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Endothelial Dysfunction in Sepsis

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Abstract: The endothelium takes part in the regulation of numerous physiological functions and lies at the interface of circulating blood and the vessel wall. Under physiological conditions, it is responsible for anticoagulant and anti-adhesive properties, and it regulates vasomotor tone and vascular homeostasis. Endothelial dysfunction has been associated with many pathophysiological processes, such as inflammation and oxidative and nitrosative stresses. Endothelial cells are precociously exposed to circulating signaling molecules and physical stresses, like in sepsis and septic shock. Septic shock is associated with hypotension and frequently with disseminated intravascular coagulation contributing to multiple organ failure and a high mortality rate. Impairment of endothelial function leads to phenotypic and physical changes of the endothelium, with deregulated release of potent vasodilators nitric oxide and prostacyclin, reduction of vascular reactivity to vasoconstrictors, associated with leukocytes' and platelets' aggregation, and increase in inducible nitric oxide synthase expression that can exert a negative feedback on endothelial nitric oxide synthase expression, with subsequent deregulation of nitric oxide signaling.

Endothelial dysfunction therefore plays a major role in the pathophysiology of septic shock and organ dysfunction, and has been suggested to be a predictor of mortality in sepsis. Thus, early detection of endothelial dysfunction could be of great interest to adapt treatment in initial stage of sepsis. Current therapeutics used in sepsis mostly aim at controlling inflammation, vascular function and coagulation. Fluid administration, vasopressors, vasodilators and recombinant human activated protein C are also part of the treatments with the ultimate goal to exert beneficial effects on organ function and survival.

Keywords: Endothelial dysfunction, inflammation, oxidative stress, sepsis.

INTRODUCTION

The endothelium could be considered as a key organ which can regulate numerous physiological processes and whose dysfunction plays a major role in the development of many diseases, like sepsis. This review will focus on the physiological role of the endothelium, followed by the endothelial activation and dysfunction in the pathophysiology of sepsis, and finally provide an overview of the different therapeutics that might contribute to restore a protective effect of the endothelium with subsequent improvement of survival.

I. ENDOTHELIUM STRUCTURE AND FUNCTION

The endothelium is composed of a monolayer of cells lining arterial and venous blood vessels and lymphatics, with an estimated total mass of about 1.5 kg and an approximate covering area of four tennis courts. Endothelial cells lie at the interface of circulating blood and the vessel wall, which is composed of several layers of vascular smooth muscle cells and the adventitia. Therefore, endothelial cells play a 'gate-keeping role' and take part in numerous important physiological functions [1]. Finally, the endothelium is characterized by an important heterogeneity in structure and function, which notably depends on the vascular bed considered.

1. Structure and Function of Endothelial Cells Vary According to Vascular Beds

Endothelial cells phenotype and functions may differ and adapt to the vessels type, the organ considered, the nature of underlying tissue's demands and the stresses the vessels will be exposed to. The vascular tree is indeed made up of different types of vessels, namely large arteries, arterioles, capillaries, venules and larger veins. The arterial system is subdivided in "conductance arteries", which are made of elastic and large vessels, and "resistance arteries" which are small or intermediate vessels, with a larger muscular component in the media, allowing them to regulate and preserve blood pressure in physiological and pathological situations. Finally, many exchanges take place in capillaries.

2. Role in the Regulation of Vascular Tone

The endothelium regulates blood pressure and blood flow by modulating the arterioles tone, *via* numerous physical and chemical factors originating from the vascular lumen and

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surrounding tissues. Thus, it has been demonstrated that in-vitro dilatation response of isolated arteries to acetylcholine is abolished when endothelial cells are removed or impaired [2]. Indeed, endothelial cells are able to secrete and/or release several vasoactive components, in response to various mechanical and chemical stimuli: shear stress, substances released from autonomic and sensory nerves (e.g. acetylcholine, norepinephrine, adenosine triphosphate ATP, substance P), circulating hormones (e.g. adiponectin, vasopressin, catecholamines, insulin, angiotensin II), coagulation- and platelets-derived products (serotonin, adenosine diphosphate ADP, thrombin), and autacoids produced by endothelial and vascular smooth muscle cells themselves (bradykinin, ADP/ATP/UTP, angiotensins, endothelin). Endothelial cells can thus release vasodilator substances like nitric oxide ($NO^{\bullet}$), endothelium derived hyperpolarizing factor (EDHF), and prostacyclin (PGI_2), but are also able to secrete vasoconstrictors like prostanoids, for example thromboxane A_2, reactive oxygen and nitrogen species (ROS and RNS), endothelin, angiotensin II, serotonin and histamine [3].

The endothelium-dependent control of vascular tone is the result of the activation, interaction and balance between several cellular pathways. The first one is the $NO^{\bullet}$ pathway. $NO^{\bullet}$ is a gaseous transmitter, constitutively produced by the endothelial nitric oxide synthase (eNOS) from L-arginine and which rapidly diffuses across membranes into the bloodstream and in the vascular smooth muscle cells. It activates soluble guanylyl cyclase (sGC), which in turn catalyzes the formation of cyclic guanosine monophosphate (cGMP), finally leading to the relaxation of the vascular smooth muscle. Moreover, $NO^{\bullet}$ assumes a major role in preserving a normal endothelial function, especially in "conductance" vessels like the aorta. Endothelium-derived relaxation and/or production of endothelium-derived $NO^{\bullet}$ by the eNOS can be used as an indicator of endothelial cell function. The $NO^{\bullet}$ pathway can be activated in response to chemical factors' binding to their endothelial specific receptors, which requires an elevation of intracellular free calcium concentration, formation of a complex with calmodulin and the removal of the inhibitory caveolin from eNOS, leading to its activation. It can also be activated in response to mechanical factors, like shear stress which activates eNOS *via* a calcium-independent phosphorylation of eNOS on the serine residue 1177; eNOS phosphorylation is mediated by phosphorylation and activation of protein kinase Akt, which is itself phosphorylated by phosphatidylinositol-3-kinase (PI_3K). This mechanism plays a major role in the regulation of eNOS in physiological and pathological conditions. There are two other forms of NOS, neuronal NOS (nNOS) and inducible NOS (iNOS), the latter one being mainly activated by pro-inflammatory cytokines. Finally, $NO^{\bullet}$ also inhibits the activation and adherence of platelets to the endothelium [4].

EDHF pathway is another important signaling pathway, which has an important role in "resistance" arteries. In response to various stimuli, EDHF induces an endothelial hyperpolarization secondary to small and intermediate calcium-induced activated potassium channel activation [5]. The transmission of endothelial hyperpolarization to vascular smooth muscle cells could either involve potassium ions efflux, activating smooth muscle potassium channels and/or sodium/potassium-ATPase, or an electrical coupling between endothelial and smooth muscle cells through myo-endothelial gap junctions. Hyperpolarization prevents voltage-dependent calcium channel activation, resulting in a decrease in intracellular calcium concentration and relaxation of vascular smooth muscle cells.

Arachidonic acid-derived products can also take part in the regulation of vascular tone by endothelial cells. The metabolism of arachidonic acid by cyclo-oxygenase (COX) results in the release of several vasoactive factors, like prostacyclin or PGI_2 which is a powerful vasodilator. PGI_2 binds to vascular smooth muscle receptors to stimulate the formation of cyclic AMP by adenylyl cyclase, with the subsequent activation of protein kinase A leading ultimately to smooth muscle cells relaxation [6]. Vasoconstrictive prostanoids may also derive from arachidonic acid metabolism [7] and are able to decrease endothelium-dependent relaxation induced by acetylcholine in normal arteries [8, 9]. Under physiological conditions, the effects induced by low levels of vasoconstrictive prostanoids are attenuated by the influence of prostacyclin, $NO^{\bullet}$ and EDHF.

In response to chemical agonists binding to their respective endothelial receptors or to shear stress, endothelial cells are also able to generate ROS such as superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_20_2). Small and transient amounts of $O_2^{\bullet-}$ can be beneficial for the endothelial function, through activation of eNOS *via* Src/PI_3-kinase/Akt pathway [10]. However, high levels of ROS/RNS generated in pathological situations may lead to vasoconstriction, since $O_2^{\bullet-}$ binds to $NO^{\bullet}$ to form peroxynitrite ($ONOO^-$), resulting in a reduced bioavailability of $NO^{\bullet}$.

In addition, in response to various stimuli, endothelial cells are also able to synthesize prohormones, such as big endothelin, and endothelin-converting enzymes to produce endothelin-1, as reported in pulmonary vasculature [11]. At their luminal surface, endothelial cells also express angiotensin conversion enzyme which can transform angiotensin 1 into angiotensin 2 [12]. These two vasoactive peptides promote vasoconstriction and smooth muscle cells proliferation, thus participating to endothelial dysfunction in various diseases. Endothelial function is not only characterized by a tightly regulated vasomotor tone, but also by semi-permeable properties.

3. Endothelial Permeability

The endothelium is semi-permeable, but the degree of permeability differs according to the organ, vessel type and situation (physiological or not) considered. The junctional composition of intercellular space indeed varies across the vascular tree. "Conductance" arteries have, for example, numerous tight junctions compared to capillaries, in which physiological regulated transfer of fluids and small molecules occurs *via* a para-cellular way. Traffic *via* a trans-cellular way is also possible thanks to trans-endothelial channels or transcytosis mediated by caveolae and vesiculo-vacuolar organelles, and is used more for macromolecules traffic [13].

4. Anti-/Pro-thrombotic Properties

The endothelium constitutes an antithrombotic surface preventing inappropriate coagulation activation and inhibiting platelets' activation [14]. Endothelial cells have to pre-

serve adequate blood viscosity and promote a clot formation when vascular wall is injured. The endothelial cells luminal surface is called glycocalyx and is made of proteoglycans, including plasminogen and sulfate glycoaminoglycans [15]; it plays a major barrier role, takes part in normal vessel wall homeostasis [16-18] and provides an anticoagulant layer, thanks to negative electrical charges that repel circulating platelets and to an interaction with vitamin K-dependent coagulation factors.

The endothelium contributes to the haemostatic balance by expressing numerous pro- and anti-coagulant proteins. Tissue factor pathway inhibitor (TFPI), heparin (an antithrombine III cofactor), thrombomoduline (which activates protein C), endothelial protein C receptor (EPCR), tissular plasminogen activator (t-PA) and ecto-ADPase are amongst the anti-coagulant/anti-aggregant factors the endothelium expresses [13]. Beside vasodilatative properties, prostacyclin and $NO^{\bullet}$ also have an anti-aggregant action. Moreover, endothelial cells can contribute to the capture and degradation of thrombogenous substances like ADP and 5-hydroxytryptophan [19]. Finally, the endothelium produces numerous factors which contribute to its pro-coagulant/pro-aggregant properties, such as tissue factor (TF), plasminogen activator inhibitor (PAI)-1, von Willebrand factor (vWF) and protease activated receptors (PAR) [13, 20].

5. Adhesion Properties

Endothelial cells express adhesion molecules that can be regulated by mechanical or biochemical stimulations and are responsible for intercellular interactions and adhesions. These processes involve three super-families of adhesion molecules, namely integrins, selectins and immunoglobulin superfamily (intercellular molecule adhesion ICAM-1 and -2 and vascular cell adhesion molecule VCAM-1). The adhesion between leukocytes and endothelium, the "rolling" phenomenon and transmigration of these leukocytes in the underlying tissue, which occurs during inflammatory process, is triggered by selectins. E-selectin is highly specifically expressed in endothelial cells after cytokines activation, whereas L-selectin is expressed in leukocytes and P-selectin is stored and released by Weibel-Palade bodies after endothelial cells' activation or expressed in megakaryocytes. ICAM-1, ICAM-2 and VCAM-1 are expressed in endothelial cells, in response to inflammatory conditions.

Adhesion between endothelial cells and others blood cells is also possible: for example, in response to a stimulus, the endothelial Willebrand factor interacts with the GP Ib-IX and GP IIb-IIIa localized on platelet surface, inducing platelets adhesion and aggregation. Finally, endothelial cells can interact with bacterial components, like lipopolysaccharide (LPS), or cytokines, leading to activation of intracellular inflammation pathways. All these phenomena contribute to the body defense against pathogen agents: endothelial cells recruit leukocytes, allow their migration to infected sites, release inflammatory mediators and promote local coagulation phenomena to prevent hematogenous spread of the infection [21].

II. ENDOTHELIAL DYSFUNCTION

Endothelial dysfunction occurs when the equilibrium described previously is altered. In many diseases, this dysfunction is characterized at varying degrees by procoagulant, proadhesive and vasoconstrictive effects [22]. The vascular tone is deregulated when vasodilators production decreases and/or vasoconstrictors production increases. It can also be the consequence of a decreasing/increasing sensibility of vascular smooth muscle cells to vasodilators/vasoconstrictors respectively. Once again, there are some differences according to vascular bed. For example, endothelial dysfunction is essentially dependent on $NO^{\bullet}$ pathway in "conductance" arteries, whereas in "resistance" arteries, EDHF takes a particular importance, especially in small vessels. Activation of angiotensin renin system or/and endothelin-1 system in systemic and pulmonary hypertension induces media proliferation, vasoconstriction and ROS/RNS productions leading to endothelial dysfunction [23].

ROS and RNS play a major role in the generation of endothelial dysfunction, through cells, DNA and proteins damages and through the deleterious effects of $O_2^{\bullet-}$ and $ONOO^-$. They indeed have vasoconstrictive effects, by potentiating other endothelium-dependent contraction factors, such as prostanoids, or by reducing $NO^{\bullet}$ bioavailability, due to $O_2^{\bullet-}$ binding [24].

Endothelial permeability increases during inflammation, inducing fluid or small molecules to transfer toward underlying tissue. Equilibrium between adhesion molecules can be deregulated, leading to interaction between endothelial cells and leukocytes and therefore to leukocytes extravasation. In endothelial dysfunction, imbalance between pro- and anti-coagulant factors is frequently responsible for the arterial thrombi or microthrombi we observe in cardiovascular diseases such as diabetes, atherosclerosis or chronic kidney diseases.

In the different pathologies in which endothelial dysfunction takes part, several mechanisms leading to endothelial dysfunction are common, while others are more specific and depend on the pathology considered. Study and detection of endothelial dysfunction are important as it is involved in the early stage of most diseases and often represents a pejorative prognostic factor. Moreover, it sometimes could be used as a therapeutic target. For example, angiotensin-converting inhibitors, used in hypertension, improve endothelial function by decreasing amounts of the vasoconstrictor angiotensin II and essentially by increasing amounts of the vasodilator bradykinin [23].

III. ENDOTHELIAL DYSFUNCTION DURING SEPSIS

Sepsis results from systemic inflammatory response of organism to severe infection. It is defined by two or more of the systemic inflammatory response syndrome (SIRS) criteria (changes in body temperature, tachycardia, tachypnea and/or hypocapnia, and changes in the number of white blood cells and/or immaturity of white blood cells) [25]. Sepsis and its complications represent a continuum in clinical and pathophysiological severity. Severe sepsis is thus associated with organ dysfunction, hypoperfusion or hypotension and septic shock is associated with hypotension despite adequate fluid resuscitation [26]. Septic shock is one of the major causes of death in the non-coronary intensive care units and is responsible for about 150000 deaths in Europe and 200000 in United States of America [27, 28].

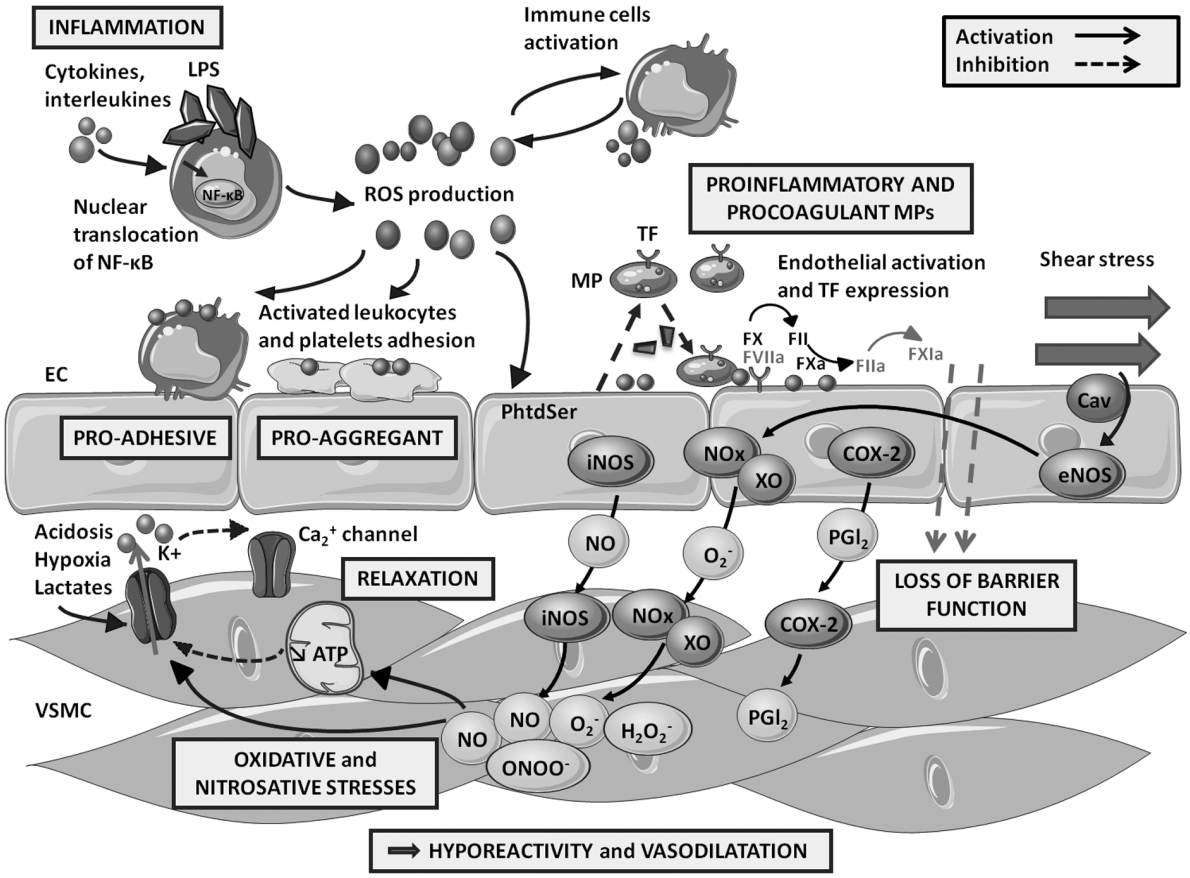

Fig. (1). Mechanisms of vascular dysfunction in sepsis. When invading an organism, pathogen agents activate numerous inter and intra cellular signaling pathways, notably leading to systemic inflammation and major oxidative and nitrosative stresses. The endothelium subsequently undergoes multiple phenotypic and functional changes, responsible for an endothelial dysfunction: the endothelium becomes pro-adhesive and pro-aggregant, supports the generation of proinflammatory and procoagulant microparticles, barrier function is lost and vascular tone is deregulated, which leads to vascular hyporeactivity and vasodilatation. ATP adenosine triphosphate, Ca^{2+} calcium, Cav caveolin-1, COX-2 cyclooxygenase-2, EC endothelial cell, eNOS endothelial nitric oxide synthase, factor VII, FII factor II, FIIa activated factor II, FVIIa activated, FX factor X, FXa activated factor X, H_2O_2 hydrogen peroxide, iNOS inducible nitric oxide synthase, K^+ potassium, LPS lipopolysaccharide, MP microparticle, NF-kB nuclear factor kappa B, NO nitric oxide, NOx nicotinamide adenine dinucleotide phosphate-oxidase, $O2^{\bullet-}$ superoxide anions, ONOO- peroxynitrite, PGI-2 prostacyclin, ROS reactive oxygen species, TF tissular factor, VSMC vascular smooth muscle cell, XO xanthine oxidase.

Death of patients is usually due to circulatory failure resistant to catecholamines, along with multiple organ dysfunction (cardiac, renal and hepatic) [29]. Pathophysiology of sepsis and organ dysfunction is not yet totally understood, but includes intense cellular activation with an overproduction of inflammatory mediators, coagulation and perfusion abnormalities and direct injuries due to infectious agents. This leads to SIRS, with subsequent multiple organ dysfunctions including, disseminated intravascular coagulation (DIC), microvascular leakage, and vascular and endothelial dysfunction [30].

1. The Inflammatory Endothelium

The endothelium is one of the first targets of the inflammatory response during sepsis and is precociously exposed to a "cytokines storm" [31]. Endothelial activation can thus be defined as an up-regulation of adhesion molecules by proinflammatory cytokines [32]. Cells undergo multiple injuries like cell swelling, vacuolization, and denudation, that may for example be mediated by LPS, known to be a critical mediator of endotoxemia [33]. Secondary to this activation, leukocytes are recruited by chemokines and adhesion molecules and extravasate into surrounding tissue; they are responsible for the production of ROS and RNS, proteases and cytotoxic enzymes [32]. Sepsis is indeed also associated with intense oxidative and nitrosative stresses, due to an excessive ROS and RNS release and a decrease in endogenous antioxidant defenses, leading to cellular and particularly endothelial and vascular smooth cells injuries [34, 35]. Inflammatory stimuli and oxidative/nitrosative stresses account for phenotypic changes on endothelium [36]. Indeed, inflammation favors molecular adhesion, leukocytes activation and *in situ* thrombosis. Altered vasomotor tone, leukocytes and platelets adhesions, increase of capillary permeability and activation of the clotting cascade with fibrin deposition lead to a procoagulant state and a heterogeneous capillary perfusion with reduced capacity for tissue oxygen extraction [37]. Endotoxemia disrupts endothelial intercellular signaling, because of a poor distribution of blood flow [38]. Endothelial cells adapt to these changes in an appropriate way (endothelial

activation) or not (endothelial dysfunction). The endothelium becomes pro-adhesive, as $NO^{\bullet}$ doesn't anymore inhibit platelets' adhesion and the expression of adhesion molecules receptors involved in platelets' and leukocytes' adhesion; it moreover becomes procoagulant because of increased expression of TF and decreased expression of thrombomodulin and activated protein C.

Mechanical forces are also profoundly modified during distributive shocks like septic shock, characterized by a major hypovolemia, due to fluid capillary leakage and endothelial dysfunction, and an intense vasoplegia. Blood flow is redistributed to the organs, while peripheral tissues undergo intense vasoconstriction, thus modifying shear stress. In a mesenteric artery ligation model in mice, Bakker *et al.* [39] showed that inflammatory response may initiate shear stress-induced arterial remodeling. After arterial ligation, the authors indeed showed fast changes in vascular tone, leading to an inflammatory response, with macrophages recruitment and increase in pro-inflammatory cytokines, and finally to arterial remodeling. They therefore suggest that inflammatory cytokines may up-regulate the expression of metalloproteinases (MMP), which can degrade matrix proteins and intercellular connections, thus taking part in vascular remodeling.

Altered endothelial properties may be involved in microcirculatory failure and more generally in organ failure [30]. Microcirculatory dysfunction is known to be a critical element of the pathogenesis of severe sepsis and septic shock [37]. One of the consequences of the activation/dysfunction of endothelial cells during sepsis phenomenon is a complex disturbance of the microcirculatory homeostasis. Without treatment, this disruption can lead to impaired oxygen transport resulting in cellular hypoxia, organ dysfunction and death [26]. Endothelial cells are impaired, with subsequent increased permeability and apoptosis [40]. The endothelium thus inhibits vasodilatation, becomes prothrombotic and antifibrinolytic and promotes platelets and leukocytes adhesion, leading to an inhomogeneous blood flow and tissue hypoxia [41]. In a septic shock model of peritonitis by cecal ligation and puncture in rats, Lam *et al.* [42] used intravital videomicroscopy and showed that sepsis altered microvascular perfusion, with increased microcirculation flow heterogeneity and reduced functional capillary density. These microcirculatory disorders may appear without any hemodynamic global effect (absence of hypotension) [43] or can be responsible for an inadequate oxygen transport and thus tissue hypoxia [44], which can persist despite achievement of normal global oxygen delivery [36]. Therefore, microcirculation's dysfunction may appear as a major mechanism in the development of multiple organ failure [45]. Arterial hypotension and the important hypovolemia during septic shock may both be explained by microcirculatory dysfunction. Hypotension may be due to an impairment of arteriolar response to vasoactive agents and hypovolemia to endothelial injuries and capillary leakage.

Endothelial cells are able to adapt very quickly to environmental stimuli, which can be either chemical (e.g pro-inflammatory like TNF-α or IL-1, LPS) or physical (e.g shear stress or hypoxia). The nuclear factor kappa B (NF-κB) (p65/p50) plays a major role in the inflammatory stimulation of endothelium; it can be activated *via* a classical pathway- and released after phosphorylation, ubiquitination and degradation of IκBα. RelA(p65)-p50 dimers are subsequently released, translocated into nucleus, and activate genes transcription of adhesion molecules, COX-2, TF, plasminogen activator inhibitor-1. In an alternative activation pathway (non-canonical pathway), NF-κB is activated after proteolysis of p100 protein, an inhibitor of RelB. Finally, in endothelial cells, NF-κB can also be activated *via* pathogens domain recognition receptors of the innate system, namely toll-like receptors 2 and 4 (TLR-2 and TLR-4), both of which are increased under inflammatory conditions [46]. Moreover, pro-inflammatory cytokines are responsible for the synthesis of numerous protein-G related factors (COX, phospholipase A2, 5-lipoxygenase and acetyltransferase), which promote inflammation *via* secretion of prostaglandins, leucotrienes and platelet-activating factor [34].

Sepsis is therefore responsible for an intense cellular activation; endothelial cells will amplify the inflammatory response by releasing proinflammatory cytokines, which contribute to spreading of microcirculatory injuries [45]. In a mouse endotoxemic shock model, Meziani *et al.* [47] demonstrated that human serum albumin reduced endotoxemia-induced inflammation, by decreasing the up-regulation of IκBα, thus blunting the activation of NF-κB. Moreover, human serum albumin was shown to have antioxidant properties, by reducing LPS-induced oxidative and nitrosative stresses, with a subsequent beneficial effect on endothelial dysfunction: albumin indeed increased eNOS activation, enhanced Nrf-2 expression and plasma glutathione concentration, and decreased circulating and tissular $0_2^{\bullet-}$ production, thus preventing its interaction with $NO^{\bullet}$ and the production of the highly toxic $ONOO^-$ [48]. These observations enhance the major role of oxidative and nitrosative stresses in diseases like sepsis.

2. Role of Oxidative and Nitrosative Stresses

Oxidative or nitrosative stress is defined by an imbalance between ROS or RNS production and antioxidative defenses. It may result from a decrease in antioxidant level and/or an increase in ROS level ($O_2^{\bullet-}$, H_2O_2…). RNS include $NO^{\bullet}$, dioxide nitrogen radicals and species which are not radicals[49]. Oxidative and nitrosative stresses may have many consequences, among them the stimulation of defense mechanisms (effective or not), cellular injuries (lipids, DNA, proteins…) or even cells death (apoptosis or necrosis) [49]. In some pathological states like sepsis, oxidative and nitrosative stresses increase, partly because of hypoxia and inflammation [50]. Main sources of ROS production are the mitochondrial respiratory chain, xanthine oxidase, immune activated cells, arachidonic acid metabolism and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [51]. LPS stimulates the assembly of the NADPH oxidase in neutrophils [52] and increases the expression of Rac2, a small protein that binds to the GTP, associated with p47phox and p67phox, two subunits necessary for the good functioning of NADPH oxidase, p91phox. The overproductions of ROS and RNS after exposure to LPS were demonstrated in various models of septic shock in macrophages and lymphocytes [51]. Neutrophils and macrophages can also produce $NO^{\bullet}$, with subsequent formation of the potent oxidant $ONOO^-$ and

$HO^{\cdot}$. ROS and RNS are also responsible for the oxidation of DNA and proteins, lipids peroxydation, changes of structural proteins and functional changes [53]. Oxidative injuries could occur in mitochondrial membranes, resulting in a membrane depolarization, a decoupling of oxidative phosphorylation and alteration of respiratory chain. It could finally lead to mitochondrial injuries, with liberation of cytochrome c, caspases activation and apoptose [54]. Finally, the overproduction of $NO^{\bullet}$ by the iNOS could contribute to hypotension, myocardial depression and vascular hyporeactivity during septic shock [55].

3. Loss of Anticoagulant Properties

Inflammation leads to a systemic activation of coagulation, impaired fibrinolysis and is associated with fibrin deposition, tissue ischemia and necrosis; moreover, it is associated with an increased risk of death in critically ill patients [40]. Septic shock is indeed characterized by a procoagulant and antifibrinolytic state, leading to DIC, pathological generation of thrombin, widespread microvascular thrombosis and hemostasis factors consumption, responsible for many sites' bleeding. The more severe the sepsis becomes, the less efficient negative regulation systems become; plasma concentration of natural anticoagulants (protein C, protein S, thrombomodulin and antithrombin III) is reduced in early stages of severe infections: fibrinolysis is particularly affected and the TFPI, which limits the activation of coagulation by interacting with the X factor, is less active. The role of coagulation in sepsis is not limited to the formation of micro-thrombi. Indeed, inflammation and coagulation are tightly linked. Inflammation-induced coagulation is mediated by pro-inflammatory cytokines, such as interleukine-6 (Il-6), playing a role in the initiation of coagulation activation, tumor necrosis factor α (TNF-α) and interleukine-1 (Il-1), which take part in the regulation of anticoagulation under physiological conditions [56]. Moreover, factor Xa, thrombin and TF/FVII complex have proinflammatory properties. They thus stimulate endothelial production of IL-6 and IL-8 by increasing the membrane expression of molecules promoting leukocytes' adhesion, such as E-selectin, which adheres to the circulating white blood cells to facilitate cell rolling, VCAM-1, ICAM-1, which solidify cellular bonds for transmigration and monocyte chemotactic protein-1 (MCP-1). In addition, protein C regulates the endothelium-mediated activation of inflammation *via* G-protein-coupled PARs, notably localized in the vasculature of endothelial cells and whose activation causes the production of cytokines responsible for inflammatory tissue damage [57]. The activation of coagulation is a consequence of inflammation, but also directly contributes to the pathophysiology of sepsis. Shapiro *et al.* [58] showed that these endothelial biomarkers in septic patients are correlated with sepsis severity, organ dysfunction and sepsis outcomes. This was reinforced by the findings of Furian *et al.* [59], who recently evaluated echocardiography-based indices of myocardial function and markers of vascular inflammation and endothelial dysfunction in the early phases of severe sepsis and showed ventricular systolic dysfunctions are directly associated with markers of endothelial dysfunction and a poor prognosis.

4. Generation of Procoagulant and Proinflammatory Microparticles

Cellular dysfunction in septic shock leads to the production of numerous mediators, including microparticles (MPs). MPs are submicron vesicles, released in the extracellular environment through a membrane reorganization and blebbing process following cell activation or apoptosis. They express several cell surface markers, which can vary according to MPs cellular origin and to the process leading to MPs formation; they are also able to acquire and transmit other antigens [60]. MPs are vectors of intercellular exchange of biologic information in health and in several diseases [61]. The endothelium is one of the main targets of circulating MPs [62, 63]. Thus, during sepsis, circulating MPs have procoagulant and pro-inflammatory properties and may activate inflammatory processes, cells apoptosis, leading to multiple organic failures [64].

MPs indeed play a major role in coagulation by exposing phosphatidylserine on their outer membrane: they constitute a catalytic surface for the assembly of coagulation factors and act as a support for thrombin generation. Moreover, monocytes derived MPs can express TF on their surface [60], thus initiating the coagulation cascade. At last, the release of GPIbα-presenting MPs by activated platelets may in turn be responsible for an increased production of thrombin following platelet FXI activation by thrombin; FXIa is thus able to cleave and activate FIX with no further dependence upon the initial TF-FVIIa complex [65-67].

MPs may also display antithrombotic activities. In severe sepsis, a treatment by recombinant human activated Protein C (rhAPC) induces the generation of endothelial MPs bearing the anticoagulant EPCR [68]. The MPs generated by activated protein C may therefore be beneficial in septic shock by decreasing the inflammatory response [69, 70], but also by modulating the haemostatic response. They indeed have anticoagulant properties, by delaying the formation of thrombin and could help restore the balance between pro-and anti-thrombotic systems and thus avoid the onset of DIC [71]. In rhAPC-treated patients, Pérez-Casal *et al.* [72] recently pointed out the clinical relevance of MP-associated APC as bioactive effectors: these MPs are stable and measurable and they express anticoagulant activity, modulate anti-apoptotic gene expression and provide an endothelial barrier protective effect through APC bound to them.

Endothelial MPs could play a role in the spreading of the sepsis inflammatory responses leading to multiple organ dysfunction [73, 74]. Barry *et al.* [75] demonstrated that the arachidonic acid presented by MPs allows them to upregulate the expression of COX-2 and intercellular adhesion molecules. They may also provoke vascular inflammation *via* lysophosphatidic acid and facilitate chemotactic migration of platelets or leukocytes to the endothelium, thus playing the role of a trigger for the production of cytokines (IL-1β, IL-8 and TNF-α) [76]. The proinflammatory cytokines themselves could contribute to the production of MPs [77], thus altering the vascular function by an amplified inflammatory process. The platelet activating factor present in endothelial cells and leukocytes is also involved in the pro-inflammatory effect of MPs [78].

5. Vasomotor Tone Abnormalities

Sepsis is characterized by a vascular dysfunction, which is partly due to impairment of endothelium-dependent vasorelaxation [79-81] caused by a loss of $NO^{\bullet}$ bioactivity in the vessel wall. Endotoxin challenge involves eNOS activity in its early phase; later, pro-inflammatory cytokines such as TNF-α, Il-1 or interferon-gamma, induce the synthesis iNOS, whose expression can exert a negative feedback on eNOS expression during endotoxemia [82-84]. The induction of iNOS leads to an overproduction of $NO^{\bullet}$ and has been shown to play a major role in endotoxemia-induced vascular hyporeactivity in several experimental models [85, 86] as well as in small vessels in patients with septic shock [87]. Abnormal endothelial-dependent vascular relaxation is attributed to alteration of endothelial cell surface receptors, dysfunction of signal transduction pathways and down-regulation and/or degradation of eNOS by pro-inflammatory cytokines such as TNF-α, as well as by ROS/RNS or bacterial endotoxin [88]. In a model of septic shock, Wang *et al.* [89] thus showed that inhibition of the biological activity of TNF-α protects vascular endothelial cells' function. Finally, during sepsis, numerous stimuli potentially activate endothelium ATP-dependent potassium channels, like $NO^{\bullet}$ or $ONOO^{-}$, hypoxia, acidosis, or hyperlactatemia. An excessive activation of potassium channels may lead to an hyperpolarization and inhibition of voltage-dependent calcium channels, with subsequent excessive cell relaxation, vasodilatation, finally leading to hypotension and vascular hyporeactivity [90]. $NO^{\bullet}$ or $ONOO^{-}$ can also activate large-conductance calcium-activated potassium channels, therefore taking part in vasoplegia during septic shock [91].

6. Loss of Barrier Function Properties

In sepsis, the endothelial barrier function is altered. The endothelium permeability is increased due to injured tight junctions and possibly injured endothelial glycocalyx [92], leading to fluid leakage from the intravascular space with subsequent edema, oxygen extraction deficit and tissue hypoxia [93]. Loss of the integrity of adherens junctions by deregulation of its components phosphorylation, due to inflammatory mediators, is responsible for the internalization of their major component, the vascular endothelial cadherin (VE-cadherin), and for endothelium leakage [94]. In mice models of sepsis, a protein, Slit2N, was shown to inhibit VE-cadherin endocytosis, thus preventing vascular endothelial growth factor (VEGF)-induced microvascular leakage and improving mice survival [95]. Furthermore, endothelial cytoskeleton plays a role in endothelial barrier disruption: members of the Rho family of guanosine triphosphatases (GTPases) regulate actin filaments, a main structural component of the cytoskeleton. In inflammatory pathologies, such as sepsis, Ras homolog gene family member A (RhoA) is activated and disrupts actin filaments structure, thus increasing the endothelium permeability [95]. During sepsis, there is also infiltration, deposition and oxidation of LDL in subendothelial space; oxidized LDL allow the expression of adhesion molecules and chemokine receptors on endothelial cells surface, causing an infiltration of vascular wall by monocytes and T lymphocytes. Leukocytes adhesion and migration are mediated by endothelial cells, *via* a tightly regulated cascade involving adhesion molecules (P-selectin, E-selectin, ICAM-1, VCAM-1) [50]. The pro-adhesive phenotype of activated endothelium is enhanced by changes in circulating cells activated by sepsis, including activated leukocytes, but also distorted erythrocytes and consequent increased blood viscosity, aggregation and adhesion [36].

IV. ENDOTHELIUM AS A THERAPEUTIC TARGET

Endothelial dysfunction is a predictor of mortality in sepsis. In an observational study, Duffy *et al.* [96] have indeed demonstrated that impaired endothelium-dependant vasodilatation, assessed by pulse wave analysis, is an independent predictor of mortality in critical illness. This *in vivo* bedside assessment of systemic endothelial function may improve stratification of the critically ill patients and help target new potential endothelial therapies. Early detection of microvascular dysfunction may indeed allow to identify hypoperfusion at an early stage of sepsis and adapt treatment before organ failure appears [45].

Direct biomarkers reflecting endothelial damage could be of great interest during sepsis, but are not yet validated and not commonly used in daily clinical routine [97]. As for imaging techniques, they remain mainly used for research purpose. Several methods can however be used to monitor microcirculatory function at the bedside of septic patients [98] and could therefore ascertain distributive alterations of oxygen transport in sepsis or assess the potential efficiency of treatments undertaken [43, 45]. They include near infrared spectroscopy (NIRS), which measures oxy- and deoxyhemoglobin in tissues and sidestream dark field (SDF), which uses reflected polarized light to visualize deeper lying microcirculation noninvasively and the flow of red blood cells in the microvessels, as polarized light is absorbed by hemoglobin. Orthogonal polarization spectral imaging technique (OPS) is more prone to artifacts and is no more used [99].

The treatments available to correct endothelial dysfunction in septic patients are rather limited and aim at controlling inflammation, vascular function and coagulation. However, in preclinical studies, some therapeutic approaches improved endothelial function and exerted beneficial effects on organ function and survival.

1. Fluid Resuscitation and Vasopressors

First of all, in the early phases of severe sepsis, fluid resuscitation with crystalloids or colloids may partly correct blood flow heterogeneity and shunting [100], through increased perfusion pressure, decreased microvascular blood viscosity and/or local vasodilatation [101]; the type of solution does not seem to influence the response to fluids. Transfusion may improve oxygen delivery [102]. Human serum albumin could also have beneficial effects on endothelial dysfunction, by reducing endotoxemia-induced inflammation and decreasing both oxidative and nitrosative stresses [47, 48]. In septic shock, inotropic and vasopressive agents are used to counteract the intense vasoplegia and may help maintain microcirculation in a perfused state; however, vasopressors can also worsen microcirculation flow heterogeneity and reduce functional capillary density, because of intense peripheral vasoconstriction [45]. Moreover, in a recent review, Boerma *et al.* [103] pointed out that there was no solid clini-

cal data available to support the benefice of increasing mean arterial pressure in term of microcirculatory perfusion and/or oxygenation. At last, although these treatments may be beneficial in term of global hemodynamic effect, they usually only have a limited impact on endothelial and microcirculatory dysfunctions, because of the complexity of these injuries [99].

2. Vasodilators

Vasodilators such as $NO^{\bullet}$ donors have been proposed to compensate for heterogeneity in microcirculatory blood flow [100]; in sepsis, $NO^{\bullet}$ may be beneficial through recruitment of the microcirculation as it takes part in both modulation of leukocyte-endothelial interactions and microvessel vasodilation. However, in a double-blind randomized placebo controlled trial bearing on seventy septic patients, Boerma *et al.* [104] concluded that intravenous nitroglycerin after a strict resuscitation protocol does not promote sublingual microcirculatory blood flow. On the other hand, although $NO^{\bullet}$ overproduction due to iNOS upregulation may contribute to arterial hypotension in sepsis, non selective NOS inhibition is known to aggravate the impairment of microvascular perfusion, by worsening leukocytes and platelets adhesion, increasing microthrombosis and microvascular permeability, causing decreased splanchnic and myocardial blood flow and defects in tissue oxygenation [36]. A phase III randomized controlled trial on non-selective NOS inhibitors was stopped early because of increased mortality in the NOS inhibition group [105]. Trzeciak therefore suggests that, in sepsis, upregulation of $NO^{\bullet}$ may be adaptive and in fact, protective [36].

As far as the regulation of vascular production and activity of $NO^{\bullet}$ is concerned, hydrogen sulfide (H_2S) is a gaseous transmitter, which has also aroused some interest in septic shock during the last decades [106, 107]. Indeed, the combination of H_2S and $NO^{\bullet}$ could form a product with little or no vascular activity in-vitro or *in vivo*, thought to be a nitrothiol [108]. The combination of sodium hydrogen sulfide (NaHS) with $NO^{\bullet}$ donor was shown to inhibit the vasorelaxant effect of acetylcholine and histamine on aorta rings of rats, while intravenous injection of NaHS in anesthetized rats increased mean arterial pressure, reduced in the presence of an inhibitor of $NO^{\bullet}$ synthase (L-NAME). Excessive levels of H_2S could contribute to the hypotension of septic shock; Hui *et al.* [109] showed that H_2S production was significantly increased in arteries of septic or endotoxemic rats and that level of endogenous H_2S were inversely correlated with blood pressure and cardiac function. Regarding the incomplete data, the use of H_2S as an adjuvant treatment in intensive care is therefore at an experimental stage and far from a clinical use for septic shock resuscitation [110].

3. Recombinant Human Activated Protein C

Another approach to improve endothelial function during sepsis would be recombinant human activated protein C (rhAPC) activated protein C is a potent anticoagulant and profibrinolytic enzyme, able to inactivate coagulation factors Va and VIIIa and TFPI [111]. Pro-inflammatory cytokines such as TNF-α decrease the activity of thrombomoduline, which interacts with the protein C to form activated protein C [34]. Recent studies show that the occurrence of DIC in septic shock is a precursor of multiple organ failure [112] and a decrease of protein C in septic patients is correlated with an increased risk of mortality [113]. During septic shock, treatment with rhAPC leads to a 19% reduction in relative risk of mortality [114], an effect attributed to its anticoagulant and anti-inflammatory properties. The PROWESS trial (Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis) [114] showed that treatment with rhAPC was associated with faster recovery of cardiovascular failure. But in rodent models of endotoxemia, rhAPC also demonstrated a protective effect on microcirculation through the inhibition of leukocyte-endothelial interaction, inhibition of subsequent leukocytes adherence to the endothelium and suppression of inflammatory cytokine production [115-117]. Moreover, Sennoun *et al.* [69] reported that rhAPC improved both endothelial dysfunction and arterial contractility induced by bacterial LPS in isolated mouse arteries, through an increase in eNOS activation, a reduction of LPS-induced upregulation of NF-κB and iNOS expression. At last, De Backer *et al.* [118] used OPS to visualize sublingual microcirculation and investigate the effects of rhAPC on septic patients; they showed an early increase of perfused capillaries. In spite of these interesting experimental data, notably endothelial properties, rhAPC would be not improve patients' survival and has therefore been recently removed from the available pharmacological tools.

CONCLUSION

Regarding the major role of microcirculatory dysfunction in the pathophysiology of sepsis and organ failure, the endothelium appears to be a key therapeutic target. So far however, no ideal treatment has been identified and further work is therefore needed to shed light on potential therapeutics of microcirculatory dysfunction during sepsis and the possibility to monitor microcirculation in critical patients. To date, the best approach seems to be a multimodal therapy targeting the different mechanisms involved in microcirculatory distress.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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