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 cells expressing P AF-R, PAF-receptor [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 bacteria to invade and cause a cytotoxic response. Pilated 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-

| Infectious agent | Mediator/effector | Reference |
|------------------|------------------|----------|
| Staphylococcus aureus | Heparan sulfate/Fibrinogen | [2] |
| | α-toxin | [45] |
| Group A Streptococcus | Cystein protease | [5] |
| | Streptolysin S | [6] |
| Group B Streptococcus | Capsular polysaccharides | [7] |
| | β-hemolysin | [8] |
| Streptococcus pneumoniae | PAF-R | [9] |
| Clostridium perfringens | MBEC1 | [48] |
| | α-toxin Phospholipase C | [47] |
| | perfringolysin O | [49] |
| Clostridium difficile | toxin B | [50] |
| Listeria monocytogenes | Internalin B | [12] |
| | Listeriolysin | [53] |
| | Phospholipase C | [11] |
| Neisseria meningitidis | Pilus protein C | [14] |
| | CD66 | [15] |
| Pseudomonas aeruginosa | pilus proteins | [17] |
| | exotoxin A | [52] |
| | pyochelin | [58] |
| Escherichia coli | outer membrane protein A | [19] |
| | ibe10 | [20] |
| | verocytotoxin | [46] |
| | hemolysin | [45] |
| Chlamydia pneumoniae | major outer membrane protein | [21] |
| | E-Selectin, VCAM-1, ICAM-1 | [23] |
| Bartonella henselae | invasome | [26] |
| Rickettsia rickettsii | autocrine IL-1 | [27] |
| Rickettsia conorii | autocrine IL-1 | [28] |
| Borrelia burgdorferi | CD14 | [30] |
| | α(v)β3, α5β1 integrin | [31] |
| | proteoglycans, heparan sulfate | [32] |
| Plasmodium falciparum | P-Selectin | [34] |
| | ICAM-1, CD36 | [35] |
| | PECAM-1 | [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 | α(v)β3 integrin | [43] |
| Ebola Virus | IL-11, IL-6⁺ | [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 chemotactic protein 1; COX-2, cyclooxygenase 2; TF, tissue factor; RANTES, regulated on activation, normal T cell expressed and secreted.
Platelet activating factor (PAF), NO products

Activation of endothelial cells by bacterial secretory and inhibits inflammatory responses [44]. Infections of endothelial cells with a transmembrane glycoprotein infects endothelial cells with a transmembrane glycoprotein. On the other hand, ebola virus by hantaviruses may be accomplished by integrin mediated expression on HUVEC [41, 42]. Hemorrhagic fever caused measles virus and cytomegalovirus increases tissue factor lymphocyte adhesion by increasing ICAM-1 [40] and proteins RANTES and IL-8 [39]. Some viral diseases are known to primarily infect Candida albicans or platelet endothelial cell adhesion molecule 1 (PECAM-1) Selectin, CD36, intercellular adhesion molecule 1 (ICAM-1) 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].

Other pathogens

Attachment of Borrelia burgdorferi, the agent inducing Lyme disease, to endothelial cells may be accomplished by CD14, alpha(v)beta3 and alpha5beta1 integrins or different classes of proteoglycans [30–32]. B. burgdorferi activates the transcription of chemokine and adhesion in 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 PGI2 secretion from HUVEC has been demonstrated by E. coli hemolysin via inositolphosphate/diacylglycerol formation and by S. aureus alpha-toxin via transmembrane Ca2+ 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 Pertussis toxin 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 sepsis containing soluble CD14-receptor because endothelial cells generally lack CD14. Alternatively, proinflammatory cytokines including tumor necrosis factor alpha (TNF-α), interleukins (IL-1, IL-4) and interferon gamma (IFN-γ) acting on endothelial cells have extensively been investigated and shown to alter endothelial in vitro functions [56, 57].

	extbf{Reactive oxygen species in endothelial biology}

The inflammatory response in endothelial cells has been linked to an alteration in reactive oxygen production including superoxide (O2·–), hydrogen peroxide (H2O2), nitric oxide (NO), hydroxyl radicals (OH) and secondary reaction products thereof. O2·– is believed to be present in unstressed conditions in less than nanomolar quantities within cells. Mitochondrial and cytoplasmatic superoxide dismutate readily reacts with O2·– forming H2O2, which has been measured in micromolar concentrations in human blood. Sources of endothelial O2·– 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 H2O2 by themselves, however interaction with the endothelium may greatly enhance cellular alterations. Streptococcal hemolysin, streptolysin S, is capable of interacting with H2O2 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 O2·– from O2 and H2O2 [58]. R. rickettsii infection of the endothelial cell line EA.hy926 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, TNF-α and IFN-γ [60–65]. TNF-α has many times been reported to increase, e.g., adhesion molecule expression inhibitable by various antioxidants [62, 66–68].

Nitric oxide, like O2·–, is chemically not very reactive at all. Endothelial production has been established by either constitutive NO-synthase (NOS) III in a Ca2+ and phosporylation dependent manner or by inducible NOS II. Direct biochemical actions of NO include metalecomplex 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 (Fe2–O2) leading to methemoglobin (Fe3+) and
Nitrate (NO\textsubscript{3}) reacts with O\textsubscript{2} forming nitrite (NO\textsubscript{2}) via intermediate NO\textsubscript{2} and N\textsubscript{2}O\textsubscript{3}. Mainly N\textsubscript{2}O\textsubscript{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\textsubscript{2}O\textsubscript{3} and may also be important in toxicity related to NO\textsuperscript{•} autoxidation. Toxicity related cellular targets of NO\textsuperscript{•} may include inhibition of cytochrome C oxidase, inhibition of catalase or DNA damage. On the other hand, NO\textsuperscript{•} 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\textsuperscript{•} 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\textsuperscript{•} formation in cultured porcine pulmonary endothelial cells [69]. Transformed mouse endothelial cells stimulated by the combination of IFN-\textgamma and TNF-\textalpha killed intracellular R. conorii by a mechanism that required the synthesis of NO\textsuperscript{•} [70]. IFN-\textgamma, TNF-\textalpha and IL-1 stimulated murine endothelial cells have been shown to kill Schistosoma mansoni through the production of nitric oxide [71]. Both NO\textsuperscript{•} and O\textsuperscript{2•} production may have a limited influence on endothelial viability under resting conditions. Cultured bovine and porcine aortic endothelial cells showed decreased NO\textsuperscript{•} production after 1 h of LPS incubation which was related to decreases in capacitative Ca\textsuperscript{2+} signals [72]. Posttranscriptional destabilization of NOS III mRNA may have accounted for this early decrease in NO\textsuperscript{•} production [73]. NOS II, which is believed to produce larger amounts of NO\textsuperscript{•} 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\textsuperscript{•}. As the amount and physiological consequences of NO\textsuperscript{•} 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\textsuperscript{•} and O\textsuperscript{2•} produced peroxynitrite (ONOO\textsuperscript{–}) which may decompose to produce HO\textsuperscript{•} like molecules and thereby kill endothelial cells [75]. At pH 7 its lifetime is in the order of a second. Half of the ONOO\textsuperscript{–} formed is rapidly equilibrated to peroxynitrous acid (ONOOH) and breaks down to NO\textsubscript{3} or NO\textsubscript{2}. The other half decomposes with reactive similarities to HO\textsuperscript{•} plus nitrogen dioxide (OH\textsuperscript{•} + NO\textsubscript{3}). The reaction of O\textsuperscript{2•} with NO\textsuperscript{•} has been shown to be very fast, being 3 times faster than O\textsuperscript{2•} reacting with superoxide dismutase. Biological actions of OONO\textsuperscript{–} may be determined in the plasma primarily by the presence of CO\textsubscript{2} (~1 mM) and within cells by the presence of thiol groups (~5 mM). ONOO\textsuperscript{–} may again react with NO\textsuperscript{•} or O\textsuperscript{2•} to form NO\textsubscript{2}. ONOO\textsuperscript{–} 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\textsuperscript{•} rather than ONOO\textsuperscript{–} and may represent the action of nitrating species. ONOO\textsuperscript{–} as a surrogate of O\textsuperscript{2•} and NO\textsuperscript{•} 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\textsuperscript{•} formation. However, beneficial effects of ONOO\textsuperscript{–} have been shown in ischemia-reperfusion.

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

| 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↑, ascoryl radical↑, lipidperoxides↑ |
| [161]     | 8              | Sepsis             | Vitamin C↓, bleomycin detectable iron↑ |
| [162]     | 30             | Sepsis             | Vitamin C↓, lipidperoxides↑ |
| [163]     | 17             | Sepsis             | NO\textsubscript{2}−/NO\textsubscript{3}− |
| [164]     | 12             | Septic shock       | NO\textsubscript{2}−/NO\textsubscript{3}− |
| [165]     | 23             | Sepsis, septic shock | NO\textsubscript{2}−/NO\textsubscript{3}− |
| [166]     | 29             | Septic shock       | NO\textsubscript{2}−/NO\textsubscript{3}− |
| [167]     | 13             | Septic shock       | NO\textsubscript{2}−/NO\textsubscript{3}− |
| [168]     | 20             | Sepsis             | NO\textsubscript{2}−/NO\textsubscript{3}− |
| [169]     | 53             | Sepsis, children   | NO\textsubscript{2}−/NO\textsubscript{3}− |
| [170]     | 46             | SIRS, sepsis, children | NO\textsubscript{2}−/NO\textsubscript{3}− |
| [171]     | 30             | Sepsis, children   | NO\textsubscript{2}−/NO\textsubscript{3}− |
| [172]     | 31             | Sepsis, children   | NO\textsubscript{2}−/NO\textsubscript{3}− |
| [173]     | 22             | Septic shock, children | NO\textsubscript{2}−/NO\textsubscript{3}− |
| [174]     | 11             | Septic shock       | NO\textsubscript{2}−/NO\textsubscript{3}− |
| [175]     | 11             | Sepsis             | Immunohistochemical endothelial nitrotyrosine↑ |
| [176]     | 3              | Septic shock       | NO\textsubscript{2}−/NO\textsubscript{3}−, plasmatic nitrotyrosine↑ |
| [177]     | 3              | Septic lung injury | Immunohistochemical endothelial nitrotyrosine↑ |

Antioxidant capacity was defined as the ability of plasma to inhibit * ferryl myoglobin production by hydrogen peroxide addition to metmyoglobin or ** o xo-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 H2O2 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 H2O2 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 O2’, has recently been demonstrated to convert NO2 into NO2, 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-α [83]. Both LPS or TNF-α usually do not cause endothelial cell death unless protein synthesis is blocked probably due to simultaneous increases in antiapoptotic protein synthesis [84]. However, postmortem 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 (NFkB). NFkB has gained wide interest as a target in inflammatory diseases as it seems to be invariably upregulated [86]. A variety of agents including cytokines and reactive oxygen stress cause IkB to dissociate from the complex after phosphorylation by IkB-kinase complex. H2O2 has been reported to activate transcription factor NFkB in porcine aortic endothelial cells [87] whereas HUVEC were unresponsive [88]. NO’ has in most cases been shown to inhibit transcription factor NFkB and its influence on transcription factor AP-1 is unclear. Inhibition of NFkB-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-α [89]. Data on activated intracellular signalling pathways in sepsis patients are scarce but include NFkB 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 Ca2+signal is induced [91], but it is unclear at present whether this has any functional consequence. It is tempting to speculate that Ca2+-signals may then in a context sensitive manner augment proadhesive 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-1α), monocyte chemoattractant proteins 1–4 and RANTES (regulated on activation, 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 endogeneous 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-γ 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↑ |
| [179] | 60, sepsis | sTM↑ |
| [180] | 19, sepsis, ARDS | sTM↑, sICAM-1↑, svWF↑ |
| [181] | 67, sepsis | sE-Sel↑ |
| [182] | 16, severe sepsis | sE-Sel↑, sICAM-1↑ |
| [183] | 25, sepsis | sE-Sel↑, sICAM-1↑, svWF↑ |
| [184] | 22, septic shock | sTM↑ |
| [185] | 71, sepsis, malaria | sICAM-1, sVCAM-1, sE-Sel↑ |
| [186] | 32, SIRS | svWF↑ |
| [187] | 42, sepsis, DIC | Endothelin↑, sTM↑ |
| [188] | 16, sepsis | sEPCR↑ |
| [189] | 12, Mediterranean spotted fever | eEC↑, sTM↑, svWF↑ |
| [190] | 40, sepsis | Endothelin↑, sTM↑, sICAM-1↑, sVCAM-1↑ |

svWF, soluble von Willebrandt Factor; sE-Sel, 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.
Permeability

Endothelium is known to regulate transvascular fluid flux, flux of nutrients, mediators and cells by either paracellular or transcellular vacular 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 antiprofibrilagin 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$_2$O$_2$ induced hyper-permeability of porcine pulmonary endothelial cells has been reported to be effectively reduced by cGMP elevating drugs including phosphodiesterase II inhibition or NO$^\cdot$ 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 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 NO$^\cdot$. NO$^\cdot$ 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, VIIIa and Xa. Xa was shown to bind to endothelial effector cell protease receptor-1 and thereby cause release of NO$^\cdot$, 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 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 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 procoagulant 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 PGI₂ 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 diamutase prolonged the action of EDRF whereas addition of O₂⁻ 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 Ferid Murad received the noble laureate in 1998 for demonstrating that NO⁻ 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⁻, 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⁻ release and subsequent relaxation via ETₐ-receptors on endothelial cells. Endothelin secretion is thought to occur abuminally leading to ETₐ-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 prostanooids like thromboxane A₂ or prostaglandin H₂ 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 concurrent 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⁻, PGI₂, angiotensin converting enzyme (ACE) activity, endothelin and adrenomedullin. Endothelium dependent vasoregulation has largely been studied in animal models (Table 4). In 1985 endothelium dependent vaso regulatory 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 Ca²⁺-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-
iolar 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 μg/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˙ 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   | 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.) | [145]     |
|             |         |                  | –EC: no effect            |           |
| Rat         | i.p. feces | Coronary arterioles | ADP↓, SNP pres. | [143]     |
| Rat         | i.p. feces | Mesenteric arterioles | Clonidine↓, ADP↓, SNP + Pinacidil pres. | [191]     |
| 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       | P. aeruginosa | 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, Ca²⁺-ionophore; COX, cyclooxygengase; 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.
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.

References

[1] Pfaller MA, Jones RN, Doern GV, Kugler K. Bacterial pathogens isolated from patients wit bloodstream infection: frequencies of occurrence and antimicrobial susceptibility patterns from the SENTRY antimicrobial surveillance program (United States and Canada, 1997). Antimicrob Agents Chemother 1998; 42: 1762–70.

[2] Lowy FD. Staphylococcus aureus infections. N Engl J Med 1998; 339: 520–32.

[3] Menzies BE, Kourteva I. Internalization of Staphylococcus aureus by endothelial cells induces apoptosis. Infect Immun 1998; 66: 5994–8.

[4] Vesga O, Groeschel MC, Otten MF, Brar DW, Vann JM, Proctor RA. Staphylococcus aureus small colony variants are induced by the endothelial cell intracellular milieu, J Infect Dis 1996; 173: 739–42.

[5] Burns EH, Lukomska S, Rurangirwa J, Podbielski A, Musser JM. Genetic inactivation of the extracellular cysteine protease enhances in vitro internalization of group A streptococci by human epithelial and endothelial cells. Microb Pathog 1998; 24: 333–9.

[6] Ginsburg I, Varani J. Interaction of viable group A streptococci and hydrogen peroxide in killing of vascular endothelial cells. Free Radic Biol Med 1993; 14: 495–501.

[7] Gibson RL, Lee MK, Soderlund C, Chi EY, Rubens CE. Group B streptococci invade endothelial cells: type III capsular polysaccharide attenuates invasion. Infect Immun 1995; 61: 478–85.

[8] Nizet V, Kim KS, Stins M, Jonas M, Chi EY, Nguyen D, et al. Invasion of brain microvascular endothelial cells by group B streptococci. Infect Immun 1997; 65: 5074–81.

[9] Cundell DR, Gerard NP, Gerard C, Idanpaan HI, Tuomanen EI. Streptococcus pneumoniae anchor to activated human cells by the receptor for platelet-activating factor. Nature 1995; 377: 435–8.

[10] Ring A, Weiser JN, Tuomanen EI. Pneumococcal cocolliection across the blood-brain barrier. Molecular analysis of a novel bidirectional pathway. J Clin Invest 1998; 102: 347–60.

[11] Schwarzar N, Nost R, Seybold J, Parida SK, Fuhrmann O, Krull M, et al. Two distinct phospholipases C of Listeria monocytogenes induce ceramide generation, nuclear factor-kappa activation, and E-selectin expression in human endothelial cells. J Immunol 1998; 161: 3010–8.

[12] Parida SK, Domanne E, Rohde M, Muller S, Darji A, Hain T, et al. Internalin B is essential for adhesion and mediates the invasion of Listeria monocytogenes into human endothelial cells. Mol Microbiol 1998; 28: 81–93.

[13] Noel-RF J, Sato TT, Mendez C, Johnson MC, Pohlan TH. Activation of human endothelial cells by viable or heat-killed gram-negative bacteria requires soluble CD14. Infect Immun 1995; 63: 4046–53.

[14] Pron B, Taha MK, Rambaud C, Forunet JC, Pattey N, Monnet JP, et al. Interaction of Neisseria meningitidis with the components of the blood-brain barrier correlates with an increased expression of PI3C. J Infect Dis 1997; 176: 1285–92.

[15] Virji M, Watt SM, Barker S, Makepeace K, Doyonnas R. The N-domain of the human CD66a adhesion molecule is a target for Opa proteins of Neisseria meningitidis and Neisseria gonorrhoeae. Mol Microbiol 1996; 22: 929–39.

[16] Quinn FD, Weyant RS, Worley MJ, White EH, Utt FA, Ades EA. Human microvascular endothelial tissue culture cell model for studying pathogenesis of Brazilian purpuric fever. Infect Immun 1995; 63: 2317–22.

[17] Plotkowski MC, Saliba AM, Pereira SH, Cervante MP, Bajolet LQ. Pseudomonas aeruginosa selective adherence to and entry into human endothelial cells. Infect Immun 1994; 62: 5456–63.

Table 5. Treatment of endothelial vaso-regulatory dysfunction in animal sepsis models.

| Species | Model/vessel | Treatment | Suggested mode of action | Reference |
|---------|--------------|-----------|--------------------------|-----------|
| Rat     | LPS/aorta    | NOS II antisense | NOS III↑ | [193]     |
| Rat     | CLP/aorta    | Pentoxifyline | ROS ↓ | [194]     |
| Rat     | CLP/aorta    | Heparin/GM1892 | NO↑ | [195]     |
| Rat     | LPS/aorta    | Tirilazad Mesylate | OH↑ | [196]     |
| Rat     | LPS/mesent. art. | hSOD, Tirilazad Mesylate | O₂↑/LPO↓ | [197]     |
| Rat     | LPS/aorta    | 3-aminobenzamide | OONO ↓ | [198]     |
| Rabbit  | LPS/aorta    | Perindopril | O₂↑ | [199]     |
[18] Plotkowski MC, Meirelles MN. Cincomitant endosome-phagosome fusion and lysis of endosomal membranes account for Pseudomonas aeruginosa survival in human endothelial cells. J Submicrosc Cytol Pathol 1997; 29: 229–37.

[19] Prasadarao NV, Wass CA, Kim KS. Endothelial cell GlcNAc beta 1–4GlcNAc epitopes for outer membrane protein A enhance traversal of Escherichia coli across the blood-brain barrier. Infect Immun 1996; 64: 154–60.

[20] Huang SH, Wass C, Fu Q, Prasadarao NV, Stins M, Kim KS. Escherichia coli invasion of brain microvascular endothelial cells in vitro and in vivo: molecular cloning and characterization of invasion gene ibe10. Infect Immun 1995; 63: 4470–5.

[21] Molestina RE, Dean D, Miller RD, Ramirez JA, Summersgill JT. Huang SH, Wass C, Fu Q, Prasadarao NV, Stins M, Kim KS. Escherichia coli invasion of brain microvascular endothelial cells in vitro and in vivo: molecular cloning and characterization of invasion gene ibe10. Infect Immun 1995; 63: 4470–5.

[22] Fryer RH, Schwobe EP, Woods ML, Rodgers GM. Chlamydia species infect human vascular endothelial cells and induce procoagulant activity. J Investig Med 1997; 45: 168–74.

[23] Krull M, Khucken AC, Wuppermann FN, Fuhrmann O, Magerl C, Seybold J, et al. Signal Transduction Pathways Activated in Endothelial Cells Following Infection with Chlamydia pneumoniae. J Immunol 1999; 162: 4834–41.

[24] Drancourt M, Mainardi JL, Brouqui P, Vandenesch F, Carta A, Lehnert F, et al. Bartonella (Rochalimaea) quintana endocarditis in three homeless men. N Engl J Med 1995; 332: 419–23. Virology 1997; 159: 1909–16.

[25] Brouqui P, Raoult D. Bartonella quintana invades and multiplies within endothelial cells in vitro. Cell Adhes Commun 1994; 2: 145–57.

[26] Hippenstiel S, Tannert OS, Vollrath N, Krull M, Just I, Aktories K, et al. Characterization of a strain of Chlamydia pneumoniae isolated from a coronary atheroma by analysis of the omp1 gene and biological activity in human endothelial cells. Infect Immun 1998; 66: 1370–6.

[27] Bryant AE, Stevens DL. Phospholipase C and perfringolysin O disrupts endothelial barrier function. Am J Physiol 1997; 272: H78–83.

[28] Dehio C, Meyer M, Berger J, Schwarz H, Lanz C. Interaction of Bartonella henselae with endothelial cells results in bacterial aggregation on the cell surface and the subsequent engulfment and internalisation of the bacterial aggregate by a unique structure, the invasome. J Cell Sci 1997; 110: 2141–54.

[29] Clifton DR, Goss RA, Sahni SK, van-Antwerp D, Baggs RE, Marder VJ, et al. NF-kappa B-dependent inhibition of apoptosis is essential for host cell survival during Rickettsia rickettsii infection. Proc Natl Acad Sci USA 1998; 95: 4646–51.

[30] Dignat GF, Teyssere N, Mutin M, Bardin N, Lesaulie G, Raout D, et al. Rickettsia conorii conorii infection enhances vascular cell adhesion molecule-1- and intercellular adhesion molecule-1-dependent monocyte cell adherence to endothelial cells. J Infect Dis 1997; 175: 1142–52.

[31] Kaplanski G, Teyssere N, Farnarier C, Kaplanski S, Lissitzky E, Brouqui P, et al. Bartonella (Rochalimaea) quintana: a new vector for Chlamydia pneu-

[32] McCormick CJ, Craig A, Roberts D, Newbold CI, Berendt AR. Intercellular adhesion molecule-1 and CD36 synergize to mediate adherence of Plasmodium falciparum-infected erythrocytes to cultured human microvascular endothelial cells. J Clin Invest 1997; 100: 2521–9.

[33] Treutiger CJ, Heddini A, Fernandez V, Muller WA, Wahlgren M. PECAM-1/CD31, an endothelial receptor for binding Plasmodium falciparum-infected erythrocytes. Nat Med 1997; 3: 1405–8.

[34] Filler SG, Pfunder AS, Spellberg BJ, Spellberg JP, Edwards-JE J. Candidate albicans stimulates cytokine production and leukocyte adhesion molecule expression by endothelial cells. Infect Immun 1996; 64: 2609–17.

[35] Ibrahim AS, Filler SG, Sandgard D, Edwards-JE J, Hube B. Secreted aspartyl proteinases and interactions of Candida albicans with human endothelial cells. Infect Immun 1998; 66: 3003–5.

[36] Avirutnan P, Malasit P, Seliger B, Bhakdi S, Husmann M, Dengue virus infection of human endothelial cells leads to chemokine production, complement activation, and apoptosis. J Immunol 1998; 161: 6338–46.

[37] Brankin B, Hart MN, Cosby SL, Fabry Z, Allen IV. Adhesion molecule expression and lymphocyte adhesion to cerebral endothelium: effects of measles virus and herpes simplex 1 virus. J Neuroimmunol 1995; 56: 1–8.

[38] Filler SG, Pfunder AS, Spellberg BJ, Spellberg JP, Edwards-JE J. Candidate albicans stimulates cytokine production and leukocyte adhesion molecule expression by endothelial cells. Infect Immun 1996; 64: 2609–17.

[39] Vercellotti GM. Effects of viral activation of the vessel wall on inflammation and thrombosis. Blood Coagul Fibrinolysis 1998; 9 Suppl 2: S3–S6.

[40] Gavrilovskaia IN, Brown EJ, Ginsberg MH, Mackow ER. Cellular entry of hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by beta3 integrins. J Virol 1999; 73: 3951–9.

[41] Harcourt BH, Sanchez A, Offermann MK. Ebola virus inhibits induction of genes by double-stranded RNA in endothelial cells. Virology 1998; 252: 179–88.

[42] Grimminger F, Rose F, Sibelius U, Meinhardt M, Potzsch B, Spriestersbach R, et al. Human endothelial cell activation and mediator release in response to bacterial exotoxins Escherichia coli hemolysin and staphylococcal alpha-toxin. J Immunol 1997; 159: 1909–16.

[43] Alvarez-Lonzi MA. The role of viral activation of the vessel wall on inflammation and thrombosis. Blood Coagul Fibrinolysis 1998; 9 Suppl 2: S3–S6.

[44] Karmali MA. Infection by verocytotoxin-producing Escherichia coli. Clin Microbiol Rev 1989; 2: 15–38.

[45] Bunting M, Lorant DE, Bryant AE, Zimmermann GA, McIntyre TM, Stevens DL, et al. Alpha toxin from Clostridium perfringens induces proinflammatory changes in endothelial cells. J Clin Invest 1997; 100: 565–74.

[46] Chen Z, Zandonatti M, Jakubowski D, Fox HS. Brain capillary endothelial cells express MBEC1, a protein that is related to the Clostridium perfringens enterotoxin receptors. Lab Invest 1998; 78: 535–63.

[47] Bryant AE, Stevens DL. Phospholipase C and perfringolysin O from Clostridium perfringens upregulate endothelial cell-leukocyte adherence molecule 1 and intercellular leukocyte adherence molecule 1 expression and induce interleukin-8 synthesis in cultured human umbilical vein endothelial cells. Infect Immun 1996; 64: 358–62.

[48] Hoffenstiel S, Tannert OS, Vollrath N, Krull M, Just I, Aktories K, et al. Glucosylation of small GTP-binding Rho proteins induces proinflammatory changes in endothelial cells. J Clin Invest 1997; 100: 565–74.

[49] Baluna R, Rizo J, Gordon BE, Ghetie V, Vitetta ES. Evidence for a structural motif in toxins and interleukin-2 that may be responsible for binding to endothelial cells and initiating vascular leak syndrome. Proc Natl Acad Sci USA 1999; 96: 3957–62.
Endothelial function in sepsis

Oswald IP, Eltoum I, Wynn TA, Schwartz B, Caspar P, Paulin D, Moutet M, d’Alessio P, Malette P, Devaux V, Chaudiere J. Glutathione peroxidase mimics prevent TNFalpha- and neutrophil-mediated endothelial cell injury. Am J Physiol 1999; 270: L931–41.

Pobes JS, Cotran RS. Cytokines and endothelial cell biology. Physiol Rev 1990; 70: 427–51.

Mantovani A, Bussolino F, Introna M. Cytokine regulation of endothelial cell function: from molecular level to he bedside. Immunol Today 1997; 18: 231–40.

Britigan BE, Rasmussen GT, Cox CD. Pseudomonas siderophore pyochelin enhances neutrophil-mediated endothelial cell injury. Am J Physiol 1994; 266: L192–L198.

Hong JE, Santucci LA, Tian X, Silverman DJ. Superoxide dismutase-dependent, catalase-sensitive peroxides in human endothelial cells infected by Rickettsia rickettsii. Infect Immun 1998; 66: 1293–8.

Privat C, Stupien O, David-Dufilho M, Brunet A, Bedioui F, Marteau P, et al. Superoxide release from interleukin-1B-stimulated human vascular cells: in situ electrochemical measurement. Free Radic Biol Med 1999; 27: 554–9.

Maziere C, Conte MA, Dantin F, Maziere JC. Lipopolysaccharide enhances oxidative modification of low density lipoprotein by copper ions, endothelial and smooth muscle cells. Atherosclerosis 1999; 143: 75–80.

Rahman A, Kefer J, Bando M, Niles WD, Malik AB. E-selectin expression in human endothelial cells by TNF-alpha-induced oxidative stress and NF-kappaB activation. Am J Physiol 1998; 275: L533–L544.

Murphy HS, Shayman JA, Till GO, Mahrourgui M, Owens CB, Ryan US, et al. Superoxide responses of endothelial cells to CsA and TNF-alpha: divergent signal transduction pathways. Am J Physiol 1992; 263: L51–9.

Bhunia AK, Arai T, Bulkey G, Chatterjee S. Lactosylceramide mediates tumor necrosis factor alpha-induced intercellular adhesion molecule-1 (ICAM-1) expression and the adhesion of neutrophil in human umbilical vein endothelial cells. J Biol Chem 1998; 273: 34349–57.

Mitchell J, Jiang H, Berry L, Meyrick B. Effect of antioxidants on lipopolysaccharide-stimulated induction of manganese superoxide dismutase mRNA in bovine pulmonary artery endothelial cells. J Cell Physiol 1996; 169: 333–40.

Arai T, Kelly SA, Brengman ML, Takano M, Smith EH, Goldschmidt CP, et al. Ambient but not incremental oxidant generation effects intercellular adhesion molecule I induction by tumour necrosis factor alpha in endothelium. Biochem J 1998; 331: 851–63.

d’Alessio P, Moutet M, Coudrier E, Darquenne S, Chaudiere J. ICAM-1 and VCAM-1 expression induced by TNF-alpha in human umbilical vein endothelial cells. Biochem Biophys Res Commun 1995; 217: 1208–15.

Suttrop N, Fuhrmann M, Tannert OS, Grimminger F, Bhadki S. Pore-forming bacterial toxins potently induce release of nitric oxide in porcine endothelial cells. J Exp Med 1993; 178: 337–41.

Walker DH, Popov VL, Croquet VP, Welsh CJ, Feng HM. Cytochrome-c oxidase inhibitor, nitric oxide-dependent, intracellular antirickettsial activity of mouse endothelial cells. Lab Invest 1997; 76: 129–38.

Oswald JP, Eltoum I, Wynn TA, Schwartz B, Caspar P, Paulin D, et al. Endothelial cells are activated by cytokine treatment to kill an intravascular parasite, Schistosoma mansoni, through the production of nitric oxide. Proc Natl Acad Sci USA 1994; 91: 999–1003.

Graier WF, Myers PR, Rubin LJ, Adams HR, Parker JL. Escherichia coli endotoxin inhibits agonistmediated cytosolic Ca2+ mobilization and nitric oxide biosynthesis in cultured endothelial cells. Circ Res 1994; 75: 659–68.

Forstermann U, Boissel JP, Kleinert H. Expression control of the “constitutive” isoforms of nitric oxide synthase (NOS I and NOS III). FASEB J 1998; 12: 773–90.

Peng HB, Spiecker M, Liao JK. Inducible nitric oxide: an auto-regulatory feedback inhibitor of vascular inflammation. J Immunol 1998; 161: 1970–6.

Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 1990; 87: 1620–4.

Chang J, Rao NV, Markewitz BA, Hoidal JR, Michael JR. Nitric oxide donor prevents hydrogen peroxide-mediated endothelial cell injury. Am J Physiol 1996; 270: L931–40.

Gupta MP, Evanooff V, Hart CM. Nitric oxide attenuates hydrogen peroxide-mediated injury to porcine pulmonary artery endothelial cells. Am J Physiol 1997; 272: L1133–L1141.

Shimizu S, Ishii M, Kawakami Y, Momose K, Yamamoto T. Protective effects of tetrahydrobiopterin against nitric oxide-induced endothelial cell death. Life Sci 1998; 63: 1585–92.

Volf T, Ioannidis I, Hensel M, deGroot H, Kox WJ. Endothelial damage induced by nitric oxide: synergism with reactive oxygen species. Biochem Biophys Res Commun 1995; 213: 196–203.

Nappi AJ, Vass E. Hydroxyl radical formation resulting from the interaction of nitric oxide and hydrogen peroxide. Biochim Biophys Acta 1998; 1380: 55–63.

Eiserich JP, Hristova M, Cross CE, Jones AD, Freeman BA, Halliwell B. Van d V. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. Nature 1998; 391: 393–7.

Ceneviva GD, Tzeng E, Hoyt DG, Yee E, Gallagher A, Engelhardt JF, et al. Nitric oxide inhibits lipopolysaccharide-induced apoptosis in pulmonary artery endothelial cells. Am J Physiol 1998; 275: L177–L178.

Haimovitz-Friedman A, Cordon-Carlo C, Bayouny S, Garzotto M, McLaughlin M, Gallily R, et al. Lipopolysaccharide induces disseminated endothelial apoptosis requiring ceramide generation. J Exp Med 1997; 186: 1831–41.

Hu X, Yee E, Harlan JM, Wong F, Karsan A. Lipopolysaccharide induces the antiapoptotic molecules, A1 and A20, in microvascular endothelial cells. Blood 1998; 92: 2759–65.

Hotchkiss RS, Swanson PE, Freeman BD, Tinsley KW, Cobb JP, Buchman TG, et al. Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. Crit Care Med 1999; 27: 1230–51.

Barnes PJ, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 1997; 336: 1066–71.

Barchowsky A, Munro SR, Morana SJ, Vincenti MP, Treadwell JF, et al. Adenovirus-mediated expression of a dominant negative mutant of p65/RelA inhibits proinflammatory gene expression in endothelial cells without sensitizing to apoptosis. J Immunol 1998; 161: 4572–82.

Bohrer H, Qiu F, Zimmermann T, Zhang Y, Jllmer T, Mannel D, et al. Role of NFkappaB in the mortality of sepsis. J Clin Invest 1997; 100: 972–85.
[91] Ziegelstein RC, Corda S, Pili R, Passaniti A, Lefer D, Zweier JL, et al. Initial contact and subsequent adhesion of human neutrophils or monocytes to human aortic endothelial cells releases an endothelial intracellular calcium store. Circulation 1994; 90: 1899–907.

[92] Baggioili M. Chemokines and leukocyte traffic. Nature 1998; 392: 565–8.

[93] Jollow KC, Zimring JC, Sundstrom JB, Ansari AA. CD40 ligation induced phenotypic and functional expression of CD80 by human cardiac microvascular endothelial cells. Transplantation 1999; 68: 430–9.

[94] Noble KE, Wickremasinghe RG, DeCorten C, Panayiotidis P, Yong KL. Monocyte stimulant expression of the Bel-2 family member, A1, in endothelial cells and confer protection against apoptosis. J Immunol 1999; 162: 1376–83.

[95] Galley HF, Webster NR. T helper cell subset ratios in patients with severe sepsis. Intensive Care Med 1999; 25: 106–9.

[96] Ozaki H, Ishii K, Horiuichi H, Arai H, Kawamoto T, Okawa K, et al. Cutting edge: combined treatment of TNF-alpha and IFNgamma causes redistribution of junctional adhesion molecule in human endothelial cells. J Immunol 1999; 163: 553–7.

[97] Newton-Nash DK, Newman PJ. A new role for platelet-endothelial cell adhesion molecule-1 (CD31): inhibition of TCR-mediated signal transduction. J Immunol 1999; 162: 682–8.

[98] Draijer R, Vaandrager AB, Nolte C, de-Jonge HR, Walter U van Oeveren W, et al. Sites of tissue factor pathway inhibitor (TFPI) expression on vascular endothelium. Trends Cardiovasc Med 1999; 9: 34–41.

[99] Gipson TS, Bess NM, Shanley TP, Crouch LD, Bleavins MR, Younkin EM, et al. Regulatory effects of endogenous protease inhibitors in acute lung inflammatory injury. J Immunol 1999; 162: 3653–62.

[100] Lennon PF, Taylor CT, Stahl GL, Colgan SP. Neutrophil-derived 5′-adenosine monophosphate promotes endothelial barrier function via CD37-mediated conversion to adenosine and endothelial A2B receptor activation. J Exp Med 1998; 188: 1433–43.

[101] Wagner JG, Roth RA. Neutrophil migration during endotoxemia. J Leukoc Biol 1999; 66: 10–24.

[102] Draijer R, Vaandrager AB, Nolte C, de-Jonge HR, Walter U van HV. Expression of CDGMP-dependent protein kinase I and phosphorylation of its substrate, vasodilator-stimulated phosphoprotein, in human endothelial cells of different origin. Circ Res 1995; 77: 897–905.

[103] Moore TM, Chetham PM, Kelly JJ, Stevens T. Signal transduction and regulation of lung endothelial cell permeability interaction between calcium and cAMP. Am J Physiol 1998; 275: L203–L222.

[104] Suttrop N, Hessz T, Seeger W, Wilke A, Koob R, Lutz F, et al. Bacterial exotoxins and endothelial permeability for water and albumin in vitro. Am J Physiol 1988; 255: C368–C376.

[105] Suttrop N, Floer B, Schnittler H, Seeger W, Bhakdi S. Role of nitric oxide and phosphodiesterase isoenzyme II for reduction of endothelial hyperpermeability. Am J Physiol 1996; 270: C778–85.

[106] Topper JN, Wasserman SM, Anderson KR, Cai J, Falb D, Gimbrone MA. Expression of the humetamide-sensitive Na-K-C1 cotransporter BSC2 is differentially regulated by fluid mechanical and inflammatory cytokine stimuli in vascular endothelium. J Clin Invest 1997; 99: 2941–9.

[107] Deng X, Wang X, Andersson R. Endothelial barrier resistance in multiple organs after septic and nonseptic challenges in the rat. J Appl Physiol 1995; 78: 2052–61.

[108] Schmidt H, Schmidt W, Muller T, Bohrer H, Gebhard MM, Martin E. N-acetylcyesteine attenuates endotoxin-induced leukocyte-endothelial cell adhesion and macromolecular leakage in vivo. Crit Care Med 1997; 25: 858–63.

[109] Schmidt H, Schmidt W, Muller T, Bohrer H, Bach A, Gebhard MM, et al. Effect of the 21-aminosteroid tirilazad mesylate on leukocyte adhesion and macromolecular leakage during endotoxemia. Surgery 1997; 121: 328–34.

[110] Hickey MJ, Issekutz AC, Reindhardt PH, Fedorak RN, Kubes P. Endogenous interleukin-10 regulates hemodynamic parameters, leukocyte-endothelial cell interactions, and microvascular permeability during endotoxemia. Circ Res 1998; 83: 1124–31.

[111] Christ F, Gamble J, Gartsieb IB, Kox WJ. Increased microvascular water permeability in patients with septic shock, assessed with venous congestion plethysmography (VCP). Intensive Care Med 1998; 24: 18–27.

[112] Irokawa M, Nishinaga M, Ikeda U, Shinoda Y, Suematsu M, Gouda N, et al. Endothelial-derived nitric oxide preserves anti-coagulant heparan sulfate expression in cultured porcine aortic endothelial cells. Atherosclerosis 1997; 135: 9–17.

[113] Wang W, Diamond SL. Does elevated nitric oxide production enhance the release of prostacyclin from shear stressed aortic endothelial cells? Biochem Biophys Res Commun 1999; 273: 748–51.

[114] Papapetropoulos A, Piccardoni P, Cirino G, Bucci M, Sorrentino R, Cicala C, et al. Hypotension and inflammatory cytokine gene expression triggered by factor Xa-nitric oxide oxidizing. Proc Natl Acad Sci USA 1998; 95: 4738–42.

[115] Hirokawa K, Aoki N. Up-regulation of thrombomodulin in human umbilical vein endothelial cells in vitro. J Biochem Tokyo 1999; 108: 839–45.

[116] Calne DS, Grinnell BW. Thrombomodulin-dependent anti-coagulant activity is regulated by vascular endothelial growth factor. Exp Cell Res 1998; 238: 294–8.

[117] Bodin P, Birnstock G. Increased release of ATP from endothelial cells during acute inflammation. Inflamm Res 1999; 48: 351–4.

[118] Robson SC, Kaczmarek E, Siegel JB, Cindimas D, Kozlak K, Millan M, et al. Loss of ATP diphosphohydrolase activity with endothelial cell activation. J Exp Med 1997; 185: 153–63.

[119] Matsumoto Y, Kawai Y, Watanabe K, Sakai K, Murata M, Handa M, et al. Fluid shear stress attenuates tumor necrosis factor-alpha-induced tissue factor expression in cultured human endothelial cells. Blood 1998; 91: 4164–72.

[120] Camerer E, Kolsto AB, Prydz H. Cell biology of tissue factor. The principal initiating factor of blood coagulation. Thromb Res 1996; 81: 1–41.

[121] Osterud B, Bajaj MS, Bajaj SP. Sites of tissue factor pathway inhibitor (TFPI) and tissue factor expression under physiologic and pathologic conditions. On behalf of the Subcommittee on Tissue factor Pathway Inhibitor (TFPI) of the Scientific and Standardization Committee of the ISTH. Thromb Haemost 1995; 73: 873–5.

[122] Matsumoto H, Ueshima S, Fukao H, Mitsui Y, Matsu O. Effects of lipopolysaccharide on the expression of fibrinolytic factors in the vascular endothelial-cadherin complex at endothelial cell-to-cell junctions. J Exp Med 1997; 186: 517–27.
an established cell line from human endothelial cells. Life Sci 1996; 59: 85–96.
[130] Okada H, Woodcock MJ, Mitchell J, Sakamoto T, Marutsuka K, Sobel BE, et al. Induction of plasminogen activator inhibitor type 1 and type 1 collagen expression in rat cardiac microvascular endothelial cells by interleukin-1 and its dependence on oxygen-centered free radicals. Circulation 1998; 97: 2175–82.
[131] Duboscq C, Quinata I, Bassiotta E, Bergonzelli GE, Porterie P, Sassetti B, et al. Plasminogen: an important hemostatic parameter in septic patients. Thromb Haemost 1997; 77: 1090–5.
[132] Johnson K, Choi Y, DeGroot E, Samuels I, Creasey A, Aarden L. Potential mechanisms for a proinflammatory vascular cytokine response to coagulation activation. J Immunol 1998; 160: 5130–5.
[133] Kaplaniski G, Marin V, Fabrigoule M, Boulay V, Benoist AM, Bongrand P, et al. Thrombin-activated human endothelial cells support monocyte adhesion in vitro following expression intercellular adhesion molecule-1 (ICAM-1; CD54) and vascular cell adhesion molecule-1 (VCAM-1; CD106). Blood 1998; 92: 1259–67.
[134] Roy SS, Saifeddine M, Loutzenhiser R, Trigger CR, Hollenberg MD. Dual endothelium-dependent vascular activities of proteinase-activated receptor-2-activating peptides: evidence for receptor heterogeneity. Br J Pharmacol 1998; 123: 1434–40.
[135] Herwald H, Morgelin M, Olsen A, Dahlback B, Muller Esterl W, et al. Activation of the contact-phase system on bacterial surfaces – a clue to serious complications in infectious diseases. Nat Med 1998; 4: 298–302.
[136] Altura BM, Gebrewold A, Burton RW. Reactive hyperemic responses of single arterioles are attenuated markedly after intestinal ischemia, endotoxia and traumatic shock: possible role of endothelial cells. Microcirc Endothelium Lymphatics 1985; 2: 3–14.
[137] Altura BM, Gebrewold A, Burton RW. Failure of microscopic metarterioles to elicit vasodilator responses to acetylcholine, bradykinin, histamine and substance P after ischemic shock, endotoxia and trauma: possible role of endothelial cells. Microcirc Endothelium Lymphatics 1985; 2: 121–7.
[138] McKenna TM, Martin FM, Chernow B, Briglia FA. Vascular endothelium contributes to decreased aortic contractility in experimental sepsis. Circ Shock 1986; 19: 267–73.
[139] Parker JL, Adams HR. Selective inhibition of endothelium-dependent vasodilator capacity by Escherichia coli endotoxins. Circ Res 1993; 72: 539–51.
[140] Myers PR, Zhong Q, Jones JJ, Tanner MA, Adams HR, Parker JL. Release of EDRF and NO in ex vivo perfused aorta: inhibition by in vivo E. coli endotoxemia. Am J Physiol 1995; 268: H955–H961.
[141] Parker JL, Myers PR, Zhong Q, Kim K, Adams HR. Inhibition of endothelium-dependent vasodilation by Escherichia coli endotoxemia. Shock 1994; 2: 451–8.
[142] Myers PR, Gupta M, Rogers S, Mattox ML, Adams HR, Parker JL. Chronic endotoxia and endothelium-dependent vasodilation in coronary arteries. Shock 1996; 6: 267–73.
[143] Wang SY, Camden EM, Fink MP, Sellek FW. Chronic septicemia alters alpha-adrenergic mechanisms in the coronary circulation. J Surg Res 1997; 69; 61–6.
[144] Camden EM, Wang SY, Fink MP, Sellek FW. Mesenteric and skeletal muscle microvascular responsiveness in subacute sepsis. Shock 1998; 9: 184–92.
[145] Curzen NP, Griffiths MJ, Evans TW. Contraction to endothelin-1 in pulmonary arteries from endotoxin-treated rats is modulated by endothelium. Am J Physiol 1995; 268: H266–H2266.
[146] Gadet M, Dignan RJ, Mullen PG, Windsor AC, Sugerman HJ, Wechsler AS. Pulmonary artery endothelial cell function in swine pseudomonas sepsis. J Surg Res 1996; 60: 186–92.
[147] Wang P, Ba ZF, Chaudhry IH. Nitric oxide. To block or enhance its production during sepsis? Arch Surg 1994; 129: 1137–42.
[148] Wang P, Ba ZF, Chaudhry IH. Endothelium-dependent relaxation is depressed at the macro- and microcirculatory levels during sepsis. Am J Physiol 1995; 269: R988–R994.
[149] Zhou M, Wang P, Chaudhry IH. Endothelial nitric oxide synthase is downregulated during hyperdynamic sepsis. Biochim Biophys Acta 1997; 1335: 182–90.
[150] Kristof AS, Noorhosseini H, Hussain SN. Attenuation of endothelium-dependent hyperpolarizing factor by bacterial lipopolysaccharides. Eur J Pharmacol 1997; 328: 69–73.
[151] Kessler P, Popp R, Busse R, Schini KV. Proinflammatory mediators chronically downregulate the formation of the endothelium-derived hyperpolarizing factor in arteries via a nitric oxide/cyclic GMP-dependent mechanism. Circulation 1999; 99: 1878–84.
[152] Corretti MC, Plotnick GD, Vogel RA. Technical aspects of evaluating brachial artery vasodilatation using high-frequency ultrasound. Am J Physiol 1995; 268: H1393–408.
[153] Bhagat K, Coller J, Vallance P. Local venous responses to endotoxin in humans. Circulation 1996; 94: 490–7.
[154] Hartl WH, Gunther B, Inthorn D, Heberer G. Reactive hyperemia in patients with septic conditions. Surgery 1988; 103: 440–4.
[155] Astiz MB, DeGent GE, Lin RY, Rackow EC. Microvascular function and rheologic changes in hyperdynamic sepsis. Crit Care Med 1995; 23: 265–71.
[156] Astiz ME, Tilly E, Rackow ED, Weil MH. Peripheral vascular tone in sepsis. Chest 1991; 99: 1072–5.
[157] Goode HF, Cowley HC, Walker BE, Howdle PD, Webster NR. Decreased antioxidant status and increased lipid peroxidation in patients with septic shock and secondary organ dysfunction. Crit Care Med 1995; 23: 1219–24.
[158] Cowley HC, Bacon PJ, Goode HF, Webster NR, Jones JG, Menon DK. Plasma antioxidant potential in severe sepsis: a comparison of survivors and nonsurvivors. Crit Care Med 1996; 24: 1179–83.
[159] Gardinali M, Padalino P, Vesconi S, Calcegno A, Ciappellani S, Concilio L, et al. Complement activation and polymorphonuclear neutrophil leukocyte elastase in sepsis. Correlation with severity of disease. Arch Surg 1992; 127: 1219–24.
[160] Galley HF, Davies MJ, Webster NR. Xanthine oxidase activity and free radical generation in patients with sepsis syndrome. Crit Care Med 1996; 24: 1649–53.
[161] Galley HF, Davies MJ, Webster NR. Ascorbyl radical formation in patients with sepsis: effect of ascorbate loading. Free Radic Biol Med 1996; 20: 139–43.
[162] Galley HF, Howdle PD, Walker BE, Webster NR. The effects of intravenous antioxidants in patients with septic shock. Free Radic Biol Med 1997; 23: 708–74.
[163] Ochoa JB, Udekwu AO, Billiar TR, Curran RD, Cerra FB, Simmons RL, et al. Nitrogen oxide levels in patients after trauma and during sepsis. Ann Surg 1991; 214: 621–6.
[164] Evans T, Carpenter A, Kinderman H, Cohen J. Evidence of increased nitric oxide production in patients with the sepsis syndrome. Circ Shock 1993; 41: 77–81.
[165] Endo S, Inada K, Nakae H, Arakawa N, Takakuwa T, Yamada Y, et al. Nitrite/nitrate nitrogen (NOX) and cytokine levels in patients with septic shock. Res Commun Mol Pathol Pharmacol 1996; 91: 347–56.
[166] Gomez-Jimenez J, Salgado A, Mourelle M, Martin MC, Segura RM, Peracaula R, et al. L-arginine: nitric oxide pathway in endotoxemia and human septic shock. Crit Care Med 1995; 23: 253–8.
[167] Arnalich F, Hernanz A, Jimenez A, Lopez J, Tato E, Vazquez JJ, et al. Relationship between circulating levels of calcitonin gene-related peptide, nitric oxide metabolites and hemodynamic changes in human septic shock. Regul Pept 1996; 65: 115–21.
[168] Takakuwa T, Endo S, Inada K, Kasi T, Yamada Y, Ogawa M. Assessment of inflammatory cytokines, nitrate/nitrite, type II phospholipase A2, and soluble adhesion molecules in systemic inflammatory response syndrome. Res Commun Mol Pathol Pharmacol 1997; 98: 43–52.
[169] Dougthy L, Carcillo JA, Kaplan S, Janosky J. Plasma nitrite and nitrate concentrations and multiple organ failure in pediatric sepsis. Crit Care Med 1998; 26: 157–62.
[170] Spack L, Havens PL, Griffith OW. Measurements of total plasma nitrite and nitrate in pediatric patients with the systemic inflammatory response syndrome. Crit Care Med 1997; 25: 1071–8.

[171] Duke T, South M, Stewart A. Activation of the L-arginine nitric oxide pathway in severe sepsis. Arch Dis Child 1997; 76: 203–9.

[172] Wong HR, Carcillo JA, Burckart G, Shah N, Janosky JE. Increased serum nitrite and nitrate concentrations in children with the sepsis syndrome. Crit Care Med 1995; 23: 835–42.

[173] Krafte JB, Brilli R, Szabo C, Denenberg D, Moore L, Salzman AL. Circulating methemoglobin and nitrite/nitrate concentrations as indicators of nitric oxide overproduction in critically ill children with septic shock. Crit Care Med 1997; 25: 1588–93.

[174] Avontuur JA, Stam TC, Jongen LM, van-Amsterdam JG, Eggermont AM, Bruining HA. Effect of L-NAME, an inhibitor of nitric oxide synthesis, on plasma levels of IL-6, IL-8, TNF alpha and nitrite/nitrate in human septic shock. Intensive Care Med 1998; 24: 673–9.

[175] Kooy NW, Lewis SJ, Royall JA, Ye YZ, Kelly DR, Beckman JS. Extensive tyrosine nitration in human myocardial infarction: evidence for the presence of peroxynitrite. Crit Care Med 1997; 25: 812–9.

[176] Fukuyama N, Takebayashi Y, Hida M, Ishida H, Ichimori K, Nakazawa H. Clinical evidence of peroxynitrite formation in chronic renal failure patients with septic shock. Free Radic Biol Med 1997; 22: 771–4.

[177] Kooy NW, Royall JA, Ye YZ, Kelly DR, Beckman JS. Evidence for in vivo peroxynitrite production in human acute lung injury. Am J Respir Crit Care Med 1995; 151: 1250–4.

[178] Rubin DB, Wiener Kronish JP, Murray JF, Green DR, Turner J, Lué JM, et al. Elevated von Willebrand factor antigen is an early plasma predictor of acute lung injury in nonpulmonary sepsis syndrome. J Clin Invest 1990; 86: 474–80.

[179] Iba T, Yagi Y, Kidokoro A, Fukunaga M, Fukunaga T. Increased plasma levels of soluble thrombomodulin in patients with sepsis and organ failure. Surg Today 1995; 25: 585–90.

[180] Moss M, Gillespie MK, Ackerson L, Moore FA, Moore EE, Parsons PE. Endothelial cell activity varies in patients at risk for the adult respiratory distress syndrome. Crit Care Med 1996; 24: 1782–6.

[181] Cummings CJ, Sessler CN, Beall LD, Fisher BJ, Best AM, Fowler AA. Soluble E-selectin levels in sepsis and critical illness. Correlation with infection and hemodynamic dysfunction. Am J Respir Crit Care Med 1997; 156: 431–7.

[182] Egerer K, Roehr U, Krausch D, Kox W. [The circulating adhesion molecules sICAM-1 and sP-selectin in patients with sepsis]. Die zirkulierenden Adhäsionsmoleküle sICAM-1 und sB-Selectin bei Patienten mit Sepsis. Anaesthesist 1997; 46: 592–8.

[183] Kayal S, Jais JP, Aguiní N, Chaudiere J, Labrousse J. Elevated circulating E-selectin, intercellular adhesion molecule 1, and von Willebrand factor in patients with severe infection. Am J Respir Crit Care Med 1998; 157: 776–84.

[184] Krafte JB, Brilli R. Increased circulating thrombomodulin in children with septic shock. Crit Care Med 1998; 26: 933–8.

[185] Turner GD, Ly VC, Nguyen TH, Tran TH, Nguyen HP, Bethell D, Wyllie S, et al. Systemic endothelial activation occurs in both mild and severe malaria. Correlating dermal microvascular endothelial cell phenotype and soluble cell adhesion molecules with disease severity. Am J Pathol 1998; 152: 1477–87.

[186] McGill SN, Ahmed NA, Christou NV. Increased plasma von Willebrand factor in the systemic inflammatory response syndrome is derived from generalized endothelial cell activation. Crit Care Med 1998; 26: 296–300.

[187] Endo S, Inada K, Nakae H, Takakuwa T, Kasai T, Yamada Y, et al. Blood levels of endothelin-1 and thrombomodulin in patients with disseminated intravascular coagulation and sepsis. Res Commun Mol Pathol Pharmacol 1995; 90: 277–88.

[188] Kurosawa S, Stearns KD, Carson CW, D’Angelo A, Della VP, Esmon CT. Plasma levels of endothelial cell protein C receptor are elevated in patients with sepsis and systemic lupus erythematosus: lack of correlation with thrombomodulin suggests involvement of different pathological processes. Blood 1998; 91: 725–7.

[189] George F, Brouqui P, Boffa MC, Mutin M, Drancourt M, Brisson C, et al. Demonstration of Rickettsia conorii-induced endothelial injury in vivo by measuring circulating endothelial cells, thrombomodulin, and von Willebrand factor in patients with Mediterranean spotted fever. Blood 1993; 82: 2109–16.

[190] Boldt J, Papsdorf M, Kumle B, Piper S, Hempelmann G. Influence of angiotensin-converting enzyme inhibitor enalapril on endothelial-derived substances in the critically ill. Crit Care Med 1998; 26: 1663–70.

[191] Cameron EM, Wang SY, Fink MP, Sellke FW. Mesenteric and skeletal muscle microvascular responsiveness in subacute sepsis. Shock 1998; 9: 184–92.

[192] Whitehurst RM, Laskey R, Goldberg RN, Herbert D, VanBreenen C. Influence of group B streptococci on piglet pulmonary artery response to bradykinin. J Appl Physiol 1999; 86: 61–5.

[193] Hoque AM, Papapetropoulos A, Venema RC, Catravas JD, Fuchs LC. Effects of antisense oligonucleotide to iNOS on hemodynamic and vascular changes induced by LPS. Am J Physiol 1998; 275: H1078–H1083.

[194] Wang P, Wood TJ, Ba ZF, Chaudry IH. Pentoxifylline maintains vascular endothelial cell function during hyperdynamic and hypodynamic sepsis. Surgery 1996; 120: 367–73.

[195] Morrison AM, Wang P, Chaudry IH. A novel nonanticoagulant heparin prevents vascular endothelial cell dysfunction during hyperdynamic sepsis. Shock 1996; 6: 46–51.

[196] McKenna R, Laskey RE, Wang Y, Jaeschke H, Mathews WR. Effect of endotoxin-enhanced hepatic reperfusion injury on endothelium-dependent relaxation in rat aorta. Shock 1996; 6: 106–11.

[197] Siegfried MR, Ma XL, Lefer AM. Splanchnic vascular endothelial dysfunction in rat endotoxemia: role of superoxide radicals. Eur J Pharmacol 1992; 212: 171–6.

[198] Szabo C, Cuzzocrea S, Zingarelli B, O’Connor M, Salzman AL. Endothelial dysfunction in a rat model of endotoxic shock. Importance of the activation of poly (ADP-ribose) synthetase by nitric oxide pathway in severe sepsis. Arch Dis Child 1997; 76: 725–7.

[199] Vallet B, Leclerc J. Endothelial cell dysfunction in septic shock. In: Vincent JL, ed. Yearbook of intensive Care and Emergency Medicine. Berlin: Springer, 1998: 133–42.

[200] Breemen C. Influence of angiotensin-converting enzyme inhibitor enalapril on endothelial-derived substances in the critically ill. Crit Care Med 1998; 26: 1663–70.