) (Fig. 1).

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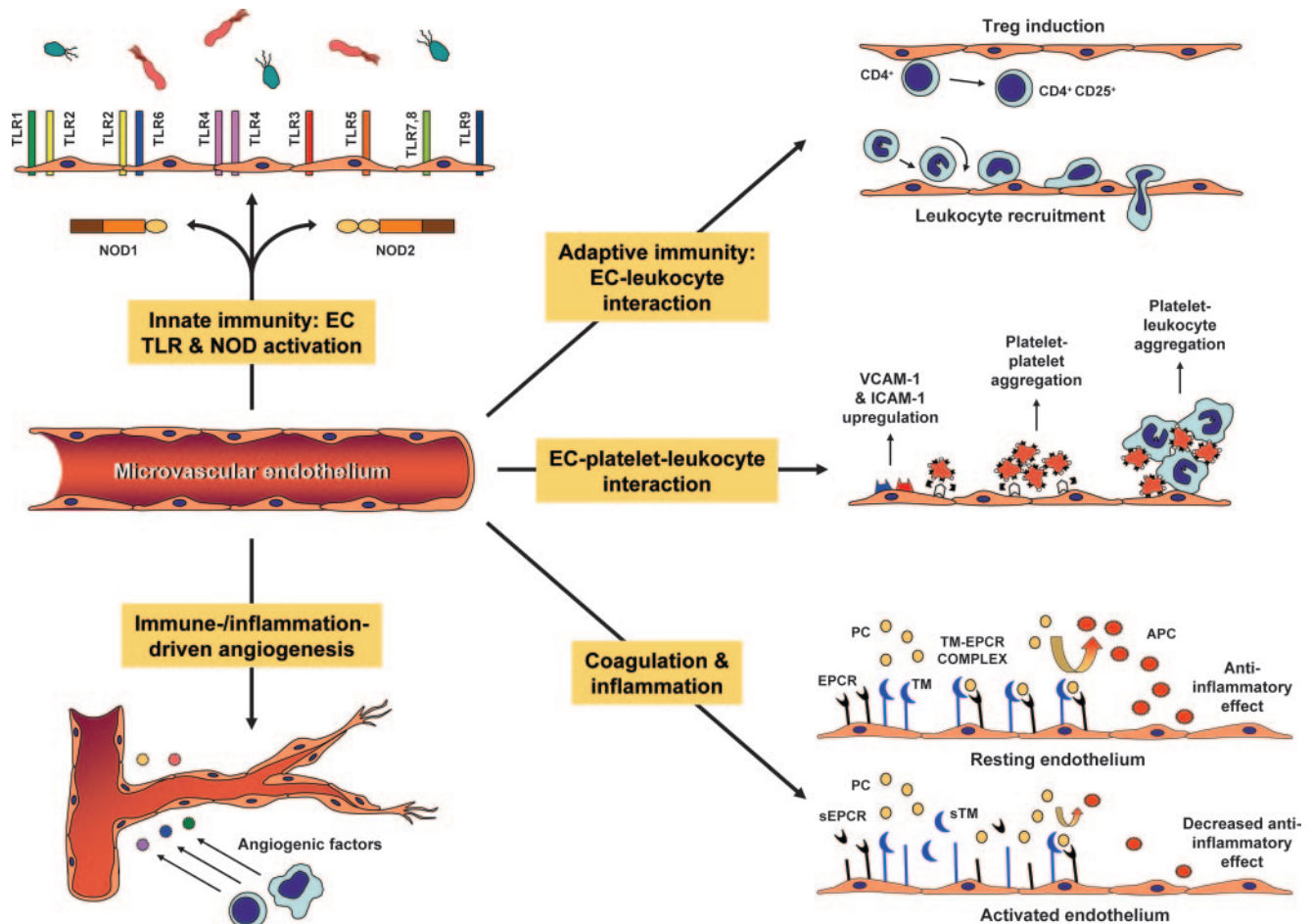
FIGURE 1. Schematic overview of the various functions and cellular interactions mediated by microvascular ECs that impact on innate and adaptive immunity, coagulation, and inflammation. PC, Protein C; APC, activated protein C; sEPCR, soluble EPCR; sTM, soluble TM.P

EC expression of TLR1 is still in question. One report showed TLR1 immunoreactivity in atherosclerotic endothelium (21), while another failed to demonstrate TLR1 expression by human microvascular EC lines in vitro, even though TLR1 transfection inhibited TLR4-dependent signaling (22).

TLR2 has been identified on atherosclerotic endothelium, expressed by von Willebrand factor-positive ECs and markedly up-regulated in vascular inflammation (21). Microvascular EC lines express low levels of TLR2 mRNA and protein, which are up-regulated upon stimulation with LPS, TNF-α, and IFN-γ in a NF-κB- and MyD88-dependent manner (23–25). Neutrophil NADPH oxidase is involved in EC TLR2 up-regulation, as neutropenic mice show decreased endothelial TLR2 expression (25). This indicates a "cross-talk" between polymorphonuclear neutrophils and ECs that would enhance vascular defenses by up-regulating TLR2. Dunzendorfer et al. (24) reported that human coronary ECs are hyporesponsive to TLR2-specific ligands. Given the current belief that TLRs are proatherogenic, flow suppression of TLR2 expression may be atheroprotective. Functional expression of TLR2 may not be universal because human dermal microvascular ECs fail to respond to TLR2 agonists such as *Mycobacterium tuberculosis* 19-kDa lipoprotein or phenol-soluble modulin unless transfected with TLR2 (26, 27).

TLR3 is spontaneously present on HUVECs, and ligation by poly(I:C) up-regulates its expression together with that of IFN-β, IL-28, IL-29, and STAT1 (28).

TLR4 expression has been demonstrated on various ECs and significantly increases under inflammatory conditions. TLR4 is expressed in coronary ECs (29) and is overexpressed and colocalizes with the p65 subunit of NF-κB in coronary atherosclerotic plaques, suggesting activation of TLR4 at this site (21). This possibility is supported by the demonstration that LPS activates NF-κB in dermal microvascular ECs and that LPS, IFN-γ, and TNF-α up-regulate TLR4 mRNA and protein (23). LPS stimulation of coronary ECs induces production of IL-6, IL-8, and MCP-1, transcription of IL-1β and TNF-α mRNA, as well as expression of ICAM-1, VCAM-1, and endothelial leukocyte adhesion molecule-1 (30). Neutrophil accumulation appears to depend on TLR4 expression by ECs rather than leukocytes as sequestration of neutrophils in the lung is deeply impaired in endothelial TLR4$^{-/-}$ mice (31). The latter observation contrasts with the significant decrease of leukocyte binding caused by LPS in human intestinal microvascular ECs, perhaps reflecting a tolerance of ECs to high levels of endotoxin to which they constantly exposed in the gut microenvironment (32). Finally, LPS can directly initiate angiogenesis through TNFR-associated factor 6-dependent signaling pathways (33).

Because epithelial bacterial translocation exposes subepithelial microvessels to bacterial products, Maaser et al. (34) studied the effect of the TLR5 ligand flagellin on HUVECs, human intestinal microvascular ECs, and dermal ECs. They found that all three ECs constitutively expressed high levels of TLR5

mRNA and protein, and *Salmonella*-infected intestinal epithelial cells induced ICAM-1 expression in cocultured ECs. The functional role of EC TLR5 was demonstrated by induction of leukocyte adhesion and transmigration by flagellin, pointing to a previously unrecognized role of endothelial TLR5 in innate immunity (34).

In the only report investigating the expression of TLR7 or TLR8 by ECs, neither TLR was found to be expressed in HUVECs (28). In contrast, TLR9 is spontaneously expressed by mouse and rat lung ECs, and exposure to CpG DNA induces an inflammatory response manifested by IL-8 and ICAM-1 induction through p38 MAPK- and NF-κB-mediated pathways (35).

Other receptors mediating innate immunity include the nucleotide-binding oligomerization domains (NODs) 1 and 2, two cytosolic proteins that function as sensors for microbial peptides and regulators of inflammation (36). Both NODs have been detected in ECs and are up-regulated in response to LPS and proinflammatory cytokines (Fig. 1). HUVEC invasion by *Listeria monocytogenes* induces IL-8 production, NF-κB activation, and p38 MAPK signaling in a NOD1-dependent fashion (37). Muramyl dipeptide enhances IL-6 release by ocular ECs spontaneously expressing NOD2 and induces NF-κB transcriptional activity in transfected HUVECs overexpressing wild-type NOD2 (38, 39).

Adaptive immunity

ECs cannot replace the regulatory and effector functions of T and B cells, but because ECs can express MHC I and II class molecules and process Ag, have the potential of acting as APC. This action has been documented in vitro, but whether it occurs in vivo is still debatable, although ECs from different species express various accessory molecules, including CD80, CD86, ICOS-L, programmed death ligand 1, programmed death ligand 2, LFA-3, CD40, and CD134L (40). Therefore, the impact of ECs on adaptive immunity may be exerted through their interaction with leukocytes and platelets. Leukocyte-endothelial interactions clearly influence T cell function and directly affect adaptive immunity; platelet-endothelial interactions and platelet-leukocyte-endothelial interactions affect T cell function indirectly, but they will be discussed here because of functional relatedness.

Endothelial-leukocyte interactions

The distribution of leukocytes is tightly regulated by numerous homing and adhesion molecules (receptor and counterreceptor pairs) on the surface of microvascular and immune cells (41). EC-mediated leukocyte distribution displays specialized features depending on the tissue where lymphoid cells are destined to reside (42). In inflammation, ECs still control the type and number of immune cells that extravasate into the interstitium but in a dysregulated fashion (5, 43). Multiple reviews are available on this subject, and the contribution of ECs to adaptive immunity through leukocyte distribution will not be discussed here. A different type of leukocyte-endothelial interaction relevant to adaptive immunity occurs in the induction of transplantation tolerance. Alloantigen-specific CD8$^+$CD28$^-$ T suppressor cells induce expression of inhibitory receptors and down-regulate adhesion molecules on ECs, rendering them tolerogenic (44). In addition, alloantigen presentation by EC to CD4$^+$ T cells induces CD4$^+$CD25$^+$Foxp3$^+$ regulatory T cells

capable of suppressing proliferation of alloreactive T cells in vitro and in vivo (45) (Fig. 1).

Endothelial-platelet interactions

Platelets normally circulate without attaching to the endothelium, but do so when ECs become activated, and platelet adherence triggers inflammation (46) (Fig. 1). The molecular pairs allowing adhesion of platelets to endothelium include P-selectin glycoprotein ligand 1/P-selectin, GPIbα/von Willebrand factor, GPIbα/P-selectin, and GPIIb/IIIa/fibrinogen/ICAM-1, respectively (47). Recently, EC-derived fractalkine has also been show to contribute to platelet activation and adhesion (48). Activated platelets produce massive amounts of proinflammatory mediators and cross-talk with and activate different cells; in turn, platelets are activated by EC-derived proinflammatory substances binding to cognate receptors on the platelets' surface (49, 50). Platelets' mediators are kept in the α-granules and dense body systems (51) and are promptly released upon activation, including histamine, serotonin, thromboxane A$_2$, platelet-activating factor, PGE$_2$ and PGD$_2$, TGF-β, platelet-derived growth factor, multiple chemokines (RANTES, epithelia-derived neutrophil-activating 78, MCP-3, growth-related oncogene α, and MIP-1α), IL-1β, and thrombocidins, all of which target immune cells (46, 52). Some of these products control vascular tone and permeability, but platelets also release trophic factors for ECs like vascular endothelial growth factor (VEGF), which promotes angiogenesis (53). In addition, platelets release heparanase, causing degradation of extracellular matrix and facilitating leukocyte extravasation (54). In addition to molecules that alter EC function, platelets produce molecules that directly impact on adaptive immunity, like membrane-bound and soluble CD40L, which engages CD40 on the surface of ECs, leading to adhesion molecule up-regulation, chemokine secretion, and leukocyte recruitment (55). In this regard, activated platelets mimic the action of activated T cells, which express and release CD40L (56). In doing so, platelet modulate the immune response by establishing a link between innate and adaptive immunity (57). Finally, CD40 ligation by platelet CD40L not only promotes immune activation and inflammation but also tissue factor induction and blood coagulation (58).

Endothelial-leukocyte-platelet interactions

Inflamed microvessels can recruit leukocytes through a platelet-dependent mechanism, but at the same time, platelet recruitment is leukocyte dependent (Fig. 1). As an example of the first process, when neutrophils are perfused on an endothelial monolayer to which thrombin-activated platelets have adhered, platelets form a bridge with the endothelium and promote leukocyte adhesion (59). As an example of the second process, using an in vivo postcapillary venule system to study leukocyte dependence of platelet adhesion in ischemia-reperfusion, anti-leukocyte strategies resulted in significantly reduced platelet recruitment (60). The molecular determinants orchestrating leukocyte-dependent platelet adhesion are being elucidated. P-selectin plays a critical role in mediating platelet adhesion to endothelium, primarily through EC- rather than platelet-expressed P-selectin (61). Leukocyte-dependent platelet adhesion involves the participation of P-selectin on both platelet and ECs, as well as CD18-ICAM-1 interaction. Once adhered, platelets create a platform onto which a leukocyte can roll and

adhere firmly through leukocyte-expressed P-selectin glycoprotein ligand 1 and platelet-expressed P-selectin (47). P-selectin is also involved in the delivery of lymphocytes to high endothelial venules even in the absence of functional L-selectin (62).

In addition to adhesion molecules, other mechanisms that mediate EC-leukocyte-platelet interactions depend on chemokines or the CD40/CD40L pathway. Various experimental systems show that release of platelet-stored chemokines that adhere to ECs allows binding and retention of monocytes or lymphocytes (63, 64). In addition to mediating EC-platelet and EC-T cell binding, platelet- and T cell-associated CD40L up-regulates the density of CD40 expression on vascular EC in vivo (65), with important immunomodulatory and proinflammatory implications.

ECs in coagulation and inflammation

In addition to an anticoagulant state, a healthy endothelium also provides anti-inflammatory defenses. This dual action is mediated by the natural anticoagulant protein C (PC) pathway, which is composed of thrombomodulin (TM) and the endothelial protein C receptor (EPCR), both abundantly expressed on the EC surface, and PC, produced in the liver but circulating systemically (66, 67). The capture of PC by the TM/EPCR complex generates activated PC, a potent anti-inflammatory molecule (68, 69), but each component also exerts anti-inflammatory actions individually (Fig. 1).

TM directly inhibits leukocyte adhesion to activated endothelium and, by sequestering proinflammatory high-mobility group-B1 proteins, prevents EC activation (70). EPCR precludes leukocyte influx by blocking the integrin CD11b/CD18, as its deficiency induces neutrophilic infiltration and enhanced chemokine production in LPS-challenged mice compared with wild-type animals (71). In experimental endotoxin-induced inflammation, activated PC inhibits pulmonary vascular injury by inhibiting TNF-α release (72) and limits accumulation of activated leukocytes (73). In human EC-based in vitro systems, activated PC modulates expression of genes related to anti-inflammatory and cell survival pathways, including inhibition of NF-κB binding to target sites and multiple NF-κB-regulated genes, such as cytokines, chemokines, and adhesion molecules (74, 75).

Inflammation counteracts the protective effects of the PC pathway. In various animal and human conditions, such as sepsis, airway inflammation, Wegener's granulomatosis, and atherosclerosis, formation of active TM/EPCR/PC complexes is impaired because TM and EPCR are released in a soluble form (Fig. 1), resulting in loss of anti-inflammatory activity (67). Moreover, inflammation induces the EC up-regulation of tissue factor, a proinflammatory molecule involved in hemostasis, thrombosis, and vascular development (76).

Another link between coagulation and inflammation is mediated by protease-activated receptors (PARs). Coagulation leads to the activation of several proteases, including factors Xa, VIIa, and IIa (thrombin), which exert deleterious or protective effects through the selective activation of PAR$_{1-4}$ (77). PARs have been shown to play a role in innate immunity in various models of airway, gastrointestinal, and joint inflammation, as well as sepsis (77). In addition, PARs regulate vascular tone and permeability, EC proliferation, and angiogenesis.

Immune-driven angiogenesis

Another link of ECs to immunity is the process of angiogenesis, a vital component of both acute and chronic inflammation and integral to most immune-mediated conditions (78). Angiogenesis is the growth of new blood vessels from pre-existing ones, whereas vascular remodeling involves structural modifications without neovessel formation. As inflammation evolves, vessels expand to supply nutrients sustaining the accumulation of activated immune cells in the affected tissues and in the chronic phase local immune cells overproduce EC growth factors (79). The contribution of ECs to inflammation is biphasic: first, functional changes prevail that include dilation, increased permeability, activation, and diapedesis; then, structural changes occur with capillary and venule remodeling (80). In chronic inflammatory disorders, infiltration by macrophages and lymphocytes ensues, tissue damage and repair occur concurrently, and the newly formed vessels become permanent (6, 81). The anatomical expansion and increased activation of the remodeled microvascular bed foster further influx of immune cells, and angiogenesis and inflammation become codependent processes (82).

ECs at sites of immune reactivity display multiple abnormalities, ranging from altered expression of surface molecules to barrier function. The hallmark of a proliferating vessel is the expression of integrins, particularly $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (83), which are essential for pathological angiogenesis (84). Angiogenic vessels up-regulate receptors for angiogenic factors produced by local immune and nonimmune cells, such as basic fibroblast factor, TGF-β, and TNF-α, but growth factors, cytokines, adhesion molecules, matrix metalloproteinases, and extracellular matrix components also contribute to EC activation and growth (78) (Fig. 1).

Both innate and adaptive immune responses promote angiogenesis. Tissue macrophages foster vessel growth, remodeling, or regression (6, 81). Their proangiogenic activity is mediated by various TLRs acting in synergy with adenosine A$_{2A}$ receptors that up-regulate VEGF production (85), but some TLR ligands, like LPS, directly stimulate ECs sprouting in vitro (33). Microbicidal peptides involved in innate immunity also display proangiogenic activity, such as angiogenin 4 and the cathelicidin LL-37/hCAP-18 (86, 87). In regard to adaptive immunity, activated T cells secrete and respond to VEGF (88), and B cells contribute to lung angiogenesis in mice with chronic *Mycoplasma* infection (89). IL-17 is a mediator of EC migration and inducer of proangiogenic factors (90), indicating that the IL-23/IL-17 pathway may be involved in immune-driven angiogenesis (91).

EC-mediated immune dysfunction and disease

Considering their wide-ranging activities, ECs play a vital role in multiple immune-mediated disorders. In rheumatoid arthritis, synovial ECs are activated and display increased leakiness, apoptosis, and angiogenesis (92), changes that contribute to leukocyte recruitment, edema, pannus formation, and joint destruction (93). Similar events occur in psoriatic skin, where ECs actively display adhesion molecule for leukocyte recruitment, form new vessels, and mediate inflammation (94). In multiple sclerosis, transendothelial migration of activated leukocytes is

one of the earliest abnormalities, and exposure of the endothelium to immune cell-derived IFN-γ, TNF-α, and IL-1β disrupts the blood-brain barrier by disorganizing cell-cell junctions, enhancing leukocyte endothelial adhesion and migration, and increasing expression of MHC class II Ags (95). The endothelium promotes gut inflammation through comparable mechanisms. Compared with normal mucosa, microvessels in chronically inflamed mucosa show major functional alterations in inflammatory bowel disease: the microvasculature is greatly expanded, displays an abnormal architecture, adheres more leukocytes, intimately interacts with platelets, is procoagulant and angiogenic, and has an impaired NO-mediated relaxation response, all features reflecting an active input of mucosal ECs in disease pathogenesis (96). Similar endothelial dysfunctions are found in other immune/inflammatory disorders, such as diabetes, atherosclerosis, and chronic lung disease.

Therapeutic implications

The evidence presented in the preceding paragraphs leaves no doubt that ECs actually dictate whether immune cells will work within the limits of homeostasis or go overboard into autoimmunity and inflammation. Given this influential role, it seems logical to look at ECs as therapeutic targets when aiming at reestablishing normal immunity and resolve inflammation. Because of EC heterogeneous functions, therapeutic strategies are equally plentiful and diverse, ranging from blockade of leukocyte adhesion and activation to antithrombotic and antiangiogenic approaches, and effectiveness has been shown in a variety of human and experimental systems (97). Thus, it is conceivable that a full and permanent recuperation of immune homeostasis, particularly in chronic conditions, can only be achieved by a dual approach that aims not only at direct immunosuppression but also the concomitant inhibition of EC activation and proliferation.

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Disclosures

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