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REVIEW

H. Ait-Oufella
E. Maury
S. Lehoux
B. Guidet
G. Offenstadt

The endothelium: physiological functions and role in microcirculatory failure during severe sepsis

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H. Ait-Oufella
Inserm U970, PAris Research Cardiovascular Center (PARCC), Paris, France

H. Ait-Oufella · E. Maury · B. Guidet · G. Offenstadt
Service de Réanimation Médicale, Hôpital Saint-Antoine, AP-HP, Paris 75012, France

E. Maury · B. Guidet · G. Offenstadt
Université Pierre et Marie Curie-Paris 6, UMR S707, 75012 Paris, France

E. Maury · B. Guidet · G. Offenstadt
Inserm U707, 75012 Paris, France

S. Lehoux
Lady Davis Institute for Medical Research, McGill University, Montreal, Canada

H. Ait-Oufella (✉)
Service de Réanimation Médicale, Hôpital Saint-Antoine, 184 rue du Faubourg Saint-Antoine, 75571 Paris Cedex 12, France
e-mail: hafid.aitoufella@sat.aphp.fr
Tel.: +33-1-49282315

Abstract The endothelium is a highly dynamic cell layer that is involved in a multitude of physiological functions, including the control of vascular tone, the movement of cells and nutrients, the maintenance of blood fluidity and the growth of new vessels. During severe sepsis, the endothelium becomes proadhesive, procoagulant, antifibrinolytic and is characterized by alterations of vasomotor regulation. Most of these functions have been discovered using in vitro and animal models, but in vivo exploration of endothelium in patients remains difficult. New tools to analyze endothelial dysfunction at bedside have to be developed.

Keywords Endothelium · Sepsis · Coagulation · Cytokines

Introduction

The endothelium consists of a single cell layer lining all vessels, from their origin at the exit of the heart to their finest ramifications, the capillaries. In spite of being both widespread and functionally complex, the importance of this structure was long disregarded. Originally described by Harvey in the seventeenth century and further characterized by Malpighi, the cardiovascular system was considered as a simple pump and tubing device designed to convey oxygen and nutriments to peripheral organs. It does not help that the clinical setting had no telltale signs by which to identify endothelial health or disease. Nowhere in the classical triad of "palpation-auscultation-percussion" would endothelial state appear, no jaundice to give away hepatic distress or crackles pointing to left ventricular insufficiency. And when tools that could be used to explore endothelial functionality finally appeared, they were mainly confined to the research world and failed to greatly impact clinical practice. In spite of all these difficulties, the endothelium has grown in regard and appreciation, on its way to being recognized as an individual organ. As of today, the endothelium figures as a main topic in more than 80,000 publications cited in PubMed.

In this review, we will describe the main functions of the endothelium in an attempt to demonstrate its importance and complexity. We will describe both its physiological properties (see Table 1) and its anomalies in pathological states. We will give special notice to

Table 1 Examples of endothelial marker distribution according to EC location

Markers	Vascular territories
Von Willebrand factor	Veins > arteries, absent in hepatic sinusoids
t-PA	Abundant in the brain and lung circulation, but not in the pulmonary circulation
TFPI	Microvascular endothelium
Protein C receptor	Vessels of large diameter
Thrombomodulin	Absent in the brain
Endothelial NOS	Arteries > veins
VCAM-1	Abundant in the heart, present in the brain and the small intestine
P-selectin	Abundant in the lung, low expression in the muscle and the brain
CD36	Low expression in the brain

sepsis, a condition where endothelial distress is extreme, and a daily preoccupation in the intensive care units.

Histological description

The endothelium is composed of a single layer of cells that lines the interior surface of all blood vessels. It is estimated to comprise around 10^{13} cells, representing a weight of 1.5 kg and covering 4,000–7,000 m^2, equivalent to six football fields [1, 2].

Endothelial cells (EC) lie at the interface of the circulating blood and the vessel wall. They are flattened cells, having a thickness of about 0.5 μm, and are 100 μm long by 10 μm wide. Their lozenge shapes are juxtaposed in a mosaic such that their long axis is oriented in the direction of blood flow. They are attached to a basal membrane rich in collagen and glycoproteins, forming a complex interface between the circulation and the platelet-activating, pro-coagulant vascular matrix.

EC appositions can be quite variable from one vascular bed to the next, ranging from juxtaposition to overlapping, allowing for an intricate regulation of protein permeability across the endothelium. At one or many points along the intercellular space, neighboring membranes form tight junctions or gap junctions. The former is multifocal membrane fusions that are perfectly tight, whereas the latter consist of a joining of two close cellular membranes around a central channel that allows the exchange of ions, various metabolites and regulatory factors.

At their surface, ECs are lined with a very fine and fragile layer called the glycocalyx. First identified in the 1960s thanks to electronic microscopy [3], it consists of glycoproteins, glycosaminoglycans and proteoglycans [4]. There are five types of glycosaminoglycans including heparan sulfate, a cofactor for antithrombin III that amplifies its anti-thrombotic properties, and dermatan sulfate, which interacts with heparin cofactor II [5]. Globally, the glycocalyx provides an anticoagulant layer because of its negative electrical charge that repels circulating platelets and that allows it to interact with and catalyze the interactions of vitamin K-dependent coagulation factors.

In forming a dense and intermeshed network, the glycocalyx provides a first-line barrier, regulating the cellular and macromolecular traffic at the forefront of the endothelium [6]. Numerous animal studies have demonstrated that destruction of the glycocalyx, using enzymatic approaches, for example, leads to increased capillary permeability [7]. In vivo, degradation of the glycocalyx with heparinase favors tight contacts between circulating leukocytes and the endothelium, through denudation of the protective barrier that normally prevents this [8].

Electronic microscopy recently revealed two characteristic elements of endothelial cells: pinocytic vesicles and Weibel-Palade bodies. Pinocytic vesicles consist of transcellular transport vesicles that shuttle from the apical to the basolateral aspect of endothelial cells. Weibel-Palade bodies are protein-stocking vesicles that appear as plentiful dense bodies, typically containing von Willebrand factor and P selectin.

Endothelial plasticity

At the interface between a liquid phase, blood plasma, and a solid structure, basal membrane, endothelial cells are highly influenced by their environment. They can rapidly modulate their structure and function in response to chemical or physical stimuli, such as a change in blood flow. For example, ECs cultured in vitro have a characteristic cobblestone morphology. However, if cells are placed in a perfusion chamber and exposed to a shear stress that mimics the frictional force of blood flow, they quickly align in the direction of flow, owing to a rearrangement in cytoskeletal proteins [9]. The same elongated morphology is observed in vivo in ECs lining arteries, where shear stress is 30–50 times greater than in veins. In fact, this effect is manifest in coronary bypass grafts in humans. The ECs from saphenous veins, once placed in the arterial context, quickly elongate in the direction of the long axis, parallel to blood flow [10]. Numerous receptors responsible for this response to shear stress, termed mechanosensors, have been identified: tyrosine kinase receptors, integrins, ion channels, G protein-coupled receptors and NADPH oxidases [11].

At the abluminal aspect, the endothelium is affixed to and interacts with the extracellular matrix constituents of the basal membrane, such that the nature of this matrix influences the form and the function of ECs [12]. Jalali et al. [13] showed that ECs grown on fibronectin- or vitronectin-coated surfaces respond differently to shear

stress; surface expression of integrins differed both in terms of quantity and distribution from one matrix to the next. ECs adapt very quickly to changes in their environment. In humans, it was shown by RT-PCR of postoperative amygdala that the genetic programming of ECs removed from their native environment changes within a few hours [14]. This should be heeded as a cautionary tale when considering the interpretation of studies using endothelial cells cultured ex vivo or in vitro.

Physiological functions of the endothelium

Primary hemostasis comprises a series of events that occur as a result of a vascular lesion and terminate with the formation of a stable platelet-rich blood clot, paving the way for tissue repair mechanisms that will be followed by fibrinolysis [15]. Endothelial cells play a role in each step of this process since they participate in platelet activation and produce factors involved in the coagulation cascade and in the fibrinolytic system.

When a vascular lesion occurs, an immediate vascular constriction occurs, which reduces blood flow locally and concentrates the repair mechanisms. This is quickly followed by platelet adhesion to the vascular wall [16]. The endothelium, focally destroyed, leaves exposed in its place to the underlying matrix rich in collagen and von Willebrand factor, to which the platelets attach through membrane glycoproteins. The glycoproteins GPIa and VI quickly and reversibly interact with collagen, whereas GPIb-IX-V binds von Willebrand factor. In the veins, where shear stress is much lower, platelets can adhere more readily to damaged endothelium via $\alpha 2\beta 3$ integrins [15]. Adherent platelets become activated, shifting from an oblong morphology to an irregular shape with multiple pseudopods, expressing adhesion receptors at their surface (P-selectin and integrins) and shedding their granulocyte contents [adenosine diphosphate (ADP), serotonin] into the extracellular space. These combined effects result in the attraction and activation of circulating platelets, conglomerating into a dense mass reinforced by a fibrin network. But ECs also oppose primary hemostasis by liberating vasodilator substances [prostacyclin, nitric oxide (NO)] and ADPases that catabolize ADP, one of the most potent platelet activators.

Any deficit in molecules involved in platelet adhesion, such as Bernard-Soulier's disease (GPIb-IX-V deficit/dysfunction) or Glanzman's thrombasthenia ($\alpha 2\beta 3$ deficit/dysfunction), is characterized by facilitated bleeding episodes, either spontaneous or elicited by minor traumas.

Anticoagulant properties

The anticoagulant activity of the endothelium is geared at restricting the generation of thrombin. Several factors are implicated in this process. Heparan sulfate and dermatan sulfate, two glycocalyx glycosaminoglycans, potentiate the activity of two anticoagulant enzymes, antithrombin III (by a factor of 100) [17, 18] and heparin cofactor II, respectively [19]. The endothelium produces tissue factor pathway inhibitor (TFPI), which binds to activated factor X and then inhibits the tissue factor-activated factor VII complex [20]. ECs produce thrombomodulin (TM) that is either attached to the membrane or released into the circulation. Its plasma levels are increased in a number of pathologies where the endothelium is injured (see sepsis). TM binds to the surface of protein C and increases its anticoagulant activity by associating with its specific cofactor, protein S [21]. Finally, the endothelium accelerates activation of protein C by expressing on its surface another receptor, endothelial protein C receptor (EPCR) [22]. Protein C, when activated by all its cofactors, inhibits factors V and VIII. Finally, TM has its own intrinsic anticoagulant activity since several works have demonstrated its ability to bind and directly inhibit activated factor X [23].

Procoagulant properties

The major step in the acquisition by the endothelium of a procoagulant phenotype involves the expression of factor tissue (TF). In vitro, TF is induced by several mediators such as thrombin, endotoxin, cytokines, shear stress, hypoxia or oxidized lipids [24, 25]. Its procoagulant activity is accentuated in the presence of anionic phospholipids that are exposed by apoptotic cells [26, 27]. In the presence of agonists, the levels of TF protein and messenger RNA decline rapidly, presumably to avoid too rapid fibrin extension. Despite these in vitro findings, it remains difficult to show an actual endothelial expression of TF.

Once expressed, TF binds and activates factor VII. The TF-VIIa complex in turn mainly activates factors IX and X. These factors are anchored by their gammacarboxic residues to the membrane phospholipids of platelets and endothelial cells. This latter precision serves to remind us that reactions of the coagulation cascade do not take place in the liquid plasma phase, but rather in the solid phase, usually on cellular membranes or developing clots. Given its position and surface area, the endothelium is the main surface on which the coagulation reaction takes place.

The contact between coagulation factors and the vascular wall occurs either via non-specific physical interactions or through specific receptors synthesized by ECs. Thrombin binds a surface receptor called protease-activating factor (PAR-1) and influences the formation of fibrin and amplifies the coagulation cascade. The PAR-1 receptor is itself activated and induces the expression of different genes (TF, NO, endothelin) [28], illustrating the complex entanglement of the multiple functions of EC,

namely coagulation, regulation of vasomotor tone and leukocyte adhesion. Other thrombin receptors such as PAR-2 [29] and PAR-3 [30], which are expressed by ECs and other cell types (platelets, bone marrow precursors), have been described.

Role in fibrinolysis

The endothelium also has physiological profibrinolytic effects. Tissue plasminogen activator (t-PA), the main activator of intravascular fibrinolysis, is a protease released by ECs that transforms plasminogen into plasmin. This reaction occurs either on the endothelial cell surface or on a platelet clot. The plasmin degrades fibrin and releases degradation products of fibrin or D-dimers into the circulation. In vitro, fibrinolytic properties have been documented in different types of ECs, and the ability to express t-PA was therefore ascribed to the endothelium as a whole. However, the in vivo situation is less straightforward. Immunohistochemistry and in situ hybridization studies have detected t-PA solely in the microcirculation, and only in certain territories [31]. Another plasminogen activator, urinary type plasminogen activator (u-PA), was located exclusively at the renal level [32]; it is expressed only in tissue repair processes and during angiogenesis, suggesting that it plays an important role in cell migration and tissue remodeling. Nonetheless, u-PA has a role in vascular homeostasis and the fight against infections since u-PA knockout mice show an exaggerated response to lipopolysaccharide (LPS) injection, characterized by widespread parietal inflammation [33] and extended thrombosis [34].

Two inhibitors control the fibrinolytic system: $\alpha 2$ antiplasmin (a plasmin inhibitor) and plasminogen activation inhibitor (PAI-1, PAI-2 and PAI-3). PAI-1, produced by the liver and activated endothelium, is the most studied inhibitor, and it seems to have the greatest affinity for plasminogen activator. PAI-1 also plays a major role in inflammation through several mechanisms, including induction of lymphocyte and neutrophil recruitment, activation of neutrophils (through Toll-like receptor 4) and clearance of apoptotic cells [35].

Regulation of vasomotor tone

The endothelium is a significant contributor to the regulation of vasomotor tone, under the influence of physical and chemical factors originating from the vascular lumen or the surrounding tissues. ECs produce and release vasodilator substances such as NO and prostacyclin, and vasoconstrictor mediators such as endothelin and platelet-activating factor (PAF). The production of NO by the endothelium is constitutive and modulated by different stimuli, whereas the synthesis of other mediators (prostacyclin, endothelin and PAF) is inducible.

NO is the most important vasodilator arising from the endothelium [36]. It is produced from L-arginine by the constitutive endothelial NO synthase (eNOS), which is activated by increased intracellular calcium and phosphorylation [37]. Agents capable of stimulating eNOS through specific receptors include ADP, bradykinin, substance P and muscarinic agonists, as well as physical forces (shear stress and pulsatile tensile strain). For example, the rise in blood flow during physical exercise increases shear stress, activating eNOS, which allows for vasodilatation [38]. There are two other forms of NOS: the calcium-dependent neuronal NOS (nNOS) and the calcium-independent inducible NOS (iNOS), which is activated mainly by pro-inflammatory cytokines. Regarding endothelium-dependent vasodilatation, tribute should be made to the discovery by Furchgott and Zawadzki, published in 1980 in *Nature*. The authors found that in precontracted vessels, acetylcholine was a vasodilator in the presence of endothelium, whereas after endothelial denudation acetylcholine had no effect. The end point of this process was the production of NO in the endothelium, which diffuses in the media and activates soluble guanylate cyclase, responsible for production of cyclic GMP in smooth muscle cells (SMC), which in turn activates protein kinase G. The latter enzyme reduces the stock of free cytosolic calcium and inhibits the contraction of SMCs by myosin dephosphorylation [39]. Finally, NO has other functions. It inhibits the activation and adherence of platelets to the endothelium, and it promotes disaggregation platelets during the formation of the hemostatic plug [40].

Endothelin (ET) is produced by the endothelium uniquely in response to stimuli such as hypoxia or shear stress [41]. As is the case for NO, ET diffuses in depth and binds to its specific receptor present at the surface of SMCs. This G protein-coupled receptor promotes the release of calcium from the endoplasmic reticulum and induces vasoconstriction. This effect persists long after the dissociation of ET from its receptor by maintenance of a high intracytoplasmic calcium concentration.

Prostacyclin is an eicosanoid derived from arachidonic acid that acts as a paracrine factor. It causes vasodilatation and inhibits platelet aggregation.

Platelet-activating factor (PAF) is a phospholipid that is also derived from the metabolism of arachidonic acid. It causes vasoconstriction and stimulates the adhesion of leukocytes to the endothelium [42, 43].

Leukocyte interactions

When there is a need to quickly eliminate a pathogen or to clean a necrotic area, ECs express adhesion molecules

that regulate leukocyte trafficking between the circulating blood and the surrounding tissue. The interaction between leukocytes and ECs follows a conventional set of steps that include short contacts, more prolonged contacts, then leukocyte rolling and strong adhesion before transendothelial migration. Different adhesion molecules intervene at each stage of this process. The selectins (E-and L-selectin) mediate the first phase, as they resist the forces of shear stress and bind (and release) very quickly [44]. The integrins $\alpha 4\beta 7$ and $\alpha 4\beta 1$ are involved in the adhesion of monocytes and polynuclear neutrophils. The subsequent rolling and firm adhesion involve the molecules of the superfamily of immunoglobulins [intercellular adhesion molecule (ICAM-1, ICAM-2) and vascular adhesion molecule (VCAM)] [45, 46], joined by platelet endothelial cell adhesion molecule (PECAM) at the time of diapedesis [47]. This latter process is a complex phenomenon involving the separation of cadherins at tight junctions [48].

In addition to its participation in the innate immune response, the endothelium participates in the adaptive response involving T lymphocytes [49]. ECs in culture express major histocompatibility complex class I (MHC I) and can present antigen to CD8+ cytotoxic T lymphocytes. Furthermore, after stimulation by interferon gamma, they express MHC class II and can interact with CD4+ lymphocytes [50]. ECs can also express LFA-3 (CD58) or B7.2 (CD86) costimulation molecules at their surface, molecules common to other antigen-presenting cells (dendritic cells, macrophages, B lymphocytes) [51]. Moreover, it is now accepted that the ECs are involved in the pathophysiology of graft rejection [52].

Endothelial heterogeneity

All ECs share common properties, but also distinct features, that are expressed both during physiological processes and in adverse conditions. This complexity, called endothelial heterogeneity, is so important that we should use the plural form, endothelia (see Table 2). This concept was first brought up by histologists, who distinguished, for example, the continuous endothelium of the brain from the fenestrated endothelium of endocrine glands. The clinicians then showed that ECs were differentially affected by various pathologies; diabetes disturbs the renal and retinal microcirculation especially [53], whereas veno-occlusive liver disease targets vessels of the hepatic sinusoids [54], and thrombotic microangiopathies affect the entire microcirculation with the exception of liver and lungs [55]. That structural differences distinguish endothelial cells according to organ function was then confirmed experimentally. Electron microscopy highlighted differences in the location of the nucleus within the cell or the composition of cytoplasm depending on the site at which ECs are found. Accordingly, in the frog the intracellular volume occupied by Weibel-Palade bodies gradually diminishes from the aortic root to the microcirculation (8% of the cytoplasm in the thoracic aorta versus 0.3% in the capillaries) [56].

Table 2 Endothelial cell implication in various physiological processes through secretion or expression of different molecules

Hemostasis	
Primary hemostasis	
Activation	Von Willebrand factor
Inhibition	Nitric oxide
Coagulation cascade	
Activation	Tissue factor
Inhibition	Tissue factor pathway inhibitor, endothelial protein C receptor, thrombomodulin, heparan/dermatan sulfate
Fibrinolysis	
Activation	Tissue plasminogen activator, urinary plasminogen activator
Inhibition	Plasminogen activator inhibitor
Vasomotor tone regulation	
Dilation	Nitric oxide, prostacyclin
Constriction	Endothelin, thomboxane A_2, platelet-activating factor
Inflammation	
Adhesion	E-selectin, VCAM, ICAM
Co-stimulation	LFA-3, CD80/86
Chemokines	MIP-1 (and -2), MCP-1
Cytokines	IL-1, IL-6, IL-8, TNFα

This heterogeneity exists for all endothelial functions, and to ignore it exposes us to errors of interpretation. For example, a decrease in the endothelial expression of TM was shown in biopsies of purpuric lesions in children with meningococcal disease [57]. In the brain, ECs do not express TM [58]. Does this mean that the cerebral endothelium was in a state of continuous activation? Of course not. The most likely explanation is that TM is not involved in the hemostatic balance of the brain.

EC heterogeneity also concerns the intensity of the endothelial response to stimuli and endothelial response cannot be likened to a simple on/off switch but rather to a dimmer switch where, for a given gene activation cascade, the expression of a protein can vary between two extremes [59]. What is most challenging is to assess this expression level and deduce the endothelial status: inactivated, activated or abnormally active (dysfunctional). Such distinctions are subtle, and unfortunately limited by the current lack of tools that allow for precise assessment of endothelial function.

Endothelial dysfunction during sepsis

The endothelium is actively involved in defending the body against pathogens by recruiting the leukocytes to

infected sites, releasing inflammatory mediators and promoting local coagulation to prevent the spread of blood-borne infection. However, this normally focused adaptative response can become generalized and amplified during sepsis, causing tissue damage. This is referred to as endothelial dysfunction.

Endothelial denudation

Sepsis is accompanied by morphological changes of the endothelium. The injection of LPS in animals induces a detachment of ECs from the basal membrane and causes sub-endothelial edema [60, 61]. Cell lesions include nuclear vacuolation, protrusion and cytoplasmic fragmentation. The time of onset varies from a few minutes, after LPS injection [62], to several hours, in the model of cecal ligation/perforation [63]. This phenomenon has also been observed in humans [64]. Using a double-tagged cell (Von Willebrand factor receptor and EGF), Mutunga et al. showed that the number of circulating ECs was higher in septic patients than in healthy subjects, and even higher in cases of septic shock. Inflammatory mediators released by leucocytes (TNF, IL1, interferon, oxygen free radicals) and hypoxia increase EC apoptosis [65–67]. Apoptotic ECs in turn express adhesion molecules (ICAM-1, VCAM) and release oxygen free radicals, and in doing so amplify the recruitment of white blood cells. Apoptotic ECs also externalize negatively charged phosphatidylserine on the cell membrane and thus expose the circulating blood to a procoagulant surface [26]. Membrane charge asymmetry coupled with the activity of certain enzymes (floppase, scramblase, etc.) allows for the budding and release of membrane microparticles in the circulation [68]. These microparticles, a combination of phospholipid membrane fragments and surface proteins (TF), are involved in spreading endothelial dysfunction [69] and participate in the disseminated intravascular coagulation [70].

Endothelial barrier impairment favors the passage of cells, inflammatory mediators and plasma into the interstitial compartment. The rapid increase in vascular permeability to albumin, which occurs within 6 h of the insult, affects the pulmonary and systemic circulation heterogeneously [71]. In terms of skin and muscles, the injection of endotoxin induces an increase in vascular permeability irrespective of oncotic and hydrostatic pressure, indicating abnormalities of the cell membrane [72].

Pro-adhesive properties

Increased expression of endothelial adhesion molecules either at the membrane level or in the plasma typify the different models of sepsis [73]. In vitro incubation of ECs with bacterial LPS induces the rapid expression of ICAM-1 and E-selectin mRNA. The same effect is obtained by replacing LPS with the plasma of healthy volunteers treated with very low doses of LPS. In this latter experiment, the addition of antibodies blocking TNFα and/or IL-1β at the same time as human plasma inhibits the transcription of mRNA, illustrating the fact that cytokines released by endotoxemia induce in turn the expression of E-selectin and ICAM-1 [74]. Moreover, there is a close relationship between plasma levels of adhesion molecules and consequences of sepsis [75]. In human sepsis, a study has shown that the higher the plasma levels of ICAM-1, the greater the number of organs damaged and the mortality. In animals, the genetic or pharmacological blockade of ICAM-1 protects against endotoxin shock [76].

As regards E-selectin, several human studies have also shown an increase in its soluble form in healthy volunteers receiving an injection of LPS [77] or in patients hospitalized for sepsis [78]. The level of E-selectin in the plasma is positively correlated with the severity of sepsis in patients, evaluated by the SAPSII score or the multiple organ failure (MOF) score [79]. Increased plasma adhesion molecules reflect an increase in the endothelial membrane expression of the proteins and can be directly related to activation and/or dysfunction of the endothelium. It promotes rolling and firm adherence of neutrophils, and ultimately results in their accumulation in organs. This accumulation is in part beneficial, eliminating infectious agents, but it may also exacerbate tissue damage through the production of inflammatory mediators such as cytokines, proteases and oxygen free radicals [80]. A recently published study illustrated this paradigm. Ye et al. [81] selectively inhibited NF-κB activity in the endothelium of adult mice. After LPS injection, these animals were characterized by a decrease in endothelial adhesion molecule expression (ICAM-1, E-selectin), a decrease of neutrophil infiltration in several organs (lung, liver, kidney) and a decrease in tissue damage. In fine, selective endothelial NF-κB inhibition improved survival in endotoxinemic mice. Platelets also contribute to the recruitment of neutrophils, illustrating the complex entanglement between inflammation and coagulation that regulates the pathophysiology of septic shock. Using a perfusion chamber model lined with ECs, where leukocytes circulate in a closed system, Blanks et al. [82] showed that the simultaneous infusion of platelets significantly increases EC-neutrophil interaction. They showed that the binding of platelet P-selectin to its receptor P-selectin glycoprotein ligand 1 (PSGL-1) on polymorphonuclear leukocytes induced the expression of VLA-4, another molecule responsible for adhesion to the endothelium. The relevance of this interaction in vivo has been documented in the cecal ligation and puncture model of sepsis in mice, where platelet depletion reduced leukocyte infiltration in the lung and lesion edema [83].

Pro-coagulant properties

Different studies undertaken in animals and humans have shown that severe sepsis is characterized by a pro-coagulant state [84]. The injection of LPS in healthy volunteers induces the rapid expression of TF by circulating monocytes [85]. Similar results were observed in patients with meningococcemia, with additive prognostic value since the cellular expression of TF was higher among patients who died [86]. The production of endothelial TF is more difficult to document. In infected animals, TF was located at the endothelium of tissue sections [87]. However, such direct evidence is scarce in humans. By analogy, one can cite the example of patients with sickle cell disease, in whom Solovey et al. [88] identified circulating endothelial cells expressing TF.

The TF expressed during sepsis activates coagulation and leads to the formation of thrombin (see Fig. 1). In parallel, counter-regulatory systems (involving the endothelium) such as protein C and TFPI are defective, maintaining intravascular coagulation. The decrease in activated plasma protein C (aPC) in sepsis is partly due to decreased hepatic synthesis of protein C and increased systemic consumption of the protein. Adding to this are complex enzymatic alterations that affect thrombomodulin (TM), whose primary function is to amplify the activation of protein C. In vitro, TNF-α and IL-1 decrease endothelial expression of TM. Moreover, the granulocytes recruited at the inflammatory site release elastases that cleave and inactivate endothelial TM [89, 90]. These experimental results have been corroborated by a study in children with purpura fulminans. From skin biopsies, Faust et al. [57] showed a decreased expression of endothelial TM and endothelial cell protein receptor. In the blood, the levels of protein C, protein S and antithrombin III were also reduced. Moreover, in another study on adults with sepsis, a low rate of aPC was identified as a factor of poor prognosis [91]. The accumulated evidence on the beneficial role of aPC in sepsis has led to serious therapeutic studies. After encouraging results in animals [92] a multicenter international study was conducted. The PROWESS study, published in 2001, showed

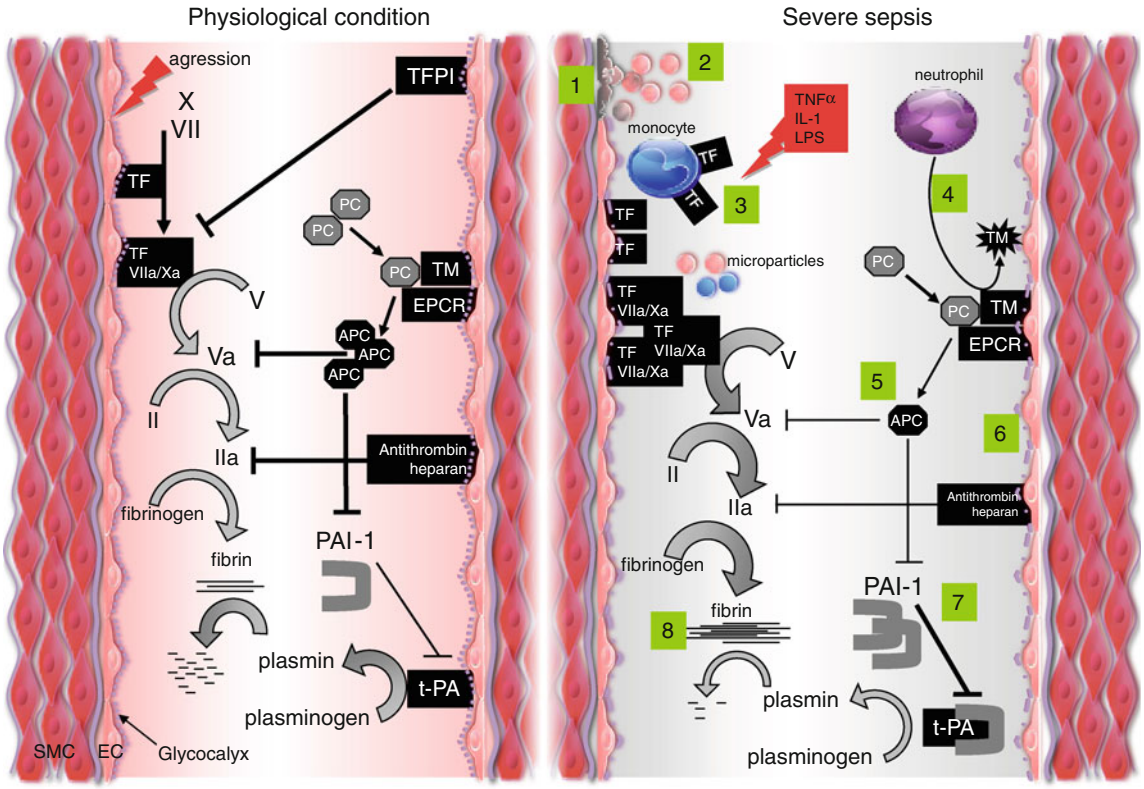

Fig. 1 Physiologically, after vascular aggression, endothelial cells express TF and initiate the coagulation cascade that leads to thrombin activation and fibrin deposition. At the same time, anticoagulant pathways and fibrinolysis are activated to avoid disseminated coagulation and limit fibrin accumulation. In severe sepsis, several disorders occur: endothelial cell apoptosis (*1*), microparticle shedding (*2*), procoagulant phenotype switch with TF expression (ECs, monocytes and microparticles) (*3*) and activation of the coagulation cascade. Alteration of anticoagulant pathways involve: TM degradation by neutrophil elastase (*4*), decreased APC levels (*5*), glycocalyx degradation (*6*), alteration of fibrinolytic pathways with increased PAI-1 activity (*7*) and consecutive fibrin accumulation (*8*)

in a cohort of 1,690 patients that administration of aPC in septic patients with at least two visceral failures reduced the relative mortality by 20% at 28 days [93]. Unfortunately, secondary studies have been disappointing. The ADDRESS study, which evaluated the role of aPC in septic patients with only one organ failure, provided no evidence of benefit in terms of survival and was stopped for "futility" [94]. The RESOLVE study in children with meningococcemia was also negative on composite criteria measuring the resolution of organ failure [95]. A number of limitations have been advanced to explain these negative results, and the controversy regarding the place of aPC in the treatment of severe sepsis remains. Patient selection provides a major point of argument because secondary analysis of the PROWESS trial showed that aPC improved survival in the more severe patients [96, 97]. It is important to note that aPC has other beneficial functions apart from its role in coagulation. In experimental models, aPC mediates anti-apoptotic effects, reduces endothelial barrier permeability and decreases pro-inflammatory cytokine production [98]. For all these reasons, the relationship between aPC and severe sepsis remains relevant.

TFPI is another powerful anticoagulant that binds to factor VIIa, TF and factor Xa. Several lines of evidence have highlighted its importance in the pathophysiology of organ failure during septic shock and especially in the intravascular coagulation that accompanies it. In rabbits, the depletion of TFPI increases intravascular coagulation [99]. Conversely, infusion of TFPI in a model of endotoxin shock in baboon decreases intravascular coagulation, reduces organ dysfunction and most importantly improves survival. TFPI also modulates the inflammatory response since it can bind directly to endotoxin and prevent its interaction with leukocyte CD14 [100]. Unfortunately, TFPI proved to be underwhelming in the clinical setting. The OPTIMIST study published in 2003, featuring critically ill patients with mild coagulopathy (international normalized ration, INR $>$ 1.2), did not reveal any reduction in mortality in the group receiving TFPI [101]. Nevertheless, TFPI had a net beneficial effect on intravascular coagulation, accompanied by an improvement in the markers of coagulation.

Anti-fibrinolytic properties

Regarding the fibrinolytic system, the first in vitro studies showed stimulation of ECs in culture with IL-1 and TNFα induced by the expression of t-PA and PAI-1 [102]. This orientation towards an anti-fibrinolytic phenotype has been difficult to confirm in humans. Some studies have shown increased levels of PAI-1 in the blood of septic patients (both adults and children) [103, 104], and PAI-1 levels are positively related to unfavorable outcomes [105]. Some gene polymorphisms of PAI-1 have been demonstrated to affect the risk of death during meningococcal infection [106]. The final result is heterogenous fibrin deposition, which exacerbates local ischemia [107]. For example, injection of LPS in mice induced deposition fibrin in the kidney, liver and heart, but not in the lung. In the baboon, the injection of lethal doses of *Escherichia coli* induced fibrin deposition in the spleen, hepatic sinusoids and renal glomeruli, but had little effect on the brain, heart and aorta.

Impaired regulation of vasomotor tone

In severe infectious states, emergency care is sought primarily to restore hemodynamic stability due to an imbalance in vasomotor tone and interstitial plasma leakage. Endothelial dysfunction and especially disturbances in NO production are the main causes of circulatory insufficiency.

Disrupted production of NO in the course of sepsis is complex and evolves over time. The first stage, called nitrosopenia, is characterized by decreased production of NO by eNOS [108]. The mechanisms underlying this effect are diverse, ranging from changes in surface receptors to altered signal transduction to quantitative or qualitative alterations in eNOS. In vitro, stimulation of ECs with TNFα or LPS induced a downregulation of eNOS mRNA expression [109, 110]. In rats, the induction of septic shock resulted in the loss of endothelial eNOS [111], whereas in rabbits, the injection of a non-lethal dose of LPS altered endothelium-dependent relaxation between 5 and 20 days [61]. In healthy volunteers, a brief exposure to endotoxin decreased endothelium-dependent relaxation for several days [112, 113]. During early nitrosopenia, the endothelial homeostatic balance therefore leans towards vasoconstriction, which affects the proximal arterioles of the first and second order of certain organs such as the digestive tract.

The second, later stage is characterized by an increase in the production of NO by iNOS. This inducible enzyme produces nanomolar concentrations of NO (1,000-fold of what is produced by eNOS), which causes diffuse microcirculatory vasodilatation and therefore a fall in blood pressure. Other effects of NO overproduction have been associated with the pathophysiology of septic shock, including the production of peroxynitrite, a potent oxidative agent that results from the interaction of NO with the superoxide anion.

The overproduction of NO in late sepsis inspired a number of studies aimed at assessing the therapeutic potential of NOS inhibition. However, results in animals have been contradictory, in part because different models of sepsis were used, coupled with diverse treatments and inhibitors [114–116]. In humans, the first attempts at blocking NOS appeared encouraging, with improved hemodynamic parameters (but no effect on mortality)

[117, 118]. Finally, a large international multi-center study was carried out in 2004. Seven hundred ninety-seven patients suffering from severe sepsis were treated with either a placebo or a non-selective blocker of NOS, L-NAME. The phase III trial was stopped prematurely because despite an improvement in circulatory parameters, administration of L-NAME was accompanied by excess mortality [119]. After the failure of this approach, several studies investigated the beneficial properties of NO. This led to the notion that it is not NO that is responsible for the circulatory failure in sepsis, but rather by its overproduction by iNOS. Therefore new research avenues today are targeting the activation of iNOS [109].

Finally, it is important to remember that advancements in the field of resuscitation are mainly based on experimental animal studies. Disappointing results obtained in the clinical trials could be partially due to significant physiopathological differences between species. At present, the tools available for the assessment of endothelial function in humans are limited and difficult to use in the bedside setting. Orthogonal polarized spectral (OPS) imaging in the sublingual area is an interesting technique that provides information about capillary density, the proportion of perfused capillaries [120] or glycocalyx size [121]. Topical application of pharmacological agents such as acetylcholine improves specific analysis of endothelial function. However, analysis of OPS data requires time and computer assistance and cannot yet help clinicians at the bedside.

Vascular oxidative stress

During severe sepsis, reactive oxygen species (ROS) such as superoxide (O_2^-) are produced in large amounts [122]. Neutrophils are the main source of ROS, but endothelial cells also synthesize ROS in response to oxidative agents or cytokines. For example, in vitro LPS/IFNγ-stimulated ECs produce superoxide within 2 h through the NADPH oxidase pathway. ROS accumulate in ECs and are modified; dismutation of superoxide forms hydrogen peroxide (H_2O_2) and nitration of superoxide forms peroxynitrite ($ONOO^-$). Both products are toxic for proteins and DNA, and could participate in endothelial cell damage [123]. ROS decrease NO availability through peroxynitrite formation and eNOS inhibition, and ultimatly affect vascular tone, platelet adhesion [124] and permeability [125]. These modifications lead to vascular occlusion and exacerbate organ hypoperfusion. Finally, to illustrate entanglement of complex phenomena in severe sepsis, experimental studies have shown that ROS could activate NF-κB, a major transcription factor for genes involved in inflammation [126], and could induce TF expression [127].

Beneficial effects of antioxidants in sepsis have been reported. In mice, vitamin C injections prevent LPS-induced edema and hypotension [128]. In a randomized, prospective, double-blind, placebo-controlled trial with 226 critically ill patients, 28-day survival was increased in the patients that received a combination of vitamin C and E. These data represent a new field of study and warrant further investigation [129].

Conclusion

After a long period in the dark, the endothelium is finally arousing interest among clinicians. Despite both its imposing dimensions and the importance of its functions, the endothelium remains difficult to grasp, as it exhibits a large degree of plasticity and heterogeneity. ECs play a role in primary hemostasis, coagulation, fibrinolysis and regulation of vasomotor tone. More recently, their role in adaptive immunity has also been demonstrated. During sepsis, most endothelial functions are disrupted, leading to a procoagulant, antifibrinolytic and proadhesive state. Production of NO decreases at first, but then it increases unduly because of the induction of iNOS. To the difficulties linked with the variability of endothelial cells, one can also add the practical complications confronted when attempting to assess them; we are not, at the bedside, properly equipped to explore endothelial function. Advances in understanding and treatment of diseases involving the endothelium will require the development of more refined tools.

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