Molecular basis of endothelial dysfunction in sepsis

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Received 17 December 2002; accepted 7 April 2003

Abstract

Sepsis is one of the major causes of mortality in critically ill patients and develops as a result of the host response to infection. A complex network of events is set into motion in the body by the infection and results in the pathogenesis of sepsis. This review article focuses on the molecular mechanisms and components involved in the pathogenesis of sepsis with a major emphasis on the endothelium. This includes sepsis-inducing bacterial components (e.g. endotoxins), cellular targets of these molecules and their responses, host reactions, intracellular and cytokine networks, individual susceptibility and new therapeutic targets in sepsis treatment.

Keywords: Sepsis; Infection/inflammation; Endothelial function; Cytokines; Endotoxins

1. Introduction

Sepsis is one of the major causes of mortality in critically ill patients and develops as a result of the host response to infection. Sepsis can be defined as a generalized inflammatory response of the entire organism and often manifests itself as the systemic inflammatory response syndrome (SIRS) [1]. The progression of SIRS usually leads to life-threatening multiple organ dysfunction culminating in multiple organ failure (MOF) [2]. The most severe hemodynamic manifestation of sepsis is a hyperdynamic shock characterized by increased cardiac output and loss of peripheral resistance. This is linked to a maldistribution of blood flow at the microcirculatory level [3] and an increased arteriovenous shunting [4]. Disseminated intravascular coagulation is often encountered in septic patients and manifests itself in the microcirculation through deposition of fibrin and the occlusion of capillaries by microthrombi [5].

The pathogenesis of sepsis is a result of a complex network of events. Components of the Gram-negative bacterial cell wall (endotoxins) are the predominant (though not exclusive) species responsible for the initiation of sepsis [6]. Endotoxins in addition to other bacterial molecules trigger a generalized response that involves both cellular and humoral pathways with the generation of pro- and anti-inflammatory mediators. These mediators include cytokines, coagulation factors, adhesion molecules, myocardial depressant substances and heat shock proteins [7–10].

The endothelium is a major target of sepsis-induced events and endothelial cell damage accounts for much of the pathology of septic shock [11]. Vascular endothelial cells are among the first cells in the body that come into contact with circulating bacterial molecules. Endothelial cells possess mechanisms that recognize structural patterns of bacterial pathogens and subsequently initiate the expression of inflammatory mediators [12].

The cellular response to bacterial toxins normally provides protection against microorganism-induced infection. However, hyperactivated cellular reactions may lead to critical injury. Under normal conditions, the biological activity of sepsis-involved mediators is under the stringent control of specific inhibitors. In sepsis this balance is disrupted and the disturbance is manifested by profound changes in the relative production of different mediators.

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doi:10.1016/S0008-6363(03)00397-3

Time for primary review 30 days.
Therefore the pathogenesis of sepsis can be described as a pro- and anti-inflammatory disequilibrium syndrome [13].

The current treatment regimen for patients with sepsis consists of a combination of antibiotic treatment, removal of the source of infection, as well as hemodynamic, respiratory, and metabolic support. Aggressive fluid resuscitation and hemodynamic support are used to restore tissue perfusion and normalize cellular metabolism. However, the mortality rates of severe septic patients or patients in septic shock remain high [14].

2. Role of endotoxin and other bacterial components

One of the molecules mainly responsible for septic complications is endotoxin, a cell wall product of Gram-negative bacteria. Endotoxin is responsible for the initiation of the septic cascade in Gram-negative bacterial infections [15] and is often liberated in large amounts in the blood stream after bacterial lysis by antibiotics [16].

Endotoxin consists of lipopolysaccharides (LPS) which can be divided into three structural units (O-specific chain, core region, lipid A component). The biological activity of LPS is mediated by a high affinity binding to a 60-kDa glycoprotein, the LPS-binding protein LBP. LBP is an acute-phase protein primarily synthesized by hepatocytes [17]. The importance of LBP in LPS-induced critical illness has been demonstrated in mice lacking the LBP gene as these mice exhibited less sensitivity to LPS [18].

The LPS–LBP complexes exert their biological activity via CD14. CD14 is expressed on monocytes and macrophages [19] and to a lower extent on neutrophils [20]. The CD14 molecule possesses a specific LPS-binding site. CD14-depleted human macrophages are unable to bind to or to be stimulated by LPS–LPB binding complexes [21]. Whether CD14 expression occurs in endothelial cells remains unclear. Two studies could not detect membrane-bound CD14 (mCD14) on endothelial cells [22,23]; however, Jersmann et al. [24] showed constitutive endothelial mCD14 expression that was further enhanced by LPS activation. Since these studies were performed in vitro, the differences observed may be due to cultivation-induced effects.

A further means by which LPS may act on endothelial cells is independent of mCD14 expression and is through soluble forms of CD14 (sCD14). sCD14 is primarily released by monocytes in the bloodstream [25] and sCD14 expression is significantly increased in sepsis patients while the expression of mCD14 is reduced [26]. sCD14 is able to mediate LPS effects in an LPB-dependent manner similar to mCD14. sCD14–LPS complexes have been shown to stimulate mCD14-negative cells such as epithelial cells and possibly endothelial cells [22,23]. However, on the basis of the contradictory data for mCD14 expression on endothelial cells, it is possible that an sCD14–LPS interaction with a very low-level expression of mCD14 on endothelial cells may be taking place [23].

Further LPS-recognizing molecules may exist since the addition of anti-CD14 antibodies cannot completely inhibit cell activation in response to high levels of LPS. One of the most likely molecule family involved are the leukocyte integrin complexes CD11/CD18 (i.e. \( \alpha_\text{I} \beta_2/\alpha_\text{IV} \beta_2/\alpha_\text{V} \beta_3 \)-integrins) which bind LPS [27]. The transfection of CD11/CD18 into LPS-nonresponsive fibroblasts imparted the ability to respond to LPS [28].

Although the Gram-negative bacterial endotoxins appear to be major compounds involved in sepsis, molecules from other bacteria give similar results. In Gram-positive bacteria no structural equivalent to LPS has been identified, although in the clinical setting, the inflammatory immune response caused by Gram-negative or Gram-positive bacteria are nearly identical. The exotoxins of Gram-negative bacteria (e.g. Escherichia coli hemolysin) [29], pore forming toxins (e.g. Staphylococcus aureus α-toxin) [30] and superantigens of Gram-positive bacteria (e.g. Staphylococcus enterotoxin B) [31] are potent inducers of a generalized inflammatory response. This property is also shared by components from the Gram-positive bacterial cell wall, i.e. soluble peptidoglycan and lipoteichoic acids (LTA). Studies by Fan et al. [32] have shown an involvement of CD14 and LPB in the signaling of LTA similar to that shown for LPS. Since the treatment with LTA does not lead to a systemic response (as shown for LPS), a more complex interaction between CD14, LBP and LTA may be taking place. Recent studies indicate that peptidoglycan, a further component of the bacterial cell wall, may in part be responsible [33]. In addition, bacterial flagellin and bacterial-derived formylated peptides, e.g. N-formylmethionylleucylphenylalanin are potent inducers of host responses [34,35].

3. Receptors for bacterial toxins

The signaling induced by bacterial components occurs primarily through toll-like receptors (TLRs). The nomenclature arises from the toll transmembrane receptor, a homologue to TLR first described in Drosophila [36]. In mammals, ten TLR subtypes have been identified to date, of which the function of only six have more or less been identified: TLR-4 mediates responses to LPS [37], a number of studies revealed an additional LPS-induced response via TLR-2 [38]. Since repurification of commercially available LPS led to the abrogation of TLR-2 responsiveness (indicating contamination with other compounds), the role of TLR-2 in LPS-recognition remains unclear [39]. However, TLR-2 recognizes a number of toxins from Gram-positive bacteria, yeast, and mycobacteria [40,41]. TLR-5 responds to bacterial flagellin [42], TLR-9 to specific patterns in bacterial DNA [43], and TLR-3 recognizes double-stranded RNA [44]. TLR-1 may
be involved in the regulation of TLR-2 [45] and TLR-4 signaling [46].

Endothelial cells express two of the known TLRs, predominantly TLR-4 and very low levels of TLR-2 [47] and the expression of both is regulated by inflammation-related factors such as LPS, tumor necrosis factor-α (TNFα) and interferon-γ (IFNγ) [48]. In addition, other factors are also involved. For TLR-2, -3, -4, -5, and -9, the stimulation with specific bacterial components leads to the recruitment of an adaptor molecule, MyD88 (myeloid differentiation protein) [49]. In MyD88-deficient mice SIRS in polymicrobial septic peritonitis was strongly attenuated implying a central role of MyD88 for the systemic immune pathology of sepsis [50]. TLR-4 requires an accessory protein, MD-2, a 20–30 kDa glycoprotein which binds to an extracellular domain of TLR-4 for an efficient response to LPS [51]. LPS-stimulated endothelial cells express high levels of MD-2 in vitro [52]. Secreted MD-2 (sMD-2) containing cell culture supernatants could confer signaling function of TLR-4-expressing cells [53]. Also TLR-2 signaling may be influenced by MD-2 [54]. Interestingly, the homologue of sMD-2 in mice (mESOP-1) is secreted at various stages of embryonic development in tissues of the hematopoietic, nervous, and reproductive systems, indicating a broader function in addition to serving as a cofactor for TLRs [55]. The exact mechanism(s) of expression of different subsets of TLRs in different cell types and the effects on the resulting signaling patterns remain unclear. Most likely, the number of TLR family members and their ligands is greater than what has been described to date.

TLRs recognize a multitude of bacterial components, however, they are not the sole receptors that have been identified with these functions. For example, the N-formylmethionylleucylphenylalanine (fMLP) receptor triggers a response to bacteria that is comparable to the TLR response [56] and mice lacking the receptors for fMLP show impaired antibacterial host defense [57]. However, the biological role of these fMLP receptors has not been fully defined. Furthermore, although much progress in identifying new signaling pathways of bacterial toxins has been made in the last few years it is likely that additional receptors for bacterial components remain to be identified [58].

4. Interleukin-1 and cytokine network

A characteristic of systemic septicemia is an inappropriate activation of inflammatory processes. Leukocyte recruitment in inflammation has been described as a multistep cascade involving soluble and membrane bound factors, and adhesion molecules of endothelial cells and leukocytes. Fig. 1 shows the cascade of LPS-induced activation of endothelial cells and leukocytes.
In the initial phase of leukocyte recruitment the loose adhesion between endothelial and leukocyte selectins and their sialylated, fucosylated receptors on the apposing cells leads to a process called leukocyte rolling [59]. The second phase of leukocyte recruitment involves the activation of different integrins (heterodimeric transmembrane receptors) on the leukocyte surface (e.g. $\alpha_\beta_2$, $\alpha_\delta\beta_2$) mediating firm adhesion to adhesion molecules on the surface of the cytokine-activated endothelial cells (e.g. ICAM-1, VCAM-1) [60]. In transmigration of leukocytes across the endothelial lining into the surrounding tissues PECAM-1 (platelet endothelial cell adhesion molecule-1) and CD99 are involved. Both molecules are located at the interendothelial contacts and in inflammation they interact with PECAM-1 and CD99 molecules, respectively, on leukocyte surfaces allowing a homophilic interaction [61,62]. Whereas the expression of ICAM-1 and VCAM-1 is strongly induced upon treatment with proinflammatory stimuli [63], the overall expression of PECAM-1 (own unpublished results) and CD99 [62] remains nearly unaltered. Studies in knockout mice have shown that deficiency of E- and P-selectin renders mice resistant to the lethal outcome of high-dose endotoxin shock [64].

The cytokine TNF$\alpha$ is one of the most important soluble mediators of inflammation. TNF$\alpha$ is mainly synthesized by activated macrophages/monocytes and is responsible for a diverse range of signaling events within cells. This leads to a proinflammatory response in neutrophils and endothelial cells [65,66] and to cell damage [67]. TNF$\alpha$ exerts many of its effects by binding, as a trimer, to either a 55-kDa cell membrane receptor termed TNFR-1 or a 75-kDa cell membrane receptor termed TNFR-2. Both belong to the so-called TNF receptor superfamily [68]. Binding of TNF$\alpha$ to its receptor leads to cell activation via the NF-$\kappa$B transcription factor [69].

Another important cytokine in host defense during sepsis is the interleukin-1 (IL-1) gene family. This family consists of three members: IL-1$\alpha$, IL-1$\beta$ (both agonists with proinflammatory character) and the IL-1 receptor antagonist (IL-1ra, anti-inflammatory counterpart). Whereas IL-1$\beta$ is solely active in its processed, secreted form, IL-1$\alpha$ is active as an intracellular precursor, membrane-associated and to a lesser extent as a secreted molecule. IL-1$\alpha$ and -$\beta$ activate numerous cell types leading to a diversity of proinflammatory events [70].

The relative contribution of IL-1 to the inflammatory cascade in sepsis is not clear. Although the plasma levels of IL-1$\beta$ are enhanced in patients suffering from septic shock [71], systemic administration of LPS in IL-1$\beta$-deficient mice does not lead to changes in levels of IL-1$\alpha$, IL-6, and TNF$\alpha$ in comparison to LPS-treated mice with normal genetic background, indicating that IL-1$\beta$ is not essential for the systemic response to LPS [72]. Further examples for equivocal response within the IL-1 family are shown in IL-1ra deficient mice, which are highly susceptible to endotoxin-induced death [73]. However, the treatment of sepsis syndrome patients with recombinant human IL-1ra compared with placebo does not lead to a statistically significant increase in survival time [74].

Both IL-1 and TNF$\alpha$ act synergistically in the initiation of the inflammatory cascade of sepsis, leading to the expression of further factors [75]. These factors include a number of proinflammatory cytokines (e.g. IL-12 and IL-18) [76,77]. The chemokines IL-8 and MCP-1 (monocyte chemoattractant protein-1) are additional proinflammatory components involved in sepsis [78,79]. IL-8 and MCP-1 are released by endothelial cells as well as other cells [80]. IL-8 is a key factor for neutrophil chemotaxis, whereas MCP-1 is involved in chemotaxis of monocytes. IL-8 also activates neutrophils to degranulate and cause tissue damage [81].

A number of cytokines IL-4, IL-10, IL-13 and IFN$\gamma$ are pleiotropic. Although IL-4, IL-10, IL-13 are potent activators of B-lymphocytes these cytokines suppress genes for the proinflammatory cytokines IL-1, TNF and the chemokines and are therefore potent anti-inflammatory agents. IFN$\gamma$ also exhibits pleiotropic characteristics, possesses antiviral activity and activates the pathway leading to the development of cytotoxic T cells. However, IFN$\gamma$ can be considered a proinflammatory cytokine, since it has been shown to augment TNF activity [81].

As mentioned above, the degree of cytokine expression in response to proinflammatory stimuli such as LPS is regulated by NF-$\kappa$B. This factor is also responsible for the regulation of transcription of adhesion molecules, immunoreceptors, procoagulatory factors (e.g. tissue factor) and acute phase proteins [63,82]. Activation and regulation of NF-$\kappa$B is tightly controlled by another transcription factor family with inhibitory functions, the I$\kappa$Bs [83]. To date, five proteins belonging to the NF-$\kappa$B family have been identified in mammalian cells [69]. Liberated NF-$\kappa$B migrates to the nucleus where it binds to specific promoter sites and activates gene transcription. The activation of NF-$\kappa$B initiates both extracellular and intracellular regulatory events resulting in the regulation of the inflammatory cascade through modulation of NF-$\kappa$B activation [84].

Repeated LPS exposure leads to diminished inflammatory responses in vitro (e.g. reduced TNF$\alpha$ expression) by monocytes, macrophages, and endothelial cells [85–87]. The acquired LPS tolerance leads not only to hyporesponsiveness in single cell types but throughout the vascular system [88]. This is associated with increased plasma levels of IL-10 and prostaglandin E2, which are known to inhibit the production of proinflammatory cytokines [89]. The tolerance to LPS is suggested as a well-controlled response in which the transcription factors NF$\kappa$B and AP-1 are involved in order to prevent excessive inflammation [90,91].

5. Endothelial dysfunction in sepsis

In the normal, physiologic state, transvascular fluid flux is tightly regulated. During septic shock the breakdown of
endothelial barrier function occurs. The loss of fluid into the extravascular space leads to life-threatening edema in the lungs, kidney, and brain of septic patients. The increase of endothelial permeability in vitro is induced by a number of sepsis-related factors (e.g., TNFα and LPS) [92,93]. There is evidence that an LPS-induced increase of endothelial permeability is achieved by enzymatic cleavage of adherens junction proteins [94]. Furthermore, structural damage to endothelial cells has been shown in a pig model of septic shock [95].

There is increasing evidence that cell death by apoptosis plays an important role in the pathogenesis of severe sepsis/septic shock. A number of in vitro studies revealed apoptotic cell death of endothelial cells in response to sepsis-related factors such as LPS and TNFα [96,97]. However, unequivocal in vivo data are missing. In addition, extensive apoptosis of lymphocytes and intestinal epithelial cells has been detected in patients who have died of multiple organ dysfunction [98].

The sepsis-associated phenomena of hypoxia and inflammation are linked to an alteration in the production of reactive oxygen (ROS) and nitrogen species, including superoxide, hydrogen peroxide, hydroxyl radicals, and nitric oxide (NO). ROS-induced oxidative stress in septic and hemorrhagic shock plays a significant role leading to endothelial and tissue injury, respectively. The initiation of lipid peroxidation, direct inhibition of mitochondrial respiratory chain enzymes, inactivation of glyceraldehyde-3-phosphate dehydrogenase, inhibition of membrane Na⁺ / K⁺ ATPase activity, inactivation of membrane sodium channels, and other oxidative protein modifications contribute to the cytotoxic effect of ROS [99].

NO functions as both an autocrine and paracrine cellular mediator. In addition to its role as a vasodilator, NO inhibits platelet aggregation and smooth muscle cell proliferation and decreases the expression of proinflammatory molecules by the endothelium [100]. NO is normally produced in the endothelium by endothelial NO synthase (eNOS), an enzyme that is constitutively expressed. Several lines of evidence suggest that the hyperproduction of NO by the inducible form of NOS (iNOS) may contribute to the hypotension, cardiodepression and vascular hyperreactivity in septic shock [101].

Normally the endothelium possesses anticoagulant/anti-thrombogenic properties in expressing e.g. tissue factor pathway inhibitors, thrombomodulin, NO, and prostacyclin [102]. Furthermore, the anticoagulation-effective activation of Protein C by the thrombin–thrombomodulin complex is controlled by the endothelium [103]. During the pathogenesis of sepsis changes in the expression of coagulation-involved factors occur (see review by Levi [104]). Tissue factor (a procoagulant glycoprotein) is released by endothelial and subendothelial cells [105], a disregulated balance of tissue-type plasminogen activator and plasminogen activator inhibitor-1 leads to increased coagulation and suppressed fibrinolytic activity [106]. In meningococcal sepsis, thrombomodulin and endothelial Protein C receptors are lacking [107]. With the occlusion of microvessels by microthrombi a lack of nutrients and hypoxic conditions develop in the tissue, contributing decisively to organ failure.

6. Individual susceptibility

Although the majority of mediators in the pathogenesis of sepsis are similar, individual case histories of sepsis show large variations in the course and outcome. The outcome of patients with sepsis does not always correlate with severity or premorbidity health status. Primary factors are chronic diseases and a poor immune status [108,109]. The patients’ age as a risk factor of fatal outcome may be related to diminished physiologic reserve, a poor immune status and a higher incidence of chronic diseases [108,110,111].

Rapid advancements in molecular biology have created insights into the genetic background of susceptibility to sepsis. A number of gene polymorphisms appear to influence the susceptibility and development of sepsis. A gene polymorphism has been identified for the human tumor necrosis factor loci [112]. Studies by Stuber et al. indicate that the amount of TNFα released in sepsis is determined genetically. They found the so-called biallelic NcoI polymorphism within the TNF locus responsible for this phenomenon [113].

A recent study revealed a polymorphism in the human interleukin-1 gene family comparable to the heterogeneity observed for the TNF gene. Patients suffering from severe sepsis showed a significantly higher frequency for a specific allele of the IL-1ra gene polymorphism (the IL-1raA2) compared to the healthy controls [114]. Recently Stassen et al. indicated that IFNγ gene polymorphisms may also exist [115].

The degree of variability in individual susceptibility is enhanced by the incidence of genetic mutations. Arbour et al. [116] were able to show that missense mutations affecting the extracellular domain of the TLR-4 are associated with a reduced response to inhaled LPS in humans. Beutler concluded from studies in meningococcal septicemia that approx. 6% of meningococcal disease in the European population can be explained by mutations altering TLR-4 structure [58].

Gender differences may play a role in sepsis and females appear to have a better outcome [117]. Wichmann et al. [118] found that a significantly smaller number of female patients required intensive care and exhibited a significantly lower incidence of severe sepsis or septic shock. A study by Angele et al. [119] indicate an involvement of sex steroids in the observed sexual dimorphism. However, the significance of these gender-specific differences remains unclear. Thus, increasing knowledge about differences in individual susceptibility may contribute to risk assessment in the pathogenesis of sepsis.

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7. New perspectives

Although much progress has been made in the treatment of inflammatory diseases, the continued high mortality rate in severe sepsis/septic shock is a sobering reflection of current therapeutic approaches. Nevertheless, increased understanding of the molecular basis of pathomechanisms should give hope that novel effective treatment regimes will become available.

The liberation of large amounts of endotoxin is often induced by antibiotic therapy and may vary depending on the type of infection, the location of infection, the virulence of strains, as well as the mode of application and the type and dosage of antibiotic [120]. Studies by Maskin et al. [121] revealed that different antibiotic chemotherapies led to variations of the host response in time and amount of TNF-α release. However, the clinical significance of antibiotic-induced endotoxin release remains to be clarified and it appears that the type of pathogen and its virulence may be more important than previously believed [120].

To date the complexity of sepsis appears to be responsible for the failure of therapies that specifically target a given mediator (e.g. treatment with TNF-α-antibody fragments or antithrombin III did not show efficacy) [122,123]. A number of novel therapeutic strategies are currently being tested. One strategy is to target the coagulopathic complications of sepsis. First clinical trials suggest a potential therapeutic use of recombinant tissue factor pathway inhibitor in patients with severe sepsis by reducing incidence and damage of disseminated intravascular coagulation [124]. Recent data also suggest recombinant human activated Protein C as an effective agent for reducing sepsis-associated mortality due to its concomitant influence on inflammatory, coagulatory, and fibrinolytic pathways [125]. Other therapeutic approaches using extracorporeal plasma filtration to reduce the amount of proinflammatory mediators are being considered. Such treatment led to improved hemodynamics and survival in a rabbit model of sepsis [126]. Finally, the TLRs present an appealing target for pharmacological intervention in specific diseases in which inflammatory consequences are a greater problem than the threat of infection itself. The TLRs may also be targets for genetic studies aimed at understanding the heritability of susceptibility to infection [58].

With the given complexity of events during sepsis it seems unlikely that a single therapeutic agent may overcome all known complications. Most strategies are targeted to points downstream of the initial complex network of events leading to sepsis. However, it remains highly desirable to further identify and therapeutically target the crucial initial steps in sepsis. Since clinical trials attempting to negate the activity of the known mediators implicated in septic shock have not proven successful there are certainly unidentified factors involved in the cellular response to bacterial components with as yet unknown roles.

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