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Host innate immune responses to sepsis

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The immune response to sepsis can be seen as a pattern recognition receptor-mediated dysregulation of the immune system following pathogen invasion in which a careful balance between inflammatory and anti-inflammatory responses is vital. Invasive infection triggers both pro-inflammatory and anti-inflammatory host responses, the magnitude of which depends on multiple factors, including pathogen virulence, site of infection, host genetics, and comorbidities. Toll-like receptors, the inflammasomes, and other pattern recognition receptors initiate the immune response after recognition of danger signals derived from microorganisms, so-called pathogen-associated molecular patterns or derived from the host, so-called danger-associated molecular patterns. Further dissection of the role of host–pathogen interactions, the cytokine response, the coagulation cascade, and their multidirectional interactions in sepsis should lead toward the development of new therapeutic strategies in sepsis.

The Mystery of Sepsis

Although sepsis was already known as a severe condition in the times of Hippocrates, the debate on what sepsis represents and how it should be delineated continues today.[1,2] Sepsis is now formally defined as a life-threatening condition that arises when the body's response to an infection injures its own tissues and organs.[3] From a more clinical perspective it has recently been proposed to include evidence of organ dysfunction in the criteria for sepsis—i.e., sepsis should be defined as a systemic response to infection with the presence of some degree of organ dysfunction.[4] Of note, this slightly differs from the definition used in the also recently published guidelines of the Surviving Sepsis Campaign in which sepsis is defined clinically as the presence (probable or documented) of infection together with systemic manifestations of infection and severe sepsis as sepsis plus sepsis-induced organ dysfunction or tissue hypoperfusion.[5] Despite an overwhelming increase in our knowledge on the pathogenesis of sepsis in the past two decades, virtually all sepsis trials have failed to show a benefit of newly developed immune-modulating drugs.[6] As a result, severe sepsis, defined as sepsis plus organ failure, remains associated with an unacceptable high mortality ranging from 15% to 50%.[7-10] What's more, the number of cases of severe sepsis is on the rise and now account for approximately 10–14% of all intensive care unit admissions in the Western world.[9-12] Evidently, there is an urgent need for new, more effective treatment strategies to counter this epidemic, and a better understanding of the pathogenesis of sepsis is imperative to unravel the sepsis mystery.[1,6,13,14] One has to realize that sepsis is too heterogeneous to treat as one disease.[13] The septic response depends on the causative pathogen, including microbial load and virulence, the makeup of the host, such as genetic composition, age, comorbidity, and medication as well as the time that has passed since initial infection. This review focuses on recent insights on host innate immune responses to sepsis. Different manuscripts in this issue will discuss the impact of sepsis on adaptive immunity and immune suppression.

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Key Role for the Pattern Recognition Receptors

The most frequently isolated pathogens in patients with sepsis include the gram-positive bacteria *Streptococcus pneumoniae* and *Staphylococcus aureus* and the gram-negative bacteria *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas aeruginosa*.[15,16] In addition, fungal sepsis, mainly caused by *Candida* species, is on the rise, at least in part due to an increase in immune compromised patients. Pathogens associated with sepsis express an imposing arsenal of virulence factors, each of which contributes to the severity of the infectious insult.[8,17]

Pattern-recognition receptors (PRR) are the central components of the innate immune system that recognize danger signals such as invading bacteria and initiate the immune response (**Fig. 1**).[18,19] PRRs recognize conserved motifs expressed by pathogens named pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS), peptidoglycan, lipopeptides (a component of many pathogens), lipoteichoic acid (a cell wall component of gram-positive bacteria), flagellin (a mobility factor of bacteria), and bacterial DNA.[8,19] PRRs can also recognize endogenous danger signals, termed alarmins or DAMPs (danger-associated molecular patterns), which are released during inflammatory stress (e.g., burns, trauma, and tissue necrosis), thereby warning the host immune system for imminent danger.[20,21] Examples of DAMPs that cause further amplification of the pro-inflammatory response through TLR4 include heat

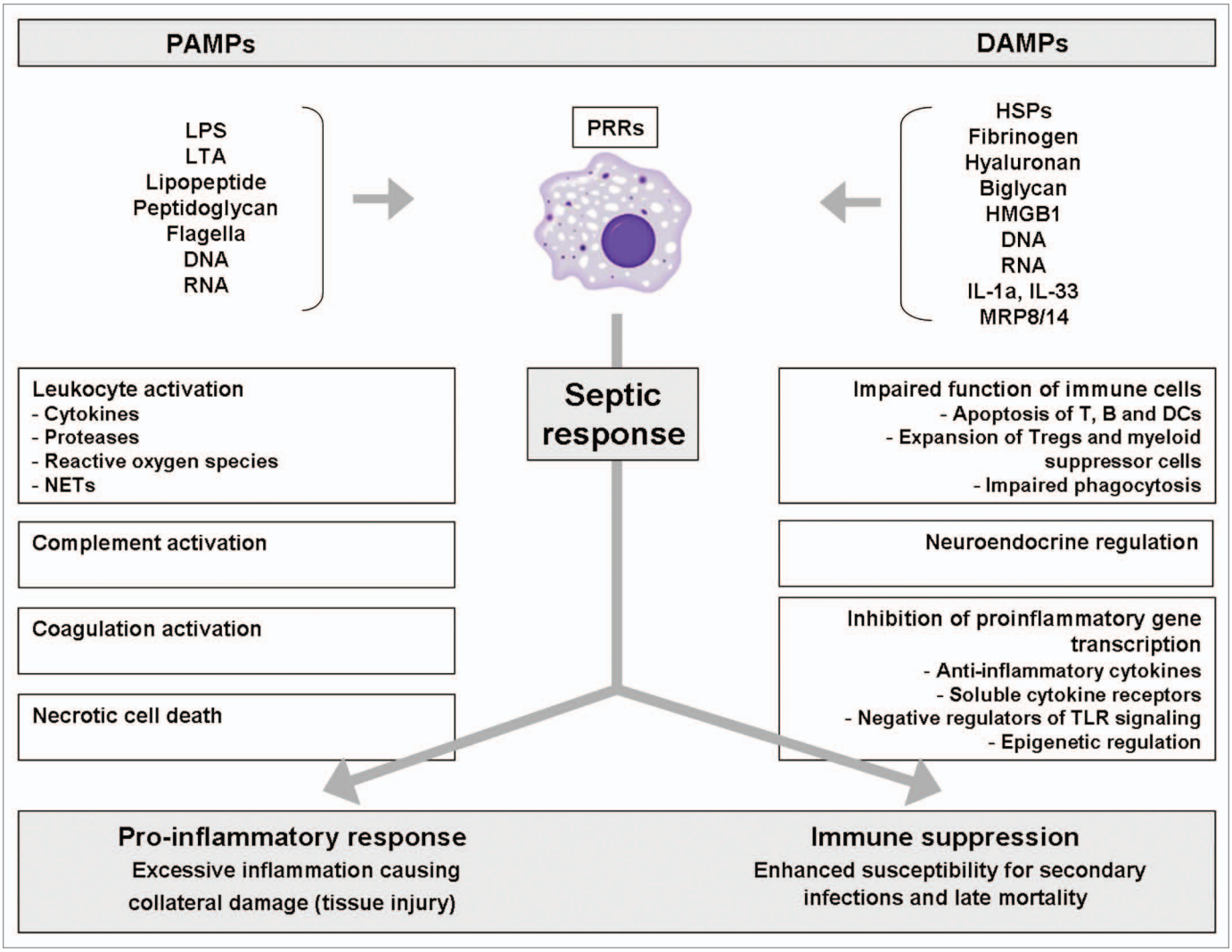

Figure 1. The host response to sepsis. The host response to sepsis is characterized by both pro-inflammatory responses and anti-inflammatory immune suppressive responses. Inflammatory responses are initiated by interaction between pathogen-associated molecular patterns (PAMPs) expressed by pathogens or endogenous danger signals (danger-associated molecular patterns, DAMPs) and pattern recognition receptors (PRR) expressed by host immune cells. Exaggerated inflammation with collateral tissue damage and necrotic cell death will result in the release of DAMPs that can perpetuate ongoing inflammation. The pro-inflammatory response is enhanced by activation of leukocytes, complement, and the coagulation system. The anti-inflammatory immune suppressive response depends on impaired function of immune cells, neuroendocrine regulation, and inhibition of pro-inflammatory gene transcription. Importantly, direction, extent, and duration of the septic response is determined by both host factors, such as genetic composition, age, comorbidity, and medication, and pathogen factors, including microbial load and virulence. LPS, lipopolysaccharide; LTA, lipoteichoic acid; HSP, heat shock protein; HMGB-1, high mobility group box-1 protein; IL, interleukin; IL-1RA, IL-1 receptor antagonist; MRP8/14, migration inhibitory factor-related protein-8/14; NETs, neutrophils extracellular traps; T, T lymphocytes; B, B lymphocytes; DC, dendritic cells; Tregs, regulatory T lymphocytes; TLR, toll-like receptor.

shock proteins, fibrinogen, S100 proteins, hyaluronic acid, and high-mobility group box-1 protein (HMGB-1).[22,23] PPRs can be categorized on the basis of their cellular localization. After the discovery of mostly cell-membrane bound TLRs in the mid-1990s, several classes of cytosolic PRRs were identified, including Nod-like receptors (NLRs), C-type lectins (CLRs), and RIG-I-like receptors (RLRs). Here we focus on PRRs that have been studied most in the context of sepsis, i.e., TLRs and NLRs.

Toll-like receptors (TLRs)

TLRs express ectodomains containing leucine-rich repeats that mediate the recognition of PAMPs; the intracellular Toll–interleukin 1 (IL-1) receptor (TIR) domain is required for downstream signal transduction, leading to the transcriptional activation of inflammatory mediators.[24] Thirteen mammalian TLRs have been identified: ten functional receptors in humans and 12 in mice; of these TLR1–9 are shared by both species, whereas TLR10 and TLR11–13 are exclusively expressed in humans and mice respectively. TLRs can be expressed on the cell surface (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10) or intracellularly (TLR3, TLR7, TLR8, and TLR9). The cellular localization of TLRs is considered to be important for ligand accessibility and the preservation of tolerance to self-molecules.

Many if not all TLRs are dimeric, with some acting as homodimers and others as heterodimers (e.g., TLR2/TLR1 and TLR2/TLR6). Some TLRs depend on other proteins to signal efficiently. For example, proficient LPS signaling requires LPS-binding protein, which transfers LPS to CD14, and the extracellular protein MD-2. The interaction between LPS and its receptor complex is further dependent on the glycosylation status of LPS: smooth LPS (with abundant O-glycosylation) requires CD14 for its detection, whereas rough LPS or lipid A do not.[25] In general, ligands of TLRs are lipoproteins, lipids, and nucleic acids. TLRs expressed at the cell surface mainly sense microbial components located on the surface or in membranes of bacteria, such as lipoproteins, lipids, and proteins. For example, the best characterized ligands for TLR2 are lipoproteins: TLR2 forms heterodimers with TLR1 and TLR6 that recognize triacyl and diacyl lipoproteins respectively. Relevant for sepsis pathogenesis, TLR2 also senses peptidoglycan (bacterial cell wall component), lipoteichoic acid (gram-positive bacteria), and zymosan (fungi). Dectin-1 (a C-type lectin) and CD36 can enhance TLR2-mediated recognition of PAMPs. TLR5 recognizes flagellin, which forms the bacterial flagella. TLRs located in endosomes and endolysosomes mostly recognize nucleic acids from pathogens. Of these, TLR3 recognizes viral double-stranded RNA, while TLR7 and TLR8 recognize viral single-stranded RNA. TLR9 senses microbial (unmethylated CpG) DNA derived from either bacteria, viruses or parasites. Cleavage and trafficking of the intracellular TLRs are necessary for signaling. Unc-93 homolog B1 (UNC93B1) binds to intracellular TLRs and mediates their trafficking to the endolysosome where signaling is initiated.[26] Of note, TLR13, which is mouse-specific, was recently shown to recognize 23S rRNA from bacteria.[27]

The entire TLR family signals via four adaptor proteins: myeloid differentiation primary-response protein 88 (MyD88), TIR domain-containing adaptor protein (TIRAP, also known as Myd88 adaptor like [Mal]), TIR domain-containing adaptor protein-inducing IFN-β (TRIF) and TRIF-related adaptor molecule (TRAM). TLR-mediated signaling can be roughly divided into two major routes: the MyD88- and the TRIF-dependent pathways. Most TLRs use MyD88 for signaling, except TLR3 that utilizes TRIF. TLR4 can utilize both MyD88 and TRIF as signaling adaptors. TIRAP connects TLR2 and TLR4 to MyD88, thus acting as a sorting adaptor in MyD88-dependent pathways. TRAM connects TLR4 to TRIF and permits TLR4 to traffic to endosomes.

TLRs play a central role in sepsis pathogenesis. TLRs on the one hand are essential for the early detection of pathogens and the initiation of an adequate innate immune response. Indeed, deficiency of TLR function leads to a strongly enhanced susceptibility for infection, as demonstrated by the vulnerability of MyD88-deficient mice for a variety of infectious diseases.[19] In accordance, children deficient for MyD88[28] or its direct downstream mediator IRAK-4[29] develop frequent purulent infections. Not surprisingly, polymorphisms in TLR encoding genes have been associated with an altered susceptibility to bacterial infections.[30] On the other hand, however, uncontrolled TLR stimulation potentially leads to disproportionate inflammation and tissue injury, as may occur during sepsis. Likely, TLR activation by DAMPs released from injured cells play a major role herein.

TLR4 has been considered an attractive therapeutic target in severe sepsis considering its role in cellular activation by several PAMPs (most notably LPS) and DAMPs. Eventually this led to the pivotal phase III ACCESS trial examining the effect of the TLR4/MD2 antagonist eritoran in patients with severe sepsis.[31] Unfortunately, although a phase II sepsis trial with this compound had shown promising results,[32] the phase III trial revealed no benefit whatsoever for patients treated with eritoran.[31]

Positive and negative regulation of the TLR signaling

TREM-1 (triggering receptor expressed by myeloid cells-1) is a receptor expressed on neutrophils and monocytes that has been shown to amplify the inflammatory cascade in response to infection.[33] Antibody-mediated activation of TREM-1 induces modest cellular activation and pro-inflammatory cytokine secretion. Importantly, co-stimulation of TREM-1 together with certain PRRs, most notably TLRs, results in a synergistic increase in inflammatory signaling. Blockade of TREM-1 in rodent sepsis models resulted in decreased systemic cytokine production and improved survival, accentuating the detrimental role of excessive inflammation in fulminant sepsis.[33] However, a recent murine study reported that TREM-1 deficiency resulted in a marked increase in mortality following induction of *Pseudomonas* pneumonia, at least in part due to deficient epithelial transmigration of neutrophils to the primary site of infection.[34] These data again are an example of the delicate balance of innate immunity, wherein pro- and anti-inflammatory processes in response to infection must be balanced to protect against infection and to avoid tissue damage and organ failure.

Several mechanisms are involved in the negative regulation of TLR signaling.[35,36] Transmembrane receptors that act as negative regulators of TLR-mediated inflammation include single immunoglobulin IL-1R-related molecule (SIGIRR and TIR8), IL-1 receptor-like 1 (ST2), and radioprotective 105 (RP105). Cells that lack either one of these receptors respond more avidly to TLR stimulation. In addition, the adaptor proteins involved in efficient signaling by TLRs (MyD88, TIRAP/Mal, TRIF, and TRAM) are targets for negative control by several mechanisms. For example, TAG (TRAM adaptor with Golgi dynamics domain) is a TRAM variant that competes with TRAM for TRIF binding, thereby inhibiting the TRIF-dependent pathway.[37] TAG also mediates internalization of TLR4 to the endosomes for subsequent degradation. Together with TMED7 (transmembrane emp24 protein transport domain containing 7) TAG disrupts the TRAM–TRIF complex, resulting in inhibition of TLR4 signaling from the endosome.[38] SARM (sterile α- and armadillo-motif-containing protein) is another TIR-domain containing protein that can bind and inhibit TRIF.[39] Moreover, inflammatory signaling can be shut down by ubiquitination, a process during which proteins are "tagged" with ubiquitin for proteasome-mediated degradation. SHP (small heterodimer partner) inhibits TLR signaling by suppressing the ubiquitination of an essential downstream signaling molecule (TRAF6).[40] Consequently, SHP-deficient cells release increased levels of pro-inflammatory cytokines upon LPS stimulation. USP4 (ubiquitin-specific

peptidase 4) also negatively regulates TRAF6, by a mechanism that involves removal of polyubiquitin chains. A20 is an essential intracellular negative regulator of TLR signaling, regulating NFκB activation via multiple mechanisms. A20-deficient mice display spontaneous inflammation in various organs, which is caused by enhanced MyD88-dependent signaling as indicated by the fact that mice with combined A20 and MyD88 deficiencies do not show aberrant inflammation.[41] Other intracellular negative TLR regulators include MyD88 short, TOLLIP (Toll-interacting protein), suppressor-of-cytokine signaling (SOCS), and IRAK-M (IL-1 receptor associated kinase-M).[36]

Inflammation following PRR activation can be further regulated by epigenetic processes.[42,43] Epigenetics is a general term encompassing mechanisms that govern gene expression patterns without modifying the underlying DNA sequence of an organism, which can include chemical modifications of DNA and/or associated histones that result in changing the physical accessibility of the DNA to transcription factors. Peripheral blood mononuclear cells of sepsis patients show increased levels of repressive histone modifications at the promoter regions of both interleukin (IL)-1 and tumor necrosis factor (TNF)-α, mediated by dimethylation of histone 3 at lysine residue 9 (H3K9me2). Additional results suggest that the early enhanced inflammatory response during sepsis may be directing the loss of specific activating epigenetic marks at promoters of pro-inflammatory genes in macrophages, such as acetylation of histone 4 (H4Ac) and lysine 4 tri-methylation of histone 3 (H3K4me3). By a distinct mechanism, posttranscriptional regulation of mRNA can be accomplished by complementary gene interference driven by micro-RNAs (miRNAs), resulting in reduced protein expression through targeted degradation of specific mRNAs.

Nod-like receptors (NLR)

Microorganisms that invade the cytosol can be recognized by cytoplasmatic PRRs, most notably the NLRs and RLRs.[18,19] NLRs are further subcategorized based on differences in the N-terminal domains.[18,44] NODs are NLRs that recognize common fragments of bacterial peptidoglycan, i.e., diamino-pimelate from gram-negative bacteria is the ligand for NOD1, while muramyl dipeptide is the ligand for NOD2. The largest NLR group, comprising 14 members, has an N-terminal pyrin domain (PYD) and is therefore called "NLRP" (previously called "NALP") of which ASC (apoptosis-associated speck-like protein containing a caspase activation and recruiting domain) serves as the central adaptor molecule. Several members of the NLR-family, including NLRP1, NLRP3, and NLRC4, can assemble multimolecular complexes termed inflammasomes in response to various activators, leading to caspase activation. Both endogenous danger signals, such as double-stranded DNA and uric acid crystals, as well as exogenous pathogen-derived molecules, such as viral RNA or bacterial peptidoglycans, can activate inflammasomes. Moreover, the NLRP3 inflammasome is assembled in response to potassium efflux, extracellular ATP, or reactive oxygen species. As such, inflammasomes can either sense pathogens directly or come to be stimulated by the intracellular alterations induced by pathogens or other PRRs. NLRP3 inflammasome activation induces caspase-1 activation, which causes the processing of the pro-inflammatory cytokines IL-1β and IL-18.[18,44]

The central role of the NLRs in the recognition of invading bacteria and the initiation of the innate immune system is by now well recognized. Activation of inflammasomes during sepsis can amplify inflammatory responses. The consequence thereof, whether beneficial or detrimental, depends on the extent and duration of inflammasome activation. The importance of inflammasomes for host defense against infection has been demonstrated in many investigations. For instance, mice lacking both NLRP3 and NLRC4 are markedly more susceptible to *Salmonella* Typhimurium infection[45] and mice lacking NLRP3 or ASC showed enhanced susceptibility to *S. pneumoniae* pneumonia.[46] In patients with septic shock inflammasome mRNA expression of ASC, caspase-1 and NALP1 are significantly downregulated when compared with critically ill control subjects, which may contribute to the state of immunosuppression observed in these septic patients.[47] On the other hand, similar to uncontrolled TLR activation, inflammasome activation, leading to caspase-1 activation and the release of IL-1β, likely contributes to organ injury during sepsis.

The Inflammatory Response in Sepsis

Moving away from the old SIRS and CARS division

In the 1990s the term systemic inflammatory response syndrome (SIRS) was introduced to describe the pro-inflammatory host response to invading pathogens, which has been considered the hallmark sign of sepsis.[48] TNF-α and IL-1 are considered the main pro-inflammatory cytokines that fuel the SIRS response. We now know however that simple inhibition of TNF-α or IL-1 does not provide clinical benefit to patients with severe sepsis.[8] Clearly, the hypothesis that excessive inflammation is the basis for an adverse outcome in sepsis requires reconsideration: the host response to sepsis involves multiple subsequent and concurrent processes that involve both exaggerated inflammation and immune suppression. SIRS has been thought to be followed by CARS or the "compensatory anti-inflammatory response syndrome", a concept introduced in the late 1990s and which is characterized by the induction of several anti-inflammatory mechanisms.[49] Recent insights show that the induction of pro- and anti-inflammatory genes in critically ill patients however occurs simultaneously, which suggests that SIRS and CARS are not two different subsequent phases of the septic response.[50] Studies describing the transcriptome of circulating leukocytes in patients with severe trauma or burn injury and healthy subjects injected with bacterial LPS (an often used model to study sepsis) show that during these severe stresses a global reprioritization of the leukocyte transcriptome affects >80% of the cellular functions and pathways, which has been called a truly "genomic storm".[50] It should be noted however that the "genomic storm" seen in this study was a monopolar, sterile inflammatory event in relatively young patients and study subjects.[50] The host response might be substantially different in a typical elderly, septic patient with a localized infection that becomes systemic over hours to

days by an invasive pathogen that continues to cause injury and tissue damage for days after onset of illness.

Cytokines: orchestrators of the septic innate immune response

The most extensively studied 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.[8] Other cytokines of known importance in regulating the septic host response include IL-6, which has both pro-inflammatory and anti-inflammatory properties, IL-8, IL-12, interferon (INF)-γ, granulocyte-colony stimulating factor (G-CSF), and the anti-inflammatory cytokine IL-10.[8] IL-17, mainly produced by Th17 cells, is a novel pro-inflammatory cytokine implicated in sepsis pathogenesis by virtue of its capacity to mediate pro-inflammatory responses by triggering the production of among others IL-1β, IL-6, and TNF-α and to provide crosstalk between lymphocytes and phagocytes.[51] Increased IL-17A levels have adverse effects during experimental sepsis: in a murine model of sepsis induced by cecal ligation and puncture IL-17A blockade was associated with reduced bacteremia, reductions of systemic pro-inflammatory cytokines and improved survival.[52]

Another cytokine involved in the septic inflammatory response is macrophage migration inhibitory factor (MIF), which regulates immune responses through modulation of TLR4. MIF-deficient mice have a defective response upon LPS challenge as a direct result of decreased TLR4 expression.[53] Inhibition of MIF activity with neutralizing anti-MIF antibodies protected mice from septic shock.[54] Plasma MIF levels are elevated in septic patients and are associated with early mortality.[55-57] Intriguingly however, polymorphisms associated with higher MIF expression may have a beneficial effect in patients with pneumosepsis 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.[58]

The pro-inflammatory cytokine HMGB-1, which is elevated during sepsis, received a lot of attention since it acts as a late mediator of sepsis and is therefore seen as an attractive treatment target.[59,60] Along with the receptor for advanced glycation end products (RAGE), HMGB-1 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.[61] HMGB-1 may do so by binding other ligands for PRRs, considering that purified HMGB-1 triggers cells to produce TNFα strictly via TLR4.[62] Treatment of mice with antibodies to HMGB-1 diminishes endotoxin lethality.[63] Taken together, it is now well established that bacterial infection leads to the activation of a whole range of pleiotropic pro-inflammatory cytokines. The balance between these mediators and anti-inflammatory cytokines or soluble inhibitors of pro-inflammatory cytokines eventually determines the net pro-inflammatory activity of the cytokine network.

Mrp8/14 as an example of DAMPs

Invasive infection and accompanying inflammatory mechanisms can cause tissue damage that is associated with release of DAMPs. A clear distinction between cytokines and DAMPs cannot be made: some cytokines act as DAMPs and some DAMPs actually are cytokines. A good example to illustrate this is myeloid related protein 8 (MRP8, S100A8) and MRP14 (S100A9), which are the most abundant cytoplasmic proteins in neutrophils.[64] These proteins function as endogenous danger proteins that promote systemic inflammation through activation of RAGE or TLR4.[64,65] They can form MRP8/14 heterodimers that are released upon cell stress stimuli. MRP8/14 has direct antimicrobial effects and has been implicated in phagocytosis.[64,66] Patients with sepsis display elevated circulating levels of MRP8/14.[67] Mice lacking MRP14 (and thereby incapable of forming biologically active MRP8/14 heterodimers) are protected from LPS-induced shock and *E. coli*-induced abdominal sepsis.[64,67] On the other hand, MRP14-deficient mice display enhanced bacterial dissemination, increased distant organ damage, and a reduced survival during *K. pneumoniae* pneumosepsis.[66] These results identify MRP8/14 as an important player in the innate immune response to sepsis with pleiotropic functions that can both harm or benefit the host depending on the causative pathogen and most probably the severity, the phase and/or compartment of the septic response.

Neutrophil extracellular traps (NETs)

Neutrophils can be regarded as the frontline soldiers against sepsis, not only because of their sheer number—they are the most abundant leukocytes—but also because of their impressive weaponry to kill invading bacteria. In addition to phagocytosis and the release of soluble anti-microbials from their granules, neutrophils are capable to entrap bacteria in ejected DNA-based structures containing anti-bacterial proteins such as elastase, cathepsin G, MRP8/14, and myeloperoxidase, which have been named neutrophil extracellular traps (NETs).[68-70] Virtually all microbes that cause sepsis are able to induce NET formation.[66,68,69,71] It was recently shown that NETs released into the vasculature are able to catch bacteria from the bloodstream and prevent dissemination in a mouse model of *E. coli* sepsis.[72] Plasma from patients with severe sepsis induces platelet–neutrophil interactions in a TLR4-dependent fashion leading to the production of NETs.[73] Interestingly, platelets seem to have a more potent NET-inductive capability than other known inducers of NETs. NET formation can be triggered even before phagocytosis which makes sense in the event of sepsis since NETs seem to be able to entangle far more bacteria simultaneously than neutrophils can by phagocytosis alone.[73,74] Some bacteria, such as certain strains of *S. pneumoniae* and *P. aeruginosa*, have developed mechanisms to circumvent NET-mediated killing.[68,71,75] Importantly, however, overwhelming NETosis or a reduced clearance capacity of NETs can be detrimental for the host and can contribute to ongoing inflammation and/or exhaustion of the immune system during sepsis.[68] Indeed, in the context of sepsis, free circulating DNA should be regarded as a DAMP by itself.[76] Of note, human DNA can only become immune stimulatory by associating with nuclear, cytoplasmic, and serum proteins upon which it can be internalized in cells and sensed by DNA receptors such as TLR9; this in contrast to bacterial DNA which CpG motifs directly act as powerful immune stimulants.[76]

Complement system

Complement factors are released as part of the inflammatory reaction to infection.[77] In experimental and clinical sepsis, elevated plasma levels of the anaphylatoxins C3a and C5a can be detected, indicative of activation of the complement system. C5a is generated from C5 following activation of complement by either the classical, alternative or lectin pathway. Preclinical research has provided evidence for a key role of C5a and its receptors (C5a receptor and C5L2) in the progression of polymicrobial abdominal sepsis induced by cecal ligation and puncture.[77] C5a binds with high affinity to its receptors, which are not only present on phagocytes (especially neutrophils) but also on several non-myeloid-derived cells, including endothelial cells. Activation of the C5a receptor by C5a results in a cascade of signaling events with responses such as priming for cell responses to a second stimulus (for example produced by a PAMP), release of cytoplasmic granule contents, reactive oxygen species production, and chemotactic responses. Clearly, these responses are part of protective immunity during infection. However, excessive C5a activity can be harmful in the setting of fulminant sepsis. Indeed, neutralization or genetic absence of C5a receptor or C5L2 improves survival during abdominal sepsis or endotoxemia in mice.[78,79] Of note, an important inhibitor of C5a and C3a is thrombin-activatable fibronolysis inhibitor (TAFI), by generation of carboxypeptidase activity, which is induced by thrombin–thrombomodulin complexes on the vascular endothelial surface.[80,81] Inhibition of C5a activity is currently considered an attractive therapeutic option in sepsis.[77]

Coagulation system

Sepsis is associated with multiple alterations in procoagulant and anticoagulant mechanisms.[81] Hemostatic disorders in patients with infection may range from subtle activation of coagulation detected by sensitive laboratory tests to fulminant disseminated intravascular coagulation (DIC). DIC is commonly seen in sepsis and in particular in septic shock where the incidence is somewhere between 30% and 50%.[82] Sepsis results in a net procoagulant state that promotes fibrin deposition through three main pathways: tissue factor (TF)-mediated thrombin generation, dysfunctional physiological anticoagulant mechanisms, and impaired fibrin removal due to depression of the fibrinolytic system.

Coagulation activation in sepsis is primarily driven by TF. TF is not exposed to circulating blood cells in a resting state, but becomes exposed on the surface of mononuclear cells and endothelial cells when they are stimulated by bacteria or by bacterial products like LPS or by pro-inflammatory cytokines such as TNF-α. TF binds and activates factor VII. The TF/factor VIIa complex that is generated after exposure of TF-presenting cells to blood initiates coagulation activation by activation of factor X, producing factor Xa and finally leading to prothrombin conversion to thrombin. Sepsis patients and healthy humans intravenously injected with LPS show enhanced TF expression on circulating mononuclear cells.[83,84] Inhibition of the TF/factor VIIa pathway abolishes coagulation activation elicited by administration of LPS or bacteria in humans[85] and non-human primates,[86,87] and in lethal sepsis models in baboons, TF inhibition prevented multiple organ failure, and mortality.[86,87] Besides in its cell-associated form, TF can reside in microparticles that can be shed from leukocytes, endothelial cells, vascular smooth muscle cells, and platelets. Microparticles can transfer TF to cells that do not generate this procoagulant protein themselves, such as granulocytes, and have been implicated in activation of both coagulation and inflammation in sepsis.[88]

Procoagulant events are controlled by three major anticoagulant proteins: antithrombin, TF pathway inhibitor (TFPI), and activated protein C (APC).[81] Antithrombin is the main inhibitor of thrombin and factor Xa. The inhibitory function of antithrombin is enhanced by endogenous glycosaminoglycans, among which heparan sulfates. TFPI is the main inhibitor of the TF-factor VIIa complex. Normally, TFPI is attached to the endothelium via proteoglycans, which facilitates its TF factor VIIa–factor X inhibiting properties on the endothelial surface. In sepsis pro-inflammatory cytokines reduce the synthesis of glycosaminoglycans on the endothelial surface, which likely impairs the function of antithrombin and TFPI. The protein C system represents an important anticoagulant mechanism by virtue of the capacity of APC to proteolytically inactivate the coagulation cofactors Va and VIIIa. APC is formed from protein C when thrombin binds to thrombomodulin, a receptor present on the vascular endothelium. The activation of protein C to APC by thrombomodulin-bound thrombin is amplified by the presence of the endothelial protein C receptor (EPCR). During sepsis the protein C system is impaired as a result of multiple factors, most notably decreased synthesis of protein C by the liver, increased consumption of protein C and impaired activation of protein C by diminished thrombomodulin and EPCR expression on endothelial cells. Many studies have supported the anticoagulant potency of the protein C system in vivo.[89] Infusion of APC confers anticoagulant, anti-inflammatory, and protective effects in multiple sepsis models. In addition, the importance of the endogenous protein C system for protection against excessive coagulation and inflammation has been firmly established by multiple studies using several strategies to inhibit the function of this pathway. The anti-inflammatory functions of APC rely on its interaction with protease activated receptor-1 (PAR1).

PARs form the crucial link between coagulation and inflammation.[90] Four PARs (1 to 4) have been identified, each of which can be activated by several proteases. Thrombin can activate PAR1, 3, and 4; these receptors can also be activated by plasmin, trypsin or cathepsin-G. PAR2 can be activated by trypsin, mast cell tryptase, leukocyte proteinase-3, and a number of bacteria-derived enzymes. TF can induce cell signaling via PAR1 or PAR2; factor Xa and APC can exert cellular effects via PAR1. Curiously, although APC and thrombin can both activate PAR1, APC affects the vascular endothelium in a way that clearly is distinct from thrombin signaling. The divergent cellular effects of APC and thrombin are especially remarkable with regard to endothelial barrier function. APC potently inhibits thrombin-induced vascular hyperpermeability by a mechanism dependent on trans-activation of the sphingosine 1 phosphate (S1P) receptor 1 (S1P1), whereas thrombin induces vascular hyper-permeability dependent on another S1P receptor, S1P3. Preclinical evidence indicates that the anti-inflammatory, rather

than the anticoagulant, effects of APC are important for protection against sepsis lethality: studies using APC mutants that lack anticoagulant properties were as protective as wild-type APC.[91,92]

Considering the abundant preclinical evidence that interference with coagulation may beneficially impact on sepsis outcome, it is not surprising that anticoagulant therapies have been studied extensively in patients with severe sepsis.[93] These investigations focused on the restoration of (supra) physiological levels of antithrombin, TFPI, and APC. Antithrombin and TFPI failed to reduce mortality in randomized clinical trials in sepsis patients.[94,95] Recombinant human APC did reduce 28-d mortality in a first pivotal phase III trial (PROWESS),[96] but did not show any benefit in a subsequent study in septic shock patients (PROWESS-SHOCK),[97] which resulted in retraction of this protein from the market by the manufacturer. Nonetheless, the debate on the possible value of APC for sepsis treatment has continued.[98] APC mutants lacking anticoagulant properties would be attractive drugs to study in clinical trials: these proteins retain the capacity to protect animals from sepsis induced death (via an effect on PAR1) but do not expose patients to the risk of bleeding complications.

Conclusion

A careful balance between the inflammatory and anti-inflammatory response is vital for a successful host response to sepsis, which can probably be seen as a PRR-mediated dysregulation of the immune system following the invasion of pathogens. Indeed, two decades of failed sepsis trials have forced one to rethink the pure hyperinflammatory sepsis paradigm. New human sepsis studies should also consider the substantial heterogeneity in the patients and type of infections included in sepsis trials as well as the predominant phenotype of the immune response, pro- or anti-inflammatory/immune suppressive, at time of inclusion.[13,99] Furthermore, in recent times it has become clear that the host response to infection and non-infectious injury is not fundamentally different; whole blood genome response in patients with trauma, burn, or sepsis result in highly similar genomic responses.[100] In depth knowledge of the interconnections between innate immune pathways should help to unravel the mystery of sepsis and identify new treatment strategies to cope with this endemic syndrome.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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