Host-pathogen interactions in typhoid fever

de Jong, H.K.

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Chapter **2**

The systemic pro-inflammatory response in sepsis

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Hanna K. de Jong, Tom van der Poll, and W. Joost Wiersinga

Abstract

The systemic inflammatory response syndrome (SIRS) is the predominantly cytokine-mediated, pro-inflammatory response of the host to invading pathogens and is considered the hallmark sign of sepsis. Molecular components of this response can be divided into cytokines, plasma cascades and acute phase proteins while the predominant cellular components are leukocytes and the endothelium. High throughput genetic profiling studies have led to increased insights into leukocyte regulation during sepsis. New players in the pro-inflammatory cytokine network include interleukin-17, high-mobility group box-1 protein, macrophage migration inhibitory factor, myeloid-related proteins MRP8 and MRP14 and soluble triggering receptor expressed on myeloid cells-1. Activation of coagulation with concurrent down regulation of anticoagulant systems and fibrinolysis are almost universally present in septic patients with SIRS. Increasing evidence points to an extensive crosstalk between inflammation and coagulation, in which the protease-activated cell receptors play an important role. Sepsis causes excessive activation of the complement system in which C5a plays a key part. Further dissection of the role of host-pathogen interactions, the cytokine network, the coagulation cascade, the complement system and their multidirectional interactions in sepsis will pave the way for new treatment targets that can modify the excessive and collective activation of all these systems.

Introduction

The systemic inflammatory response syndrome (SIRS) is a hallmark sign of sepsis and is characterized by a hyper-inflammatory response of the host to invading pathogens that is primarily mediated by cytokines. The systemic pro-inflammatory response comprises activation of multiple pathways, including cytokines, plasma coagulation and complement cascades, and acute phase protein release, while the cellular components are in particular leukocytes and the vascular endothelium. This review focuses on the new insights in the pathogenesis of this pro-inflammatory response that is offered by the impressive amount of exciting research that has been conducted in this field over the last years.

Definition and epidemiology of the systemic pro-inflammatory response in sepsis

Sepsis is regarded as the response of the host towards invading pathogens or its toxins[1]. Since this often overwhelming systemic pro-inflammatory response, which can lead to fatal multi-organ failure (MOF) and septic shock, is regarded as the key feature of sepsis, sepsis is clinically defined as a confirmed infection (or a strong suspicion of) plus the reactive systemic response called SIRS. To full fill the SIRS criteria two or more of the following conditions should be present: temperature >38°C or <36°C; heart rate >90 beats/min; respiratory rate >20 breaths/min, $PaCO_2$ <4.3 kPa, or indication for mechanical ventilation and a white blood cell (WBC) count >12 x 10^9/l or <4 x 10^9/l or > 10% immature (band) forms[1]. In a recent cohort of critically ill patients admitted to the intensive care unit (ICU) it was shown that organ system failure and mortality increase as the number of SIRS criteria increase[2]. Severe sepsis is defined as sepsis plus organ dysfunction, whereas septic shock refers to severe sepsis with refractory hypotension[1]. Septic shock is defined as sepsis with arterial hypotension (systolic pressure <90 mmHg or a mean arterial pressure <60 mmHg) despite adequate fluid resuscitation and in the absence of other causes of hypotension[1].

In the last two decades the incidence of sepsis increased annually by 9% to reach 240 cases per 100,000 population in the United States by 2000[3]. In a European cohort of 3,147 adult critically ill patients who were admitted to an ICU it was shown that almost 25% had sepsis on admission[2]. The increasing incidence of sepsis and its associated mortality is probably mainly caused by an increase in the number of immunocompromised patients, the increase of antibiotic resistance and the aging population[4,5]. In this respect it is of interest that the majority of the 750,000 patients who develop sepsis each year in the

United States are above 65 years. Sepsis is associated with a high mortality: in a cohort of 192,980 patients in the United States with severe sepsis the mortality was 28.6%[4].

Initiation of the pro-inflammatory response

Pathogen recognition receptors (PRRs), such as toll-like receptors (TLRs) and the nucleotide binding oligomerization domain (NOD)-like receptors (NLRs), are central in host defense against pathogens. TLRs recognize pathogen associated molecular patterns (PAMPs) of invading microorganisms, initiate the immune response and are a crucial link between adaptive and innate immunity (reviewed in detail in[5,6]). Humans have 10 closely collaborating TLRs[6]. TLRs and NLRs are also able to recognize endogenous danger signals, called alarmins or danger associated molecular patterns (DAMPs). For instance, heat shock proteins, fibrinogen, hyaluronic acid and high-mobility group box-1 protein (HMGB-1) are DAMPs that are released during inflammation and cause further amplification of the pro-inflammatory response through TLR4. TLRs recognize pathogens at either the cell surface or lysosome/endosome membranes, suggesting that the TLR system is not used for the detection of pathogens that have invaded the cytosol[6]. These pathogens can be further detected by various cytoplasmic PRRs, including NLRs. Differences in the N-terminal domains of NLRs are used to further subcategorize the NLR protein members[7]. The largest group, comprising 14 members, has an N-terminal pyrin domain (PYD) and is therefore called "NLRP" (previously called "NALPs")[7]. Several members of the NLR family, including NLRP1 and NLRP3, can assemble multimolecular complexes termed "inflammasomes" in response to various activators, leading to the activation of inflammatory caspases. Activation of the NLRP3 inflammasome by PAMPs or DAMPs induces activation of caspase-1, which causes the processing of the pro-inflammatory cytokines interleukin (IL)-1β and IL-18[6,7] Overproduction of IL-1β and IL-18 will contribute to the detrimental effects of the pro-inflammatory response syndrome in sepsis. The innate immune system needs to be strictly controlled: the host needs to be protected from invading pathogens by activation of the immune response, at the same time however too much activation of the TLRs and NLRs can contribute to the detrimental effect of systemic inflammation, MOF and disseminated intravascular coagulation (DIC) (Figure 1).

Gene-expression profiling of leucocytes in sepsis

In recent years the application of high-throughput genomic technologies has permitted a more complete dissection of the host response during sepsis. Calvano et al. took the lead in this field when in 2005 the global reprioritization of the leukocyte transcriptome

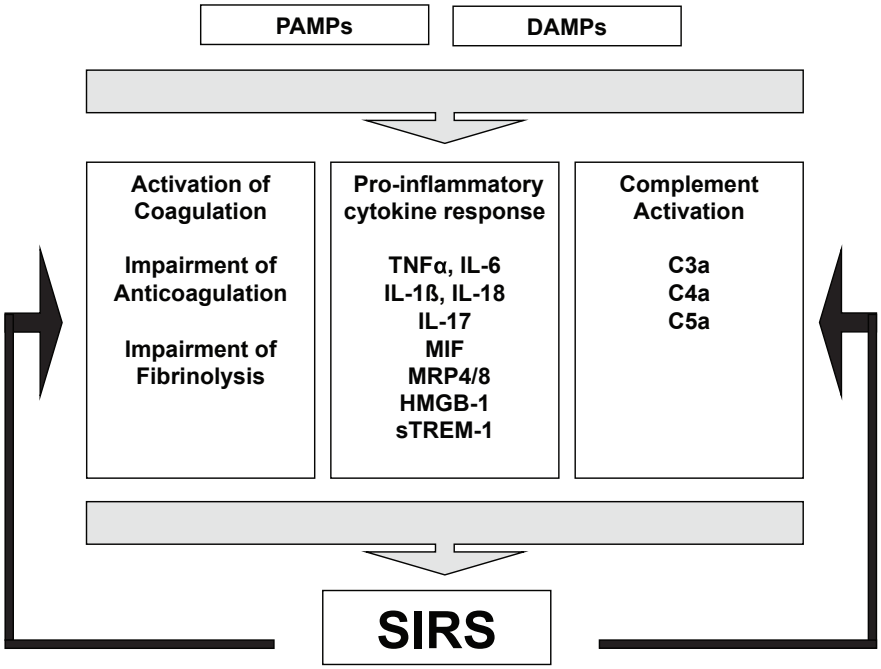

Figure 1. SIRS in sepsis

The systemic inflammatory response syndrome (SIRS) is the predominantly cytokine- mediated, pro-inflammatory response of the host to invading pathogens and is considered the hallmark sign of sepsis. This response is initiated by activation of pathogen recognition receptors, such as toll-like receptors and the nucleotide binding oligomerization domain-like receptors, that recognize both pathogen associated molecular patterns (PAMPs) of invading microorganisms and endogenous danger signals, called alarmins or danger associated molecular patterns (DAMPs). The molecular components of SIRS during sepsis can be divided into pro-inflammatory cytokines, acute phase proteins and plasma cascades such as the complement system and the coagulation system. Once released, many of these pro-inflammatory proteins are able to further amplify the pro-inflammatory response leading to an exacerbation of SIRS.

Abbreviations: TNF-α: tumor necrosis factor-α; IL: interleukin; MIF: macrophage migration inhibitory factor; MRP: myeloid-related protein; HMGB-1: high-mobility group box-1 protein; sTREM-1: soluble triggering receptor expressed on myeloid cells-1.

was revealed in vivo in human volunteers receiving lipopolysaccharide (LPS) as an inflammatory stimulus[8]. LPS administration to healthy subjects caused changes in expression in over 4000 genes during the first 24 hours, while surprisingly the expression of over half of the genes declined[8]. This reprioritization of the leukocyte transcriptome caused changes in over 300 functional modules or pathways[8,9]. Of note, the observed genomic response during SIRS seems to be cell specific, since less than 10% of the genes of which expression changed were common to both blood monocytes and T cells[8,9]. More recently, the characteristics of gene expression profiles during sepsis caused by different microorganisms have been reported. In sepsis caused by the Gram-negative bacterium *Burkholderia pseudomallei* specific monocyte and granulocyte mRNA profiles were identified which could be correlated with clinical outcome[10]. High monocyte IL-1ß,

IL-1 receptor antagonist, macrophage inflammatory protein (MIP)-1α, nuclear factor kappa-B (NFκB)-1, NFκB1A and tumor necrosis factor (TNF)-receptor-1 mRNA were correlated with mortality in these patients[10]. Additional studies have suggested that gene expression profiling of blood leukocytes can discriminate between sterile critical illness and severe sepsis[11]. Furthermore, in murine sepsis potential differences in the gene-expression profiling of Gram-negative and Gram-positive sepsis have been investigated: changes in gene expression were monitored after inoculation with *Escherichia coli* or *Staphylococcus aureus* using a DNA microarray system: 4.8% of 6,144 assessed genes were shown to be differentially regulated with a greater than twofold change across all time points[12]. Studies that investigated the potential use of molecular signatures expressed in blood leukocytes in identifying the causative pathogen in humans are inconsistent. In the largest study performed thus far, distinctive gene expression patterns were identified in peripheral blood mononuclear cells of children with acute infections caused by either influenza A virus, Gram-negative bacteria (*Escherichia coli*) or Gram-positive bacteria (*Staphylococcus aureus* or *Streptococcus pneumoniae*)[13]. However, two smaller studies performed in adult sepsis patients were unable to find an association between molecular signatures in blood leukocytes and causative pathogens[14,15].

New insights in the pro-inflammatory cytokine network

Cytokines are small molecules that, despite their short half-life of a few minutes up to a few hours, play a central role in the septic response. During sepsis their concentrations can jump from picograms per milliliter in plasma towards nanogram or even microgram per milliliter. The most extensively studied pro-inflammatory cytokines in sepsis are TNF-α and IL-1 both of which are capable to activate target cells and induce the production of more inflammatory mediators[5,16]. Other cytokines that have been implicated in the pathogenesis of sepsis include IL-6, which has both pro-inflammatory and anti-inflammatory properties, IL-12, and interferon-γ (IFN-γ)[5,16]. In patients with severe sepsis high levels of IL-1β, IL-4, IL-6, IL-8, monocyte chemotactic protein (MCP)-1 and granulocyte-colony stimulating factor (G-CSF) are associated with mortality[17]. More recently, the IL-17 cytokine family has emerged as important mediators of immune regulation[18]. The pro-inflammatory cytokine IL-17A is mainly produced by Th17 cells and is involved in mediating pro-inflammatory responses by triggering the production of many other cytokines such as IL-1β, IL-6 and TNFα and provides crosstalk between lymphocytes and phagocytes[18]. It has recently been shown that increased IL-17A levels have adverse effects during experimental sepsis: in a murine model of sepsis induced by cecal ligation and puncture (CLP), IL-17A blockade was associated with reduced levels of bacteremia, reductions of systemic pro-inflammatory cytokines together with a markedly improved survival[19]. Taken together, it is now well established that bacterial infection leads

to the activation of the cytokine network, which comprises pro-inflammatory cytokines, anti-inflammatory cytokines, and soluble inhibitors of pro-inflammatory cytokines. The balance between these counter regulatory pathways eventually determines the net pro-inflammatory activity of the cytokine network (Figure 2).

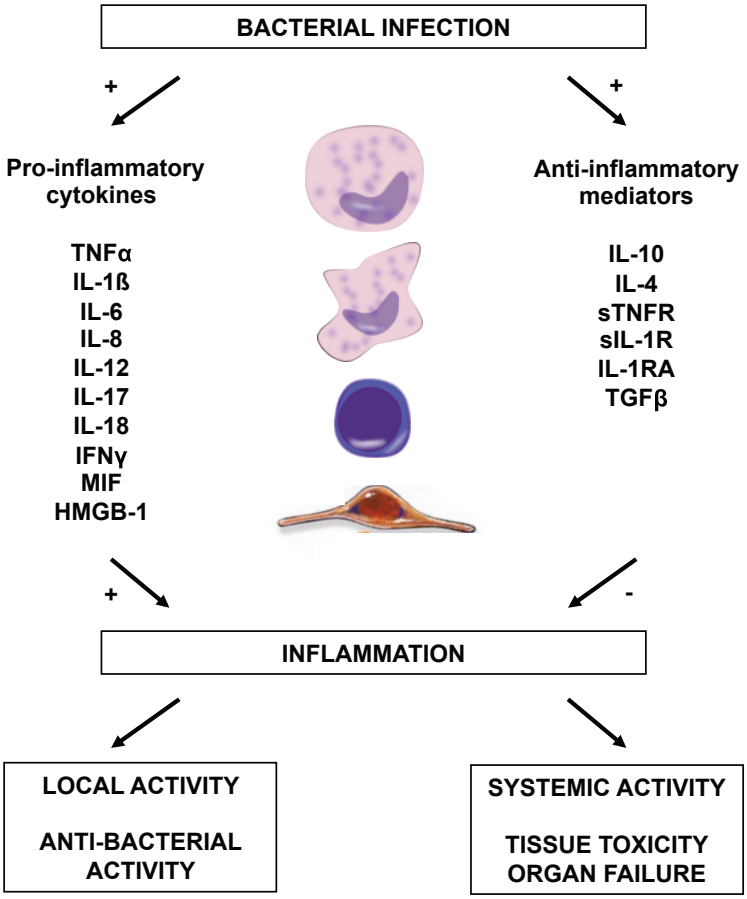

Figure 2. The inflammatory cytokine network during sepsis

Bacterial infection leads to the activation of pro-inflammatory cytokines and a number of counter-regulatory mechanisms. The balance between pro- and anti-inflammatory mechanisms determines the degree of inflammation. Local pro-inflammatory activity is required for an adequate host defense against bacterial infection while excessive systemic activity of pro-inflammatory cytokines can lead to a detrimental systemic response and organ failure. Major cytokines are mainly produced by monocytes, macrophages, lymphocytes, endothelial cells and epithelial cells.

Abbreviations: TNF-α: tumor necrosis factor-α; sTNFR; soluble TNF receptor; IL: interleukin; IL-1RA: IL-1 receptor antagonist; IFN: interferon; MIF: macrophage migration inhibitory factor; HMGB-1: high-mobility group box-1 protein; TGFβ: transforming growth factor-β.

Macrophage migration inhibitory factor (MIF)

MIF, a classical pro-inflammatory cytokine, was one of the first cytokines to be discovered almost half a century ago[20,21]. In recent years MIF has emerged as a pivotal regulator of innate immunity and is thought to be important in the pathogenesis of sepsis[20,21]. MIF is constitutively expressed by many tissues with environmental contact such as the lung and the gastrointestinal tract, and by numerous cell types, among others T- and B-lymphocytes, monocytes and macrophages[20,21]. MIF regulates innate immune responses through modulation of TLR4: when MIF-deficient mice were challenged with LPS they showed a defective response as a direct result of decreased TLR4 expression[20]. Recently it was shown that blood levels of MIF are elevated in patients with sepsis and able to predict early mortality[22-24]. Similarly, MIF is increased in patients with meningococcal disease and highest in the presence of shock[25]. MIF-directed therapies might offer a new treatment opportunity for sepsis. Inhibition of MIF activity with neutralizing anti-MIF antibodies protected mice from septic shock[21]. Intriguingly however, it was recently shown that polymorphisms associated with higher MIF expression may have a beneficial effect in patients with community-acquired pneumonia prompting caution in the clinical application of anti-MIF strategies in infectious diseases in order to avoid placing patients at increased risk of adverse outcomes[26].

High-mobility group box-1 protein

HMGB-1 is recognized as a pro-inflammatory cytokine that functions as a late mediator of sepsis and is elevated in the majority of septic patients[27,28]. It is secreted by activated immune cells and, along with the receptor for advanced glycation end products (RAGE), interacts with TLR2 and TLR4, which may provide an explanation for the ability of HMGB-1 to generate inflammatory responses that are similar to those initiated by LPS[29]. In addition to the release of cytokines HMGB-1 induction will cause activation of coagulation and neutrophil recruitment[30]. LPS stimulation was found to mediate the release of HMGB-1 from macrophages at a considerably later stage than the release of the pro-inflammatory cytokines TNFα and IL-1[28]. Although systemic HMGB-1 levels are elevated in patients with severe sepsis they do not differ between survivors and non-survivors and cannot predict hospital mortality[31,32]. Administration of HMGB-1 itself is lethal to mice, whereas the administration of antibodies to HMGB-1 diminishes lethality induced by endotoxin and CLP[28]. Not surprisingly, HMGB-1 is considered to be a new treatment target in sepsis.

Myeloid-related protein (MRP)8 and MRP14

MRP8 (also called S100A8) and MRP14 (S100A9) are members of the S100 protein family that serve as alarmins and have recently been described to be important mediators of the septic response[33]. MRP8 and MRP14 can form heterodimers that elicit a variety

of inflammatory responses. MRP8/14 complexes amplify the endotoxin-triggered inflammatory responses of phagocytes by mediating the recruitment of inflammatory cells to sites of injury[33]. The MRP8/14 complex is known to be a ligand for TLR4 of which MRP8 is the active component causing the increased expression of TNFα. In patients with sepsis and in healthy humans injected with LPS elevated MRP8/14 plasma levels are observed[34]. Furthermore it was shown that MRP8/14 is released locally during severe infection in patients with peritonitis[34]. Investigations seeking to provide insight into the functional role of MRP8/14 revealed that MRP14 contributes to bacterial dissemination and liver injury during abdominal sepsis[34]. In addition, mice lacking MRP8/14 complexes are protected from endotoxin-induced lethal shock and *E. coli*–induced abdominal sepsis[33]. Quite possibly, inhibition of MRP8/14 could be a useful adjunctive therapy for severe sepsis.

Soluble triggering receptors expressed on myeloid cells (TREM)-1

TREM-1, which is a member of the TREM family of cell surface proteins, is seen as a critical amplifier of inflammatory signaling[35]. Whereas activation of this receptor alone (through crosslinking; the natural ligand remains unknown) elicits modest cellular activation, TREM-1 synergistically enhances cellular responses induced by activation of PRR, most notably TLRs and NLRs[35,36]. TREM-1 is expressed on neutrophils, monocytes, macrophages, endothelial and epithelial cells[35]. Inhibition of TREM-1 by either a soluble recombinant TREM-1 (TREM-1/IgG1) or a small peptide named LP17 resulted in reduced inflammation and an improved survival in murine models of sepsis[35,37,38]. Of note, inflammation and infection are associated with the release of the soluble form of TREM-1[38-40], which likely is generated through proteolytic cleavage of membrane bound TREM-1 by matrix metalloproteinases[35]. Increased soluble TREM-1 levels in patients with sepsis caused by *B. pseudomallei* at admission are associated with poor outcome[38].

Cross talk between inflammation and coagulation

Coagulation abnormalities are almost universally present in critically ill patients with a systemic pro-inflammatory response[41-43]. Amongst the most prominent features of sepsis, is activation of coagulation with concurrent down regulation of anticoagulant systems and fibrinolysis. More precisely, patients with sepsis often show strong activation of the coagulation system, as reflected by high plasma levels of soluble tissue factor (TF), the prothrombin fragment F1+2 and thrombin-antithrombin complexes (TATc), and consumption of coagulation factors resulting in a prolonged prothrombin time and activated partial thromboplastin time. Concurrently, the anticoagulant mediators protein C, protein S, and antithrombin levels are down regulated in sepsis. Lastly, sepsis is often accompanied by activation and inhibition of fibrinolysis, as reflected by elevated

concentrations of tissue-type plasminogen activator (tPA), plasminogen activator inhibitor type (PAI)-1, plasmin-antiplasmin complexes (PAPc) and D-dimer. High TATc/PAPc ratios in septic patients point to a predominance of the prothrombotic pathway[43,44]. The extent of coagulation activation significantly contributes to mortality[41,44,45].

Increasing evidence points to an extensive cross talk between inflammation and coagulation, whereby inflammation leads to activation of coagulation, and coagulation also considerably affects inflammatory activity[41]. Activation of coagulation and deposition of fibrin as a consequence of inflammation can be considered instrumental in containing inflammatory activity to the site of infection. However, inflammation-induced coagulation may be detrimental in those circumstances when the triggered blood coagulation system is insufficiently controlled, which can lead to the clinical signs of DIC and microvascular thrombosis in severe sepsis[41]. The main mediators of inflammation-induced activation of coagulation are the pro-inflammatory cytokines TNFα, IL-1 and IL-6[41-43]. Importantly, although anti-TNF-α treatment is highly protective against mortality in experimental sepsis induced by intravenous administration of live bacteria, elimination of TNFα does not influence activation of coagulation in models of endotoxemia and sepsis[5,46]. These data indicate that mortality and activation of coagulation are not necessarily linked phenomena.

Tissue factor (TF)

The pivotal initiator of inflammation-induced activation of coagulation is TF. Interaction of TF with factor VIIa, which circulates at low levels in the bloodstream, results in the activation of factor X either directly, or indirectly through the activation of factor IX[41,43]. Activated factor X converts prothrombin, also called factor II, to thrombin, which finally induces the conversion of fibrin to fibrinogen, which will result in the formation of a blood clot. TF is found on the surface of various cells. As a consequence of a disruption of the vascular integrity, TF-expressing cells located in the underlying cell layers will get into contact with bloodstream. In addition, during severe inflammation cells present in the circulation will also start expressing TF. Blocking TF activity completely inhibits inflammation-induced thrombin generation in models of experimental endotoxemia or bacteremia[41,43].

The essential role of TF in activation of coagulation during a systemic inflammatory response syndrome, such as produced by endotoxemia or severe sepsis, has been established by many different experiments. Generation of thrombin in humans intravenously injected with LPS, documented by a rise in the plasma concentrations of the prothrombin fragment F1+2 and of TATc, was preceded by an increase in TF mRNA levels in circulating blood cells, enhanced expression of TF on circulating monocytes and the release of TF containing microparticles[47,48]. In line with this observation, baboons

infused with a lethal dose of *E. coli* demonstrated a sustained activation of coagulation, which was associated with enhanced expression of tissue factor on circulating monocytes, and patients with severe bacterial infection have been reported to express TF activity on the surface of peripheral blood mononuclear cells[41,44].

Anticoagulant pathways

Blood clotting is controlled by three major anticoagulant proteins, TF pathway inhibitor (TFPI), antithrombin and activated protein C (APC), which are all down regulated during sepsis resulting in a shift toward a net pro-coagulant state[41,42]. TFPI is an endothelial cell derived protease inhibitor that inactivates factor VIIa bound to TF. Antithrombin inhibits factor Xa, thrombin and factor IXa, as well as factor VIIa bound to TF. The protein C system provides important control of coagulation by virtue of the capacity of APC to proteolytically inactivate factors Va and VIIIa, thereby preventing the procoagulant activities of factors Xa and IXa. In addition, thrombomodulin, which is expressed on the vascular endothelium, inhibits coagulation by conversion of thrombin into an activator of protein C and by accelerating the inhibition of thrombin. In patients with severe meningococcal sepsis, thrombomodulin was down regulated resulting in an impaired activation of protein C in vivo[49]. Hemostasis is further controlled by the fibrinolytic system. Plasmin is the key enzyme of this system, which degrades fibrin clots. Plasmin is generated from plasminogen by a series of proteases, most notably t-PA and urokinase-type plasminogen activator (u-PA). The main inhibitor of plasminogen activator is PAI-1, which binds to t-PA and u-PA. Fibrinolysis is impaired in sepsis, primarily due to exaggerated release of PAI-1. Elevated PAI-1 levels are associated with poor outcome in patients with sepsis[44,50].

Protease-activated cell receptors (PARs)

In linking coagulation to inflammation, PARs seem to play a crucial role. The PAR family consists of four members, PAR-1 to PAR-4, which are localized on endothelial cells, mononuclear cells, platelets, fibroblasts, and smooth muscle cells[43,51]. A typical feature of PARs is that they serve as their own ligand. Proteolytic cleavage by an activated coagulation factor or other protease leads to exposure of a neo-amino terminus, which activates the same receptor, initiating transmembrane signaling. Thrombin activation of PAR-1 has been shown to induce the expression of pro-inflammatory cytokines and chemokines in vitro. In addition, LPS and TNFα induction of IL-6 expression by cultured endothelial cells is enhanced by the activation of PAR-1 and PAR-2[41,43]. LPS and inflammatory cytokines also induce PAR-2 and PAR-4 expression in cultured endothelial cells. Most probably, the activation of multiple PARs by coagulation proteases enhances inflammation during sepsis. Taken together, current data suggest that activation of

multiple PARs by coagulation proteases may contribute to the pro-inflammatory response in patients with sepsis.

C5 and the complement system

The complement system, which can be activated by the classic, alternative and lectin-binding pathways, is composed of more than 30 plasma proteins and receptors, which act as an enzymatic cascade through a variety of protein–protein interactions[52]. The complement system is an archetypical pro-inflammatory response system that bridges innate and acquired immunity by opsonizing invading pathogens, augmenting antibody responses, lysing foreign cells, clearing of apoptotic cells and stimulation of chemotaxis. Sepsis causes excessive activation of the complement system: markedly increased plasma levels of the complement constituents C3a, C4a, and C5a are seen in septic patients[5,52]. The importance of C5a for the outcome of sepsis has been underscored by several experimental investigations. Infusion of anti-C5a antibodies reduced mortality in primates with *E. coli* sepsis, and improved survival in rats subjected to CLP[52,53]. Blockade of C5a is considered to be a promising new treatment strategy in patients with sepsis in order to try to reduce the harmful effects of the overwhelming inflammatory response while trying to retain the complement's role in host defenses.

The systemic pro-inflammatory response and MOF

The pro-inflammatory septic response is considered to be directly involved in the pathogenesis of MOF in severe sepsis. An exaggerated immune response will cause hypoperfusion, organ dysfunction and tissue hypoxemia. Certain cytokines are known to induce oxygen- and nitrogen-reactive species resulting in mitochondrial dysfunction. Indeed, in the event of sepsis the increased production of pro-inflammatory mediators probably directly causes a decline in organ function by mediating the production of nitric oxide leading to mitochondrial anergy and cytopatic hypoxia[16]. In addition, sepsis induced activation of coagulation that is insufficiently contained by physiologic anticoagulant pathways and amplified by impaired endogenous fibrinolysis will give rise to DIC which is involved in the pathogenesis of microvascular dysfunction and an important contributor to organ failure[41]. Not surprisingly, MOF is a main cause of death among patients with sepsis despite the wide use and availability of powerful antibiotics and increasingly sophisticated techniques of organ support. Hopefully, a better understanding of the pro-inflammatory response – and a better insight in how one could intervene in this system – will help to reduce the high mortality in sepsis.

Conclusion

Sepsis is regarded as the response of the host towards invading pathogens or its toxins and is characterized by a systemic pro-inflammatory response. High throughput genetic profiling studies have provided us with a tremendous gain in insight into the regulation of global leukocyte activities during sepsis. It is without doubt that our knowledge on the pro-inflammatory immune response during sepsis will further increase in the very near future. Further unraveling of the role of host-pathogen interaction, the cytokine network, the coagulation cascade, the complement system and their multidirectional interaction in sepsis will pave the way for new treatment targets in sepsis that can modify the excessive and collective activation of all these systems.

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