Reviews

Endothelium function in sepsis

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Abstract. Endothelial cells can be the prime target for an infection and infected endothelial cells may serve as an initiating system for a systemic response as these cells are able to secrete many mediators known to be of paramount importance. Endothelial cell functions in turn are regulated by these circulating mediators. Cellular interactions with leukocytes revealed protective and destructive functions. Single cell and animal studies indicate that endothelial permeability is increased and apart from clinical obvious edema formation in septic patients, the endothelial component remains unknown. Endothelial coagulation activation has been shown in vitro, however human data supporting an endothelial procoagulatory state are lacking. Defects in endothelium dependent vasoregulation in animal models are well known and again human studies are largely missing.

An imbalanced production of reactive oxygen species including nitric oxide has been found to be involved in all endothelial functions and may provide a common link which at present can be supported only in animal studies.

Key words: Endothelium – Sepsis – Permeability – Vasoregulation – Coagulation

Introduction

Sepsis is considered the leading cause of death in non-coronary intensive care units. It has been defined as a systemic inflammatory reaction to an infection. Among many cellular disturbances endothelial cells play a major role in the pathogenesis of this disease as these cells are critically involved in maintaining a delicate balance between vasoconstriction and vasodilation, blood cell adherence and non-adherence, anticoagulation and procoagulation, permeability

and tightness. All these functions are believed to be imbalanced and impairment is believed to precede clinically recognizable alterations (e.g. bleeding, edema, organ dysfunction and shock) but it is by no means clear whether these changes are the cause or consequence of endothelial dysfunction in sepsis. Endothelial functions have largely been studied in vitro from the first successful attempts of culturing isolated human endothelial cells dating back to the early 70ties. Endothelial cells from and within different organs or different species behave differently and tend to change properties the longer they are kept in culture. To overcome isolation variabilities several endothelial cell lines are currently available which have retained at least some features. However, experiments from single cell cultures are flawed in many ways and results from these may never be of any relevance to medical practice. It is inherent to any cell culturing that data obtained from these experiments often are restricted to cell type, time of culture and general working conditions. In vitro stressed endothelial cells almost uniquely tend to activate a functional program leading to a proinflammatory, procoagulatory and hyperpermeable phenotype.

In this review we want to summarize investigations of disturbed endothelial functions including infection, mediator and cellular interactions, permeability, coagulation and vasoactive properties from molecular findings to patient care.

Endothelial infection by gram positive bacteria

Staphylococcus aureus is the most prevalent bacterial pathogen isolated from patients with blood stream infection in north america [1]. *S. aureus* has been reported to directly infect human umbilical vein endothelial cells (HUVEC) thereby inducing secretion of cytokines and functional upregulation of adhesion molecules [2]. Internalized *S. aureus* may lead to apoptosis in HUVEC [3] or persistence as small colony variants [4]. Group A streptococci can enter HUVEC [5], a process which may render these cells particularly sen-

sitive to otherwise subtoxic concentrations of hydrogen peroxide [6]. Group B *streptococci* (GBS) are the most common cause of neonatal sepsis and pneumonia. GBS-induced endothelial cell injury can be confirmed by histological findings at autopsy, in animal studies and in vitro [7]. GBS invasion and subsequent damage of endothelial cells may be inhibited by cytochalasin D in HUVEC implicating that cytoskeletal interactions are important for toxicity. However, invasion of brain microvascular endothelial cells by GBS may be dose dependently cytotoxic due to beta-hemolysin production [8]. *Streptococcus pneumoniae* may enter activated endothelial cells using PAF-receptors [9]. This receptor engagement may also serve as a sorting signal for endothelial transcytosis [10]. Infection and activation of endothelial cells by *Listeria monocytogenes* is believed to be a critical component of the pathogenesis of this disease and includes ceramide generation, transcription factor activation and increases in adhesion molecule expression on HUVEC [11]. *Listeria* have been described to enter HUVEC either directly via internalin B or by a cell-to-cell spread from infected monocytes [12].

Gram negative endothelial infection

Gram negative bacteria have lipopolysaccharides (LPS) within their cell wall. Cellular binding of LPS usually is accomplished by CD14. Endothelial cells lack CD14 receptors and LPS effects on endothelial cells generally require the presence of CD14 in the serum. LPS effects from gram negative live bacteria (*B. fragilis*, *E. cloacae*, *H. influenzae*, *K pneumoniae*) on endothelial cells have been demonstrated by transcription factor activation and subsequent surface expression of E-Selectin and tissue factor which was not seen from viable or heat-killed gram-positive bacteria (*S. aureus*, *E. faecalis*, *S. pneumoniae*) [13]. *Neisseria meningitidis* adherence on endothelial cells has been reported to be influenced by Pilus protein C expression and CD66 on endothelial surfaces [14, 15] and may cause tissue factor expression due to the presence of LPS within the cell wall. *Haemophilus influenzae* generally is not toxic to endothelial cells except three clones of biogroup *aegyptius* causing Brazilian purpuric fever [16]. Toxicity has been reported to be independent of endotoxin, phagocytosis and replication since irradiation, cycloheximide, cytochalasin D and methylamine have no effect on the ability of the bacterium to invade and cause a cytotoxic response. Piliated *Pseudomonas aeruginosa* adheres to and enters human endothelial cells leading to progressive damage [17] or may persist by lysis of endosomal membranes [18]. *Escherichia coli* may invade human brain microvascular endothelial cells involving specialized proteins [19, 20].

Endothelial infection by *Chlamydia pneumoniae* also activates endothelial cells to produce cytokines and adhesion molecules and become procoagulant [21–23]. *Bartonella quintana*, the cause of trench fever transmitted by the body louse, has recently been implicated in culture-negative endocarditis and bacteraemia amongst homeless people [24]. Infection and damage of endothelial cells by *B. quintana* has been demonstrated in vitro and in vivo [25]. Interaction of *Bartonella henselae* with endothelial cells may result in bacterial aggregation on the cell surface and the subsequent internalisation of the bacterial aggregate by a unique struc-

Table 1. Endothelial infection by sepsis inducing pathogens.

Infectious agent	Mediator/effector	Reference
Staphylococcus aureus	Heparan sulfate/Fibrinogen Autocrine IL-1 α -toxin	[2] [45]
Group A Streptococcus	Cystein protease Streptolysin S	[5] [6]
Group B Streptococcus	Capsular polysaccharides β -hemolysin	[7] [8]
Streptococcus pneumoniae	PAF-R	[9]
Clostridium perfringens	MBEC1 α -toxin Phospholipase C perfringolysin O	[48] [47] [49]
Clostridium difficile	toxin B	[50]
Listeria monocytogenes	Internalin B Listeriolysin Phospholipase C	[12] [53] [11]
Neisseria meningitidis	Pilus protein C CD66	[14] [15]
Pseudomonas aeruginosa	pilus proteins exotoxin A pyochelin	[17] [52] [58]
Escherichia coli	outer membrane protein A ibe10 verocytotoxin hemolysin	[19] [20] [46] [45]
Chlamydia pneumoniae	major outer membrane protein 1 E-Selectin, VCAM-1, ICAM-1	[21] [23]
Bartonella henselae	invasome	[26]
Rickettsia rickettsii	autocrine IL-1	[27]
Rickettsia conorii	autocrine IL-1	[28]
Borrelia burgdorferi	CD14 $\alpha(v)\beta3$, $\alpha5\beta1$ integrin proteoglycans, heparan sulfate	[30] [31] [32]
Plasmodium falciparum	P-Selectin ICAM-1, CD36 PECAM-1	[34] [35] [36]
Candida	E-Selectin, ICAM-1, VCAM-1, IL-6, IL8, MCP-1, COX-2 Phospholipase B	[37] [38]
Dengue virus	RANTES, IL-8	[39]
Herpes Virus	ICAM-1	[40]
Measles virus	ICAM-1, TF	[41]
Cytomegalovirus	prothrombinase, TF, P-Selectin	[42]
Hantavirus	$\alpha(v)\beta3$ integrin	[43]
Ebola Virus	IL-1 $\downarrow$, IL-6 $\downarrow$	[44]

PAF-R, platelet activating factor receptor; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; PECAM-1, platelet endothelial cell adhesion molecule 1; MCP-1, monocyte chemoattractant protein 1; COX-2, cyclooxygenase 2; TF, tissue factor; RANTES, regulated on activation, normal T cell expressed and secreted.

ture, the invasome [26]. *Rickettsia rickettsii*, an obligate intracellular gram-negative bacterium which causes Rocky Mountain spotted fever, inhibits endothelial apoptosis allowing to remain inside the host endothelium [27] but is also known to cause a proinflammatory endothelial phenotype.

Rickettsia conorii causes Mediterranean spotted fever which causes endothelial infection with secretion of cytokines, increases in adhesion molecules and induction of surface tissue factor [28, 29].

Other pathogens

Attachment of *Borrelia burgdorferi*, the agent inducing Lyme disease, to endothelial cells may be accomplished by CD14, $\alpha(v)\beta_3$ and $\alpha_5\beta_1$ integrins or different classes of proteoglycans [30–32]. *B. burgdorferi* activates the transcription of chemokine and adhesion molecule gene expression in endothelial cells [33]. *Plasmodium* infected erythrocytes may bind to endothelial cells via P-Selectin, CD36, intercellular adhesion molecule 1 (ICAM-1) or platelet endothelial cell adhesion molecule 1 (PECAM-1) on the endothelial surface [34–36]. Phagocytosed live *Candida albicans* stimulates cytokine secretion and inducible cyclooxygenase expression in endothelial cells [37, 38]. Some viral diseases are known to primarily infect endothelial cells and alter their function. Dengue virus infection of HUVEC leads to production of chemoattractant proteins RANTES and IL-8 [39], herpes and measles virus infection of brain microvascular endothelial cells increases lymphocyte adhesion by increasing ICAM-1 [40] and measles virus and cytomegalovirus increases tissue factor expression on HUVEC [41, 42]. Hemorrhagic fever caused by hantaviruses may be accomplished by integrin mediated endothelial infection [43]. On the other hand, ebola virus infects endothelial cells with a transmembrane glycoprotein and inhibits inflammatory responses [44].

Activation of endothelial cells by bacterial secretory products

Exotoxins are known to directly activate endothelial cells. Platelet activating factor (PAF), NO^* and PGI_2 secretion from HUVEC has been demonstrated by *E. coli* hemolysin via inositolphosphate/diacylglycerol formation and by *S. aureus* alpha-toxin via transmembrane Ca^{2+} entry [45]. There is increasing evidence that hemolytic uremic syndrome results from the systemic action of Verocytotoxin producing *E. coli* on vascular endothelial cells [46]. Alpha toxin from *Clostridium perfringens* is a phospholipase C and has been reported to induce adhesion molecule expression and secretion of chemokines from endothelial cells [47]. Brain capillary endothelial cells have been reported to express MBEC1, a protein that may serve as the *C. perfringens* enterotoxin receptor [48, 49]. The activity of small GTPase Rho has been shown to be altered by *C. difficile* toxin B [50] and *Pasteurella multocida* toxin [51], which leads to alterations of endothelial permeability. *P. aeruginosa* exotoxin A may directly injure endothelial cells by a motif shared by many toxins [52]. Listeriolysin and phosphatidylinositol-specific phospholipase C secreted from *L. monocytogenes* has been shown to induce phosphatidylinositol metabolism and diacylglycerol formation in the absence of bacterial uptake by the endothelial cells [53]. Some bacterial exotoxins have been used for years as pharmacological tools like Pertussis toxin for

studying G-protein dependent endothelial cell functions. Lipoteichoic acid and peptidoglycan from cell wall components of gram positive bacteria have been shown to induce sepsis in animal models. While lipoteichoic acid has been reported to directly activate endothelial cells [54], peptidoglycan seems monocyte dependent [55].

Most in vitro experiments, however, were performed with different sources of LPS as a surrogate activator modelling gram negative bacterial infection. Endothelial stimulation using LPS in relevant concentrations are usually performed in the presence of serum containing soluble CD14-receptor because endothelial cells generally lack CD14. Alternatively, proinflammatory cytokines including tumor necrosis factor alpha ($\text{TNF-}\alpha$), interleukins (IL-1, IL-4) and interferon gamma ($\text{IFN-}\gamma$) acting on endothelial cells have extensively been investigated and shown to alter endothelial in vitro functions [56, 57].

Reactive oxygen species in endothelial biology

The inflammatory response in endothelial cells has been linked to an alteration in reactive oxygen production including superoxide ($\text{O}_2^{\cdot -}$), hydrogen peroxide (H_2O_2), nitric oxide (NO^*), hydroxyl radicals (OH^*) and secondary reaction products thereof. $\text{O}_2^{\cdot -}$ is believed to be present in unstressed conditions in less than nanomolar quantities within cells. Mitochondrial and cytoplasmatic superoxide dismutase readily reacts with $\text{O}_2^{\cdot -}$ forming H_2O_2 which has been measured in micromolar concentrations in human blood. Sources of endothelial $\text{O}_2^{\cdot -}$ production apart from mitochondrial leakage may include metabolism of CytP450 or other metabolic byproducts and production by a NADH oxidase system or by nitric oxide synthase in the absence of L-arginine. Some bacterial pathogens are known to be able to produce H_2O_2 by themselves, however interaction with the endothelium may greatly enhance cellular alterations. Streptococcal hemolysin, streptolysin S, is capable of interacting with H_2O_2 to injure vascular endothelial cells [6]. Iron bound to the *P. aeruginosa* siderophore, pyochelin, augments oxidant-mediated endothelial cell injury by modification of transferrin to form iron complexes capable of catalyzing the formation of OH^* from $\text{O}_2^{\cdot -}$ and H_2O_2 [58]. *R. rickettsii* infection of the endothelial cell line EA.hy 926 and HUVEC has been demonstrated to cause glutathione depletion, a major intracellular antioxidant, and reduced glutathione peroxidase activity leading to increased amounts of intracellular peroxide [59]. An increase in reactive oxygen species production has been shown in endothelial cells after incubation with LPS, IL-1, $\text{TNF-}\alpha$ and $\text{IFN-}\gamma$ [60–65]. $\text{TNF-}\alpha$ has many times been reported to increase, e.g., adhesion molecule expression inhibitable by various antioxidants [62, 66–68].

Nitric oxide, like $\text{O}_2^{\cdot -}$, is chemically not very reactive at all. Endothelial production has been established by either constitutive NO-synthase (NOS) III in a Ca^{2+} and phosphorylation dependent manner or by inducible NOS II. Direct biochemical actions of NO^* include metalcomplex containing proteins, other radical species, oxygen and oxygen derivatives leading to oxidation, nitrosation and nitration. Relevant concentrations in vivo have been reported to range from nM to 100 μM . NO^* directly reacts with oxy-hemoglobin ($\text{Fe}^{2+}\text{-O}_2$) leading to methemoglobin (Fe^{3+}) and

nitrate (NO_3^-). NO^* reacts with O_2 forming nitrite (NO_2^-) via intermediate NO_2 and N_2O_3 . Mainly N_2O_3 is participating in N- or S-nitrosylations leading to nitrosamines or nitrosothiols, the latter may serve as a circulating source or as a transporter across cell membranes. Reduced glutathione has a high affinity to N_2O_3 and may also be important in toxicity related to NO^* autooxidation. Toxicity related cellular targets of NO^* may include inhibition of cytochrome C oxidase, inhibition of catalase or DNA damage. On the other hand, NO^* has been reported to inhibit iron catalysed Fenton reaction and to inhibit lipid peroxidation. Iron nitrosyl formation in heme containing proteins are the best characterized reactions for NO^* in biology. This type of interaction includes very sensitive stimulation of guanylate cyclase and inhibition of cytochrome c oxidase. *E. coli* hemolysin and *S. aureus* alpha toxin induce NO^* formation in cultured porcine pulmonary endothelial cells [69]. Transformed mouse endothelial cells stimulated by the combination of IFN- γ and TNF- α killed intracellular *R. conorii* by a mechanism that required the synthesis of NO^* [70]. IFN- γ , TNF- α and IL-1 stimulated murine endothelial cells have been shown to kill *Schistosoma mansoni* through the production of nitric oxide [71]. Both NO^* and $\text{O}_2^{\cdot -}$ production may have a limited influence on endothelial viability under resting conditions. Cultured bovine and porcine aortic endothelial cells showed decreased NO^* production after 1 h of LPS incubation which was related to decreases in capacitative Ca^{2+} signals [72]. Posttranscriptional destabilization of NOS III mRNA may have accounted for this early decrease in NO^* production [73]. NOS II, which is believed to produce larger amounts of NO^* is also known to be regulated by many proinflammatory stimuli with significant cell type variabilities. At sites of endothelial involvement in an inflammatory process both vascular non-endothelial and

non-resident cells are known to produce NO^* . As the amount and physiological consequences of NO^* produced from NOSs at the microcirculatory level is not known, it may well serve also to reduce inflammatory processes as recently implicated from a coculture experiment [74].

That an increase in reactive oxygen species is present in human sepsis has been documented by almost any study trying to quantify secondary reaction products by several methods, however the cellular sources remain speculative (Table 2).

In 1990 Beckman et al. showed that the presence of both NO^* and $\text{O}_2^{\cdot -}$ produced peroxynitrite (ONOO^-) which may decompose to produce HO^* like molecules and thereby kill endothelial cells [75]. At pH 7 its lifetime is in the order of a second. Half of the ONOO^- formed is rapidly equilibrated to peroxynitrous acid (ONOOH) and breaks down to NO_3^- or NO_2^- . The other half decomposes with reactive similarities to HO^* plus nitrogen dioxide ($\text{OH}^* + \text{NO}_2^*$). The reaction of $\text{O}_2^{\cdot -}$ with NO^* has been shown to be very fast, being 3 times faster than $\text{O}_2^{\cdot -}$ reacting with superoxide dismutase. Biological actions of ONOO^- may be determined in the plasma primarily by the presence of CO_2 (~1 mM) and within cells by the presence of thiol groups (~5 mM). ONOO^- may again react with NO^* or $\text{O}_2^{\cdot -}$ to form NO_2 . ONOO^- and ONOOH are potent oxidants and are known to initiate lipid peroxidation and DNA chain breakage. Tyrosine nitration has been advocated to be useful as a marker for ONOO^- production in vivo, however this process may also occur by NO_2 rather than ONOO^- and may represent the action of nitrating species. ONOO^- as a surrogate of $\text{O}_2^{\cdot -}$ and NO^* interaction is currently investigated as a major mediator in radical stress induced toxicity and challenges the prevailing view of metal catalysed damaging effects of OH^* formation. However, beneficial effects of ONOO^- have been shown in ischemia-reperfusion

Reference	No. of patients	Clinical condition	Indicator
[157]	16	Septic shock	Retinol(A)↓, tocopherol(E)↓, β -carotene↓, TBARS↑
[158]	15	Severe sepsis	*antioxidant potential ↓
[159]	26	Sepsis	TBARS↑
[160]	14	Sepsis	XOD↑, ascorbyl radical↑, lipidperoxides↑
[161]	8	Sepsis	Vitamin C↓, bleomycin detectable iron↑
[162]	30	Sepsis	Vitamin C↓, lipidperoxides↑
[163]	17	Sepsis	$\text{NO}_2^-/\text{NO}_3^-$
[164]	12	Septic shock	$\text{NO}_2^-/\text{NO}_3^-$
[165]	23	Sepsis, septic shock	$\text{NO}_2^-/\text{NO}_3^-$
[166]	29	Septic shock	$\text{NO}_2^-/\text{NO}_3^-$
[167]	13	Septic shock	$\text{NO}_2^-/\text{NO}_3^-$
[168]	20	Sepsis	$\text{NO}_2^-/\text{NO}_3^-$
[169]	53	Sepsis, children	$\text{NO}_2^-/\text{NO}_3^-$
[170]	46	SIRS, sepsis, children	$\text{NO}_2^-/\text{NO}_3^-$
[171]	30	Sepsis, children	$\text{NO}_2^-/\text{NO}_3^-$
[172]	31	Sepsis, children	$\text{NO}_2^-/\text{NO}_3^-$
[173]	22	Septic shock, children	$\text{NO}_2^-/\text{NO}_3^-$
[174]	11	Septic shock	$\text{NO}_2^-/\text{NO}_3^-$
[175]	11	Sepsis	Immunohistochemical endothelial nitrotyrosine↑
[176]	3	Septic shock	$\text{NO}_2^-/\text{NO}_3^-$, plasmatic nitrotyrosine↑
[177]	3	Septic lung injury	Immunohistochemical endothelial nitrotyrosine↑

Table 2. Indicators of reactive oxygen and reactive nitrogen species in human sepsis.

Antioxidant capacity was defined as the ability of plasma to inhibit * ferryl myoglobin production by hydrogen peroxide addition to metmyoglobin or ** oxo-iron induced damage to deoxyribose, phospholipids and DNA. TBARS, thiobarbituric acid reactive substances; XOD, xanthine oxidase.

models and therefore question the view that this molecule solely is detrimental. Toxicity induced by an interaction between hydrogen peroxide and nitric oxide has led to conflicting results. Rat lung microvascular endothelial cells and porcine pulmonary artery endothelial cells exposed to H_2O_2 have been reported to be protected by NO^* donors [76, 77], whereas toxicity in bovine aortic endothelial cells [78] and in rat liver microvascular endothelial cells [79] was increased in the presence of NO^* donors. NO^* in the presence of H_2O_2 may also produce OH^* like molecules independent of the presence of iron [80]. Neutrophils may add to the complexity of toxic reactions. Myeloperoxidase from activated neutrophils, which produces $HOCl$ and OH^* molecules in the presence of O_2^- , has recently been demonstrated to convert NO_2^- into NO_2 , thereby damaging endothelial cells [81]. High doses of reactive oxygen species (including NO^*) have been shown to cause apoptosis of endothelial cells, whereas low doses were protective [82]. In mice disseminated endothelial apoptosis has been suggested to be responsible for organ failure and shock induced by endotoxin or $TNF-\alpha$ [83]. Both LPS or $TNF-\alpha$ usually do not cause endothelial cell death unless protein synthesis is blocked probably due to simultaneous increases in antiapoptotic protein synthesis [84]. However, postmortal investigation in humans deceased from or with sepsis did not confirm these results [85].

Most of the genes activated *in vitro* during the endothelial stress response are controlled by at least two transcription factor families: activator protein 1 (AP-1) and nuclear factor kappa B ($NF\kappa B$). $NF\kappa B$ has gained wide interest as a target in inflammatory diseases as it seems to be invariably up-regulated [86]. A variety of agents including cytokines and reactive oxygen stress cause $I\kappa B$ to dissociate from the complex after phosphorylation by $I\kappa B$ -kinase complex. H_2O_2 has been reported to activate transcription factor $NF\kappa B$ in porcine aortic endothelial cells [87] whereas HUVEC were unresponsive [88]. NO^* has in most cases been shown to inhibit transcription factor $NF\kappa B$ and its influence on transcription factor AP-1 is unclear. Inhibition of $NF\kappa B$ as a therapeutic means has been suggested. However, as this transcription factor is also involved in protective gene regulation, its inhibition can make cells sensitive to e.g. $TNF-\alpha$ [89]. Data on activated intracellular signalling pathways in sepsis patients are scarce but include $NF\kappa B$ within mononuclear cells [90].

Cellular interactions

Usually any blood cell is kept off endothelial surfaces. This is believed to be accomplished by net electrical charge, biomechanical characteristics of flowing blood and the secretion of NO^* . If blood cells touch the endothelium a Ca^{2+} -signal is induced [91], but it is unclear at present whether this has any functional consequence. It is tempting to speculate that Ca^{2+} -signals may then in a context sensitive manner augment pro-adhesive processes or antiadhesive endothelial properties. Activated endothelial cells are potent producers of cytokines like IL-1, a major proinflammatory cytokine, and chemotactic peptides including IL-8 for neutrophils, macrophage inflammatory protein-1 alpha (MIP-1 α), monocyte chemoattractant proteins 1–4 and RANTES (regulated on activa-

tion, normal T cell expressed and secreted) for monocytes, T-lymphocytes and dendritic cells, growth related protein (GRO) and gamma-interferon-inducible protein (IP-10) for activated T-lymphocytes, epithelial neutrophil activating peptide 78 (ENA-78), vascular monocyte adhesion-associated protein (VMAP-1) and endothelial monocyte-activating polypeptide II (EMAP-II) for monocytes [92].

Many adhesion molecules are expressed on the surface of endothelial cells in a highly complex yet regulated manner. P-Selectin, E-Selectin, ICAM-1, vascular cell adhesion molecule 1 (VCAM-1), PECAM-1 are well known for their stimulus-, cell-, time- and organ specific dependence of expression and their importance in regulation of leukocyte-endothelial interactions. Moreover, apart from being passive adhesion molecules, all of the above mentioned molecules have been shown to signal inside endothelial cells upon receptor engagement. Shed receptors from endothelial surfaces may also serve as endogenous antiadhesive molecules demonstrating even more the dynamic and complex nature of these processes. Soluble forms of adhesion molecules have been shown to be present in high amounts in human sepsis (Table 3), however whether this is beneficial or detrimental is not known and the assumption that these parameters may serve as practical indexes remains to be established.

Particularly $IFN-\gamma$ has been repeatedly shown to increase endothelial HLA-DR expression rendering them capable of MHC class II restricted interactions with $CD4^+$ T-cells. Costimulatory $CD80$ (B7-1) and $CD86$ (B7-2) are usually not present on endothelial surfaces leading to the conventional view that endothelial cells are semiprofessional antigen presenting cells. However, under certain conditions both costimulatory molecules and $CD40$ can be upregulated on endothelial surfaces indicating excessive antigen presentation [93]. Migration of lymphocytes into inflamed mouse tis-

Table 3. Soluble markers for endothelial involvement in human sepsis.

Reference	Patients	Indicator
[178]	45, sepsis	svWF $\uparrow$
[179]	60, sepsis	sTM $\uparrow$
[180]	19, sepsis, ARDS	sTM $\uparrow$, sICAM-1 $\uparrow$, svWF $\uparrow$
[181]	67, sepsis	sE-Selectin $\uparrow$
[182]	16, severe sepsis	sE-Selectin $\uparrow$, sICAM-1 $\uparrow$
[183]	25, sepsis	sE-Selectin $\uparrow$, sICAM-1 $\uparrow$, svWF $\uparrow$
[184]	22, septic shock	sTM $\uparrow$
[185]	71, sepsis, malaria	sICAM-1, sVCAM-1, sE-Selectin $\uparrow$
[186]	32, SIRS	svWF $\uparrow$
[187]	42, sepsis, DIC	Endothelin $\uparrow$, sTM $\uparrow$
[188]	16, sepsis	sEPCR $\uparrow$
[189]	12, mediterranean spotted fever	eEC $\uparrow$, sTM $\uparrow$, svWF $\uparrow$
[190]	40, sepsis	Endothelin $\uparrow$, sTM $\uparrow$, sICAM-1 $\uparrow$, sVCAM-1 $\uparrow$

SvWF, soluble von Willebrandt Factor; sE-Selectin, soluble E-selectin; sVCAM-1, soluble vascular cell adhesion molecule 1; sICAM-1, soluble intercellular adhesion molecule 1; sTM, soluble thrombomodulin; sEPCR, soluble endothelial protein C receptor; eEC, circulating endothelial cells.

sues has been reported to depend on PSGL-1 and ESL-1 binding endothelial P-selectin and E-selectin, respectively [94]. This phenotype was found to be restricted to Th1 lymphocytes, but human sepsis seems to be predominated by a Th2 type [95]. Both TNF- α and IFN- γ are able to promote transmigration of leukocytes, whereas coapplication of both cytokines inhibit this process [96]. T-cells migrating through the endothelial barrier in a PECAM-1-dependent manner are subject to inhibitory signals which may limit their activation in tissues [97]. TNF bound to monocytes has been shown to inhibit endothelial apoptosis, whereas this process is promoted in the presence of lymphocytes [98]. Unperturbed endothelial cells express Fas-ligand which has been implicated as a means of signalling apoptosis to constitutively Fas receptor (CD95) bearing cells, whereas TNF- α treated endothelial cells decrease Fas ligand expression thereby allowing leukocyte survival during extravasation [99]. How the endothelium might interact specifically with lymphocytes or monocytes in septic patients can only be speculated on.

Neutrophils interacting with endothelial cells have repeatedly been shown to cause toxicity due to the release of enzymes and reactive oxygen species. If uncontrolled, these cells are believed to mediate significant tissue damage. However whether uncontrolled activation or rather deactivation is present in human sepsis is a matter of debate. Endothelial cells have been reported to inhibit some neutrophil functions [100]. Transmigrated neutrophils may actively participate in the endothelial resealing process by the secretion of adenosin precursors [101]. However, massive leukocyte extravasation as one would expect from most animal studies has never been shown in septic patients [102].

Permeability

Endothelium is known to regulate transvascular fluid flux, flux of nutrients, mediators and cells by either paracellular or transcellular vacuolar channel related pathways. Lateral junction proteins including the vascular endothelial cadherin-complex, platelet-endothelial cell adhesion molecule-1, occludin, zona occludens-1, recently described junctional adhesion protein, CD151/platelet endothelial tetraspan antigen and CD81/target of antiproliferative antigen are known to participate in this process. Paracellular permeability is achieved by either an active contraction or the controlled release of an intrinsic tone mediated in most cases by the action of myosin light chain kinase (MLCK) acting on non muscle myosin. Generally an increase in the concentration of cAMP keeps cultured endothelial monolayers tight and cGMP has been reported to assist this function in human aortic and foreskin vessels [103]. Endothelial retraction may be initiated by increases in intracellular Ca^{2+} concentration, but elevation of Ca^{2+} in the presence of maintained cAMP-kinase dependent phosphorylation is not edemagenic. These antagonistic effects of Ca^{2+} and cAMP in endothelial permeability regulation have recently been reviewed by Moore et al. [104].

Many reports documented increases in endothelial permeability involving exotoxins like *S. aureus* alpha-toxin and *P. aeruginosa* cytotoxin [105, 106] or endotoxins [107]. For example, *P. multocida* toxin has been shown to activate

Rho/Rho kinase, which inactivates MLC phosphatase. The resulting increase in MLC phosphorylation caused endothelial cell retraction and a rise in endothelial permeability [51]. LPS induced increases in paracellular permeability by caspase activated cleavage of adherens junction proteins [108]. Counteracting lipid peroxidation during LPS activation may inhibit increases in permeability [109]. TNF- α stimulated endothelial cadherin complex is disrupted in a proteasome dependent manner [110]. The resulting increase in permeability has been reported to lower cAMP and activate phosphodiesterase II and IV [111]. H_2O_2 induced hyperpermeability of porcine pulmonary endothelial cells has been reported to be effectively reduced by cGMP elevating drugs including phosphodiesterase II inhibition or $\text{NO}^{\bullet}$ donors [112]. The electroneutral Na-K-Cl cotransport system is thought to function in the maintenance of a selective permeability. IL-1, TNF- α and LPS upregulate the expression of a bumetanide-sensitive Na-K-Cl cotransporter subtype in HUVEC and in murine lung and kidney endothelial cells [113].

Septic rats show different increases in albumin flux across several endothelial beds [114]. Increases in venular permeability have been shown to be preventable by the antioxidants N-acetyl-cystein or Tirilazad mesylate in *E. coli* infused rats [115, 116]. IL-10, an antiinflammatory cytokine not produced by endothelial cells, was shown to participate as an inhibitor of endothelial permeability induced by LPS in mice [117].

Clinically, increases in endothelial permeability may be obvious in many septic patients, but only recently venous congestion plethysmography showed a selectively elevated filtration capacity as a measure of endothelial dysfunction in septic patients [118]. Which of the many pathways of increased permeability might be turned on and whether it persists remains unknown.

Endothelial coagulatory system

In principle endothelial cells are believed to be anticoagulatory by virtue of their surface expression of glycosaminoglycan-antithrombin III complex, thrombomodulin, heparin releasable tissue factor pathway inhibitor and production of adenosine by ecto-ADPases, their secretion of protein S, prostacyclin and $\text{NO}^{\bullet}$. $\text{NO}^{\bullet}$ production was shown to participate in heparan sulfate preservation in porcine aortic endothelial cells [119] and may be responsible for prostacyclin secretion by activating cyclooxygenase-1 under resting conditions [120]. Endothelial release of plasminogen activator (t-PA) and plasminogen activator inhibitor 1 (PAI-1) may determine the fibrinolytic potential of plasma. Endothelial cells specifically bind coagulation factors XII, IIa, IX, VIIa and Xa. Xa was shown to bind to endothelial effector cell protease receptor-1 and thereby cause release of $\text{NO}^{\bullet}$, IL-6, IL-8, MCP-1 and functional upregulation of ICAM-1, E-Selectin and VCAM-1 [121].

When endothelium is perturbed by physical or chemical factors transformation to a prothrombotic surface is invariably seen in in vitro models. Thrombomodulin surface expression on HUVEC can easily be downregulated by LPS, IL-1 and TNF- α and upregulated by increasing cAMP [122].

A TNF- α induced decrease in surface Thrombomodulin has been suggested via activation of phosphodiesterase II and IV thereby decreasing cAMP in BAEC [111]. Vascular endothelial growth factor may counteract IL-1, TGF- β and LPS induced suppression of both thrombomodulin surface antigen and mRNA [123]. Adenosine nucleotides are released from damaged as well as LPS stimulated and shear stressed HUVEC [124]. Endothelial cells have ATP diphosphohydrolase (CD 39) on their surface to degrade ATP via ADP and AMP to Adenosine. Adenosine is known to have antiaggregatory properties due to stimulation of prostacyclin and NO $^{\circ}$ production. However, activating endothelial cells with TNF- α has been reported to cause loss of ATP diphosphohydrolase activity, which was preventable in the presence of antioxidants [125]. Tissue factor (TF) expression on endothelial cells by bacteria, LPS, IL-1 and TNF- α is well known. Tissue factor pathway inhibitor (TFPI), a serine protease inhibitor of Xa and Xa/VIIa/TF complex on endothelial surfaces, which immediately blocks tissue factor activation, has been shown to be decreased under proinflammatory conditions. Another counterbalancing mechanism includes shear stress in TNF- α stimulated HUVEC [126]. However, an anticipated increase in endothelial tissue factor expression has not convincingly been demonstrated in animal or human sepsis [127, 128]. Fibrinolytic systems on endothelial surfaces are also believed to be altered in sepsis. Increases in PAI-1 has been reported after stimulation with IL-1 or LPS [129, 130]. However soluble PAI-1 in septic patients was not found to be different from nonseptic patients [131]. Once thrombin formation has occurred, its cleavage of endothelial proteinase activated receptor (PAR) in turn may lead to secretion of IL-8 and IL-6 [132], upregulation of ICAM-1 and VCAM-1 [133], relaxation via NO $^{\circ}$ production or to vasoconstriction by an as yet unidentified factor [134]. These data may demonstrate an interconnection between coagulation activation, inflammation and vasoregulation mediated by the endothelium. Coagulation activation is clearly present in septic patients, however endothelial participation in this process is unclear. Potent procoagulatory sources may well include bacterial surfaces per se [135] or monocytes [128].

Endothelium dependent vasoregulation

Up to the late 60ies the endothelium was viewed as a passive organ, which at best was able to remove vasoactive hormones in the lung. In 1976–1978 Sir John Vane's group (noble laureate 1982) reported that endothelial cells can synthesize I series prostaglandins (like prostacyclin) and thereby relax arteries and inhibit platelet aggregation. However, whether PGI $_2$ regulates basal vascular tone is unclear. After a new technician used an unintended vessel preparation Robert Furchgott realized after a series of contradicting results that acetylcholine (ACh) was no longer able to relax precontracted arteries when endothelium was removed and termed the nonprostanoid mediator an endothelium derived relaxing factor (EDRF). In 1986 superoxide was shown to participate in vasoregulation. The presence of superoxide dismutase prolonged the action of EDRF whereas addition of O $_2^{\circ -}$ inactivated EDRF. Even direct effects of several reactive oxygen species have been suggested to be relevant in cerebral

or coronary circulation. Collectively Robert Furchgott, Louis Ignarro and Ferrid Murad received the noble laureate in 1998 for demonstrating that NO $^{\circ}$ is a major EDRF. Endothelial cells produce more relaxing factors which pharmacologically can be separated from nitric oxide action and these were termed endothelium derived hyperpolarizing factors (EDHFs). These factors may particularly be important in coronary and gastrointestinal vessels. Epoxyeicosatrienoic acids, anandamide, the endogenous ligand of cannabinoid receptors or simply the release of K $^+$ may constitute EDHFs. Conceptually shear may in larger vessels primarily determine production of NO $^{\circ}$, whereas cyclic strain determines physiological EDHF release. Soon after the discovery of EDRF endothelin-1 (ET-1), a vasoconstricting peptide produced from endothelial cells was isolated. Low doses of ET-1 can induce NO $^{\circ}$ release and subsequent relaxation via ET $_B$ -receptors on endothelial cells. Endothelin secretion is thought to occur abluminally leading to ET $_A$ -receptor activation on smooth muscle cells and subsequent vasoconstriction. Many other factors clearly contribute to endothelial control of vasoregulation by e.g. transcellular production of vasoconstricting prostanoids like thromboxane A $_2$ or prostaglandin H $_2$ or the enzymatic conversion of angiotensin I to angiotensin II by angiotensin converting enzyme.

A hallmark of sepsis is the heterogeneous pattern of vasoconstriction and vasodilatation in different organs, culminating in a fall in total peripheral vascular resistance concomitant with regional maldistribution of blood flow. Vasoactive substances produced by the endothelium under experimental septic conditions are known to be altered by factors such as NO $^{\circ}$, PGI $_2$, angiotensin converting enzyme (ACE) activity, endothelin and adrenomedullin. Endothelium dependent vasoregulation has largely been studied in animal models (Table 4). In 1985 endothelium dependent vasoregulatory failure was seen as a defect in reactive hyperemia related vasodilator release [136] and decreased dilatation of arterioles induced by ACh [137]. In a rat model of cecal ligation and puncture decreased vasoconstriction was found after administration of norepinephrine in septic animals which was largely reversible by removal of the endothelium [138]. Parker et al. [139] showed in explanted coronary arteries and aortas of guinea pigs treated with LPS intraperitoneally for 16 h that endothelium dependent relaxation induced by acetylcholine and ADP was depressed, whereas relaxation induced by substance P or receptor independent relaxations by Ca $^{2+}$ -ionophore A23187 was unaffected. EDRF release and bioactivity from explanted aortas of these animals was decreased after ADP or ACh stimulation, whereas A23187 induced EDRF release was unaltered [140]. This group also demonstrated reduced ADP and ACh responses after 4 h of LPS endotoxemia in guinea pigs and that ADP may produce constricting thromboxane in septic animals [141]. In contrast, coronary arteries of rabbits treated for 5 weeks with low doses of LPS stimulation with ACh but not ADP showed increased relaxation of explanted vessels [142]. Wang et al. [143] isolated subepicardial arterioles from rats treated 48 h intraperitoneally with feces containing live *E. coli* and showed in a pressurized no-flow chamber that relaxation by alpha 2 agonist clonidine and ADP was reduced in an endothelium dependent manner, which could be inhibited by the NOS inhibitor LNMA. Also, in this model mesenteric arter-

Species	Model	Explanted vessel	Stimulus	Reference
Rat	LPS i.v.	Mesent. artery	ACh↓, Bk↓, SP↓, Hist↓	[137]
Rat	LPS i.v.	Mesent. artery	Reactive hyperemia↓	[136]
Rat	LPS	Pulm. artery	+EC: ET-1↓ (COX + ETB-R dep.) -EC: no effect	[145]
Rat	i.p. feces	Coronary arterioles	ADP↓, SNP pres.	[143]
Rat	i.p. feces	Mesenteric arterioles	Clonidine↓, ADP↓, SNP + Pinacidil pres.	[191]
		Skeletal muscle	ADP constr., SNP + Pinacidil pres.	
Rat	CLP	Aorta	+EC: NE↓, -EC: NE pres.	[138]
Rat	CLP	Aorta	ACh↓, SNP pres.	[147]
Rat	CLP	Aorta, sup. mesent. a.	ACh↓, NTG pres.	[148]
Rat	CLP	Aorta	ACh↓, NTG pres., NOS III↓	[149]
Guinea pig	LPS i.p.	Aorta, coronary artery	ACh↓, ADP↓ SP, A23187, SNP pres.	[139]
Guinea pig	LPS i.p.	Aorta	ADP↓, ACh↓; SNP pres.	[141]
Guinea pig	LPS i.p.	Aorta	ACh↓, A23187 pres.	[140]
Rabbit	LPS i.p.	Coronary artery	ACh↓, SNP pres.	[142]
Rabbit	LPS, TNF- α , Ifn- γ	Carotid artery	ACh-, SP-, A23187-EDHF↓	[151]
Rabbit	LPS, TNF- α , Ifn- γ	Coronary artery	BK-, A23187-EDHF↓	[151]
Piglet	GBS	Pulmonary artery	BK↓	[192]
Swine	<i>P. aeruginosa</i>	Pulm. artery	ACh↓, BK pres., SNP pres.	[146]
Swine	LPS	Coronary artery	BK-EDHF↓	[150]

+/-Ec: presence or absence of endothelium; NE: norepinephrine; ACh: acetylcholine; ADP, adenosine diphosphate; SP, substance P; A23187, Ca^{2+} -ionophore; COX, cyclooxygenase; SNP, sodium nitroprusside; NTG, nitroglycerine; ET, endothelin; BK, bradykinin; EDHF, endothelium derived hyperpolarizing factor; pres., preserved.

Table 4. Endothelium dependent relaxation is impaired in animal sepsis models.

olar relaxation after ADP and clonidine was decreased, whereas in skeletal muscle these agonists caused vasoconstriction [144]. Pulmonary arteries of rats treated with LPS showed depressed endothelin-1 induced contractions which were even augmented in endothelium denuded vessels. The authors [145] concluded that a vasoconstrictor eicosanoid is produced in LPS treated animals by pulmonary endothelium upon ET-1 stimulation. Swine infused with live *P. aeruginosa* showed no alteration in endothelium dependent bradykinin and endothelium independent nitroprusside relaxation, whereas ACh induced relaxation was found to be reduced in explanted peripheral arteries [146]. Chaudry's group investigated endothelium dependent relaxation in rats treated with cecal ligation and puncture. A time dependent alteration was demonstrated with increased ACh induced vasorelaxation early after challenge whereas depressed vasodilatation after 5–20 h was found in explanted aortas with no alteration in nitroglycerine induced relaxation [147]. The decreased endothelium dependent response to ACh was also found in superior mesenteric arteries and small intestinal arteries [148]. In the same model this group demonstrated a reduction of immunodetectable NOS III in explanted aortas [149]. Porcine coronary arterioles incubated for 20 h with *E. coli* LPS (100 $\mu\text{g/ml}$) decreased bradykinin induced EDHF secretion [150]. Carotid and coronary arteries from rabbits also showed decreases in EDHF-release in an ex-vivo assay

after treatment with LPS, TNF- α and IL-1 [151]. Collectively these data indicate that the majority of sepsis models consistently show a disruption of receptor coupled relaxation mechanism leading to an intraendothelial signalling deficit.

To quantify endothelial function in humans several methods are available. Endothelium dependent relaxation after pharmacological stimulation or flow dependent relaxation after vessel obstruction are frequently quantified by high resolution ultrasound techniques [152], alternatively flow and size can be determined angiographically. However, definite functional measurements in human sepsis are scarce. Endothelium dependent relaxation has been investigated in isolated superficial hand veins of healthy volunteers after LPS exposure. Reduced vasorelaxation by bradykinin and arachidonic acid in noradrenalin precontracted vessels were noted which persisted for more than 2 days [153]. Reactive hyperemia is believed to mainly test endothelial NO $^{\circ}$ production upon shear stress if vessel diameters and flow is monitored. Implications from indirect measurements support an endothelial dysfunction in septic patients [154–156]. However, data on pharmacological stimulation of endothelium dependent relaxation in humans are currently not available.

Species	Model/vessel	Treatment	Suggested mode of action	Reference
Rat	LPS/aorta	NOS II antisense	NOS III $\uparrow$	[193]
Rat	CLP/aorta	Pentoxifylline	ROS $\downarrow$	[194]
Rat	CLP/aorta	Heparin/GM1892	NO $\uparrow$	[195]
Rat	LPS/aorta	Tirilazad Mesylate	OH $\downarrow$	[196]
Rat	LPS/mesent. art.	hSOD, Tirilazad Mesylate	O $_2^{\cdot-}$ $\downarrow$ /LPO $\downarrow$	[197]
Rat	LPS/aorta	3-aminobenzamide	OONO $\downarrow$	[198]
Rabbit	LPS/aorta	Perindopril	O $_2^{\cdot-}$ $\downarrow$	[199]

CLP, cecal ligation and puncture; LPO, lipid peroxidation; ROS, reactive oxygen species.

Conclusion

A normal response to infection or other insults is a self limiting process that through temporal expression of regulators and effector molecules causes resolution. The failure to resolve the causative infection may lead to sepsis. Cellular and animal models of sepsis using bacteria, endotoxins, exotoxins, cytokines or some peptides all consistently produce endothelial impairment which is usually regarded as dysfunctional. Blocking the majority of pathways used by these inducing agents has often lead to the inhibition of such endothelial alterations. Many aspects of these induced alterations can be expected to reveal exciting new pathways and complex interactions at various molecular and cellular level. The past has taught us that inhibitors of presumably activated pathways consistently failed to improve survival in septic patients. This has stimulated many researchers to reconcile the results of experimental and clinical models in sepsis. Compared to activating pathways, considerably less is known about how an inflammatory response is endogenously counterregulated. Cytokines induce a whole host of signal inhibiting proteins and endogenous counterregulating systems are just beginning to be elucidated. Activation of endogenous counterregulatory systems may become the predominant feature of the so-called compensatory antiinflammatory response syndrome. Endothelial responses to endogenously present antiinflammatory mediators have hardly been investigated in sepsis models. Almost all endothelial cell studies in sepsis indicated that an imbalance in reactive oxygen species production is associated with the above described dysfunctions. Animal studies in which endothelial function could be improved pharmacologically also consistently indicate that reversal of imbalanced reactive oxygen production may be a common link (Table 4). It seems that at times an adequate production of nitric oxide is lacking whereas superoxide and/or derivatives are overproduced. However, as endothelial functional measurements in septic humans become available, we will hopefully get a clearer picture of what might happen in our patients.

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