

Pathogenesis of Sepsis

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Published Date: May 20, 2016

THE ROLE OF MACRO- AND MICROORGANISMS

The pathogenesis of sepsis can be characterized as the interaction of two subjects, *i.e.*, macro- and microorganisms. A crucial role in this interaction is played by the patient's immune response but also the microorganism's virulence, which varies between pathogens, is important [1]. In this context, mention should be made of "quorum sensing", which is a system of intercellular communication between bacteria resulting in coordinated activation of gene expression and the production of virulence factors. Quorum sensing regulates the expression of genes associated with synthesis and release of a large number of virulence factors. Thus armed, the pathogen is then capable of initiating and developing the process of infection. In the pathogenesis of sepsis, the key player is the dysregulation of the mechanisms of the innate and adaptive immunity. A localized inflammatory response progresses into a systemic response, reflecting a failure of the immunological compensatory mechanisms.

RECOGNITION MECHANISMS OF THE INNATE IMMUNE SYSTEM

In the process of evolution, the innate immune system has developed many mechanisms that are capable of recognizing a pathogenic organism and responding to its presence accordingly. Furthermore, these mechanisms are capable of identifying homeostasis-disrupting stimuli of a non-infectious nature (e.g., ischemia, trauma). For this purpose, immunocompetent cells possess pattern recognition receptors (PRRs) that are capable of recognizing characteristic pathogen-associated molecular patterns (PAMPs). There are three families of receptors that distinguish infectious agents, Toll-like receptors (TLRs), nucleotide oligomerization domain (NOD)-like receptor (NLRs), and the retinoic acid-inducible gene-1 (RIG-I) -like receptors (RLRs) [2]. The immune system is believed to be able to recognize 10^3 PAMPs, including lipopolysaccharide (LPS), peptidoglycan (PGN), bacterial lipoproteins, lipoteichoic acid (LTA), and bacterial and viral nucleic acids. In addition, PRRs are capable of distinguishing signals transmitted by alarmins, which are endogenous molecules that are rapidly released in response to ischemia, trauma, or necrosis. Together with PAMPs, alarmins make up the damage-associated molecular patterns (DAMPs). Alarmins include the high-mobility group box 1 (HMGB1) protein, heat-shock proteins (HSPs), S100 proteins, and many others.

Based on their function, PRRs are divided into two groups called endocytosis and signaling receptors. Examples of the endocytosis receptors include opsonin, scavenger, and mannose receptors, which bind microbes and mediate their subsequent phagocytosis. Signaling receptors are present both outside and inside the cell, and their binding to a PAMP activates the innate and adaptive immune systems. The most important are the TLRs, a family of transmembrane receptors that is crucial for transmitting information about the presence of an infectious agent, thus leading to the production of proinflammatory cytokines. To date, 10 human TLRs have been identified, with each specifically distinguishing various microbial structures. The subfamily of TLR1, TLR2, TLR6, and TLR9 recognize nucleic acids, TLR4, in conjunction with MD-2, recognizes lipopolysaccharide (LPS), and TLR5 binds bacterial flagellin. TLR11 has recently been found to recognize a profilin-like molecule of *Toxoplasma gondii*. While some TLRs (TLR1, TLR2, TLR4, TLR5, TLR6, TLR10) that recognize bacterial and fungal components are localized to cell surfaces, other TLRs that detect nucleic acids of viruses and some microbes (TLR3, TLR7, TLR8, and TLR9) are localized within endosomes. These TLRs allow detection of nucleic acids released by apoptotic cells, microbes, or virions once they have been phagocytized and ingested. Toll-like receptors are currently regarded as the main mediator of interaction with the infectious agent and the innate immune system. TLRs are not only involved in identifying infectious agents, as they also play a major role in the development of systemic inflammatory response syndrome (SIRS) of non-infectious origin [3]. Other receptors that are capable of recognizing PAMPs make up the family of NOD-like receptors (NLR), retinoic acid-inducible gene (RIG)-like helicases, and C-type lectin receptors (CLR). The family of cytoplasmic NLR proteins form a protein complex that is responsible for the activation of caspases 1 and 5, and subsequent production of the proinflammatory cytokines

interleukin-1 β (IL-1 β), IL-18, and IL-33. This protein complex is called the inflammasome and is one of the key effector mechanisms of the innate immune system when it detects the presence of infectious pathogens [4]. Upon interaction of PAMPs and PRRs, adaptor proteins come into play whose task is to activate transcription factors. Essentially, two pathways are used. The first, the myeloid differentiation primary response protein (MyD88)-dependent pathway, is employed by all TLRs except for TLR3. The second pathway, mediated by the Toll/IL-1-receptor domain-containing adapter inducing interferon (TRIF) protein, is indispensable for type I interferons (interferons α and β). The family of adaptor proteins includes TIR-associated protein (TIRAP) and Toll-receptor associated molecule (TRAM). Adaptor proteins activate the transcription factors, including nuclear factor κ B (NF- κ B), activation protein-1 (AP-1), and interferon response factor (IRF). The activation results in the expression of genes for pro- and anti-inflammatory cytokines.

THE HOST-INFECTIOUS AGENT INTERACTION

Sepsis is defined as a systemic inflammatory response to infection. A model example is infection with Gram-negative bacteria. The outer membrane of Gram-negative bacteria invariably contains a unique component, lipopolysaccharide (LPS or endotoxin), which is responsible for the pathogenic effects [5]. Detectable endotoxin levels were reported in 75% of patients in intensive care units. In addition to the presence of a Gram-negative infection in the host, endotoxemia may also be due to bacterial translocation whereby microbes and/or their products cross the mucosal barrier. Bacterial translocation occurs in individuals in the presence of specific conditions, such as immunosuppression, liver disease, acute pancreatitis, or impaired intestinal mucosal integrity. Enhanced bacterial translocation has been documented in athymic mice, while an association with a surgical procedure for intestinal obstruction was reported in a human. From the perspective of clinical practice, a landmark study demonstrated an effect of morphine on the intestinal mucosal integrity, thus causing bacterial translocation [6]. Effects similar to those of endotoxin are exerted by Gram-positive bacterial structures, e.g., teichoic and lipoteichoic acids, capsular lipopolysaccharide, and group-specific carbohydrates [7]. A major role is played by exotoxins, which act as superantigens, in Gram-positive bacterial sepsis. What makes this mechanism unique is the direct binding of the superantigen to the major histocompatibility complex (MHC) class II antigens, which are expressed on antigen-presenting cells, and reacting directly with T-cell receptors (TCRs). A corresponding clinical nosological unit is the toxic shock syndrome. A list of toxic shock syndromes is provided in the Table 1.

Table 1: Responsible toxins of Etiological agents.

Etiological agent	Responsible toxin
<i>Staphylococcus aureus</i>	TSST-1 (Toxic shock syndrome toxin 1)
	Enterotoxins A, B, C, D
<i>Streptococcus hemolyticus</i> group A	Pyrogenic toxins A, B, C
	Streptogenic mitogenic exotoxin

A Model Example of Development of the Immune Response to the Presence of Infection

The substances implicated in the development of the pro- and anti-inflammatory responses by the macroorganism are endotoxin in Gram-negative sepsis and lipoteichoic acid in Gram-positive sepsis. The key role in the development of this inflammatory cascade is played by two important cytokines: tumor necrotizing factor- α (TNF- α) and interleukin-1 β (IL-1 β). These cytokines stimulate the immune response, including arachidonic acid production, integrin expression, complement and neutrophil activation, and nitric oxide production. In addition, the above cytokines increase the procoagulation activity of endothelial cells, activate neutrophils, and increase the gene expression of adhesion molecules, thus resulting in tissue injury. The primary role of the inflammatory response is localization of the injury. Release of proinflammatory mediators, i.e., leukotrienes, complement components, and cytokines, and the formation of antigen-antibody complexes, results in neutrophils and monocytes accumulating at the site of injury. Monocytes and neutrophils locally release a host of proinflammatory cytokines, e.g., TNF- α , IL-1, IL-2, IL-6, and interferon (IFN)- γ . Production of proinflammatory cytokines is opposed by a compensatory anti-inflammatory response through the production of IL-4, IL-10, IL-11, IL-13, and soluble TNF- α receptors. However, in contrast to their beneficial reparatory function, neutrophils are also the villain of the piece in organ damage.

MECHANISMS OF IMMUNOSUPPRESSION IN SEPSIS

The Hyperinflammatory Response and Compensatory Anti-Inflammatory Response Syndrome (CARS)

The characteristic features shared by the syndrome of systemic inflammatory response and sepsis include inflammation and homeostasis disruption, both being a set of reactions in response to the body's impaired integrity. The pathogenesis of the sepsis is largely intertwined through the impaired functions of the humoral and cell-mediated immunity. Initially, both sepsis and SIRS are characterized by increased blood levels of inflammatory mediators, which are paralleled by a compensatory anti-inflammatory immunosuppressive response (CARS) [8]. The mechanisms of adaptive immunity act primarily through cell-mediated immunity. In the 1980s, Mosman characterized two subgroups of CD4⁺ cells with an antagonistic profile of the cytokines produced: type 1 T-helper (Th1) cells, which produce predominantly TNF- α , IFN- γ , and IL-2

(proinflammatory), and Th2 cells, which produce IL-4, IL-5, IL-10, and IL-13 (anti-inflammatory) cytokines. Th1 cell-produced IFN- γ and TNF- α boost the ability of phagocytes to kill intracellular infectious agents, and are responsible for delayed hypersensitivity, whereas Th2 cells have been implicated particularly in the hormonal response to extracellular pathogens. As to which type of response (pro- or anti-inflammatory) eventually prevails depends on the predominant type of mediator produced. The intensity of the response is another critical factor for immune response compartmentalization, i.e., whether the inflammatory response will be localized or systemic. Sepsis is characterized by a shift from a Th1 to Th2 response [9], resulting in immunosuppression. An important role in the process of inducing immunosuppression is played by IL-10, thus inhibiting antigen-specific T lymphocyte proliferation.

In connection with the role played by T lymphocytes in the immune response, mention should be made of subpopulations of regulatory T lymphocytes (TREGs) and Th17 lymphocytes. TREGs are characterized by the expression of CD25 (interleukin 2 receptor) and the intracellular presence of Foxp3 (a transcription factor). TREGs are involved in the control and/or suppression of the development of autoimmune disease. In addition, they have been shown to effectively regulate the intensity of the inflammatory response in infection. Generally, TREGs have been suggested to have a largely immunosuppressive function by modulating the response of lymphocytes and antigen-presenting cells [10]. However, though clinical trials have documented an increased number of TREGs, views on their role in patients with sepsis have to date been inconsistent. Another interesting lymphocyte subpopulation related to sepsis is the Th17 lymphocytes. These lymphocytes are characterized by production of IL-17 and, in addition to their major role in autoimmune diseases, they are significantly involved in host defense against bacteria and fungi. An important link between the innate and adaptive immunity is the co-stimulatory molecules. These proteins, which are expressed on the surface of antigen-presenting cells, are important for activation of antibody- and cell-mediated immunity. Co-stimulatory molecules provide a second signal leading to activation and proliferation or, conversely, inhibition of T lymphocytes, followed by anergy and apoptosis. Typical co-stimulatory molecules include the CD28, CD80, CD86, and programmed cell death (PD-1) protein. Sepsis is associated with overexpression of inhibitory co-stimulatory molecules, together with decreased expression of stimulatory molecules. Blocking of inhibitory co-stimulation molecules resulted in reduced mortality in an experimental model of sepsis.

Necrosis, Necroptosis, Apoptosis, Pyroptosis, and Their Immunomodulatory Effects

The action of physical and chemical effects, as well as bacterial or viral infectious agents, results in the death of affected cells. While the mechanisms differ, their ultimate result, in all cases, is induction of the immunomodulatory response of the macroorganism through triggering the inflammatory response. Based on the mechanisms involved, the immunomodulatory activity is either immunostimulatory (necrosis, necroptosis) or immunosuppressive (apoptosis).

Necrosis develops due to extreme physical or chemical stress (heat, mechanical damage, osmotic damage), with the consequences being loss of the cell membrane integrity and cell death, while the cell nucleus remains more-or-less intact. The intracellular substances released from the damaged cell, i.e., the damage-associated molecular patterns (DAMPs), induce an inflammatory response. The DAMPs include DNA fragments, IL-1, HMGB1, and ATP. The action of these mediators is ultimately immunostimulatory. Necroptosis means regulated cell necrosis mediated by the receptor-interacting protein kinase 1 and 3 (RIPK1-RIPK3) complex, which forms upon the interaction of PAMP of infectious etiology (bacteria or viruses) and the PRRs. Again, the consequence is initiation of the inflammatory response [12].

Apoptosis, or programmed cell death, is one of the most important regulatory mechanisms of the immune system. A large number of lymphocytes and intestinal epithelial cells die during sepsis through the process of apoptosis. Sepsis is associated with increased apoptosis of immunocompetent cells, whose effect is ultimately immunosuppressive. While a dramatic decrease in B lymphocyte and CD4⁺ cell counts has been documented, sepsis did not result in a decrease in CD8⁺ T lymphocyte and NK cell counts. The rates of apoptosis in individual organs vary. The largest counts of apoptotic cells have been demonstrated in the spleen and intestine. The decrease in the immunocompetent cell counts is necessarily reflected in a decreased ability of the body to respond to infection. What's more, apoptotic cells actively suppress the inflammatory response, and induce anergy and anti-inflammatory cytokine production, while necrotic cells exert an immunostimulatory effect and enhance the body's defense.

Pyroptosis is another mechanism of genetically determined programmed cell death. The triggers are similar to those seen in apoptosis, i.e., bacterial and viral infection, but also non-infectious triggers, such as cellular substances that are formed during myocardial infarction. Unlike apoptosis, which is mediated by caspases 3, 6, and 8, the course of pyroptosis depends on the effect of caspase 1 [13].

The Effect of Neuroregulation on Immunosuppression

The stress response plays a major role in inducing immunosuppression not only in infection but also after injury/trauma or blood loss. The regulatory role of the hypothalamic-pituitary-adrenal (HPA) axis, which involves stimulation of corticosteroid production, is well characterized. Studies have shown the effect of the autonomic nervous (sympathetic and parasympathetic) systems on the immune response. The neuroendocrine system regulates the anti-inflammatory role of monocytes and macrophages (TNF- α production) both directly and indirectly via induction of immunomodulatory cytokines, e.g., IL-10, whose action results in Th1 deactivation. These interactions are critical for preventing an excessive inflammatory response. However, the increasing intensity of the inflammatory response during massive infection or trauma is associated with enhanced activation of the HPA stress axis leading, in turn, to increased immunosuppression and, consequently, increased risk of systemic infection. High levels of corticosteroids and

catecholamines result in increased apoptosis rates, while the function of adaptive immunity mechanisms is reduced to anergy. Heidecke showed that reduced Th1 function without increased Th2 cytokine production is associated with the state of anergy, and this reduced T-lymphocyte function correlated with mortality rates.

Immunosuppression and Immunoparalysis

A landmark study documenting immunosuppression in sepsis patients was published in 2011 [11] by Boomer et al. [14] who conducted a functional study to determine the status of the immune system in patients dying from sepsis. The authors isolated cells of the lungs and spleen of patients to compare them with those of patients dying from trauma. They immunophenotyped the cells to determine their functional status as defined by cytokine production following LPS stimulation. There was functional immunosuppression in patients dying from sepsis, manifesting itself by, among other things, substantially reduced production of pro- and anti-inflammatory cytokines following LPS stimulation and decreased expression of HLA-DR on antigen-presenting cells. Importantly, the state of sepsis-induced immunosuppression can be reversed by moving the cells away from the sepsis “milieu” [14]. The immune response of patients with sepsis is typically a hyperinflammatory systemic state and/or a state of “immunoparalysis” characterized by low monocyte HLA-DR expression and decreased TNF- α production *ex vivo* following LPS stimulation. The hyperinflammatory phase is characterized by the high production of cytokines, e.g., TNF- α , migration-inhibiting factor (MIF), and HMGB-1 protein. In contrast, the compensatory anti-inflammatory response syndrome, which may progress to immunoparalysis, is typically associated with production of IL-4, IL-10, IL-11, IL-13, transforming growth factor β (TGF- β), granulocyte- and macrophage-colony stimulating factors (G-CSFs and GM-CSFs), soluble receptors of TNF-, and IL-1 receptor antagonists (IL-1ra). Both phases – pro and anti-inflammatory occur simultaneously as early as the onset of sepsis [15]. In addition to infection, immunoparalysis can be induced by non-infectious causes such as trauma, ischemia, and burns. Besides the term immunoparalysis, some authors refer to “leukocyte reprogramming” [16]. In this context, mention should be made of the phenomenon of endotoxin tolerance, which is synonymous with the immunosuppressive phase of the inflammatory response. Experimental models with mice and *in vitro* experiments have shown that monocytes and macrophages exposed to a priming dose of endotoxin do not respond to the next one. The result is a substantially decreased ability to produce TNF- α following the second stimulation with endotoxin [17]. A study using monocytes isolated from healthy volunteers demonstrated the development of endotoxin tolerance that lasted for 5 days after endotoxin priming, i.e., a period putting the patient at high risk of secondary superinfection before returning to the state of a hyperinflammatory systemic response [18]. However, endotoxin tolerance is not only characteristic of patients with sepsis, and similar findings have been reported in patients with trauma, conditions associated with liver and kidney ischemia, acute coronary syndrome, and cystic fibrosis. Although perceived as a defense mechanism in patients with sepsis, endotoxin tolerance is associated with a high risk of secondary infection.

Table 2: Overview of mechanisms responsible for the status of immunosuppression in sepsis.

1. Increased rates of innate and adaptive immunity cell apoptosis
2. T-cell depletion
3. Monocyte deactivation
4. Increase in TREG count
5. Increase in negative and decrease in positive co-stimulatory molecule counts
6. Shift from Th1 to Th2 response
7. Regulatory effect of the central nervous system on the immune system

To describe immunopathogenesis in simple terms, it is in terms of function—a state of immunosuppression with a typical decrease in immunocompetent cell counts in individual organs and the circulation and dramatically reduced production of pro- and anti-inflammatory cytokines by these cells. This immunosuppression is a critical factor in potentially fatal and ongoing infection. Further in-depth research in this area is crucial for potential immunotherapy of sepsis, with the current options being immunostimulation therapy [19] or, conversely, anti-inflammatory therapy in a subpopulation of patients [20].

MULTIPLE ORGAN FAILURE AS A SEQUELA OF SEPSIS

Patients diagnosed with dysfunction of two or more organ systems meet the criteria for multiple organ dysfunction syndrome (MODS). Should the condition progress and organ function loss occur, it is referred to as multiple organ failure (MOF), with sepsis as the primary cause. Furthermore, MOF can develop in patients with severe trauma, pancreatitis, burns, after ischemia/reperfusion injury (I/R injury, e.g. after cardiopulmonary resuscitation or aortic aneurysm rupture), generally as a consequence of SIRS or circulatory failure [21]. Patients with MOF/MODS show clinical and laboratory signs of multiple organ dysfunction even though the primary insult did not cause any direct injury to the failing organs. Development of this syndrome has been traditionally perceived as the result of release of circulating inflammatory mediators, which triggers changes in the circulation, presenting as hypotension with subsequent global and tissue hypoxia, and eventually leading to the cellular injury of affected organs. Organ failure secondary to ischemia is believed to be associated with cell necrosis or apoptosis, such as cardiomyocyte necrosis occurring, for example, in acute myocardial infarction. However, the microscopic study of organs of patients dying from sepsis shows almost normal findings with minimal or no signs of apoptosis or necrosis [22]. Furthermore, survivors have shown complete recovery of function over time even in organs with limited regeneration capacity. What then are the mechanisms responsible for the onset and development of MODS? Why do some patients, despite the maximum level of care and organ support or replacement therapy, show progression to death, while about two-thirds of patients with MODS due to sepsis survive, with the vast majority of survivors not requiring further long-term therapy or support?

PATHOPHYSIOLOGY OF SYSTEMIC ALTERATIONS

The Role of Hypoxia

The role played by tissue hypoxia in septic shock patients in the pathophysiology of MODS is not easy to assess. While individual cells can, to an extent and for a certain period of time, cope with reduced oxygen availability, severe or prolonged hypoxia results in activation of mechanisms intended to maintain cellular integrity. The initial response is inhibition of protein synthesis and other major processes associated with high ATP consumption [23]. Further, a decrease in mitochondrial function occurs accompanied with *is* reduced ATP consumption. As humans have normally high ATP consumption, severe hypoxia in them leads to metabolic (energetic) failure with subsequent cell death. The originally widely accepted perception of MODS as the consequence of inadequate systemic oxygen supply to tissues in septic shock had to be modified. In the early 1990s, Kreymann reported that, in patients with uncomplicated sepsis, systemic oxygen consumption (VO_2) is increased by about 30% when compared with normal basal metabolism, whereas the VO_2 in patients with severe sepsis and those in septic shock is significantly reduced [24]. The same trend could thus be presumed in the disease course in a specific patient, i.e., in the progression of uncomplicated sepsis through severe sepsis and MODS to the development of MOF. All these findings, together with the surprising values of normal to increased partial oxygen pressure in muscle tissue of septic patients, which becomes normalized in the regeneration phase of sepsis, suggest impaired utilization of oxygen rather than inadequate oxygen supply [25]. Because more than 90% of oxygen is consumed in the process of oxidative phosphorylation in the mitochondria, it is logical to assume that it is in fact impaired mitochondrial function that plays an important role in the pathophysiology of sepsis-related MOF.

ALTERATIONS AT THE ORGAN LEVEL

Endocrine System

Once the body is exposed to a dangerous situation or harmful stimuli, it activates a typical, deeply preserved and coordinated stress response called, by Hans Selye in the 1930s, the “general adaptation syndrome” [26]. It is an alarm response aimed at restoring homeostasis and increasing the chance of survival. The response activates the sympathoadrenal system, thus resulting in direct release of catecholamines and, at the same time, increases the secretion of the stress hormone cortisol from the adrenal cortex via the HPA axis. This is mediated predominantly by two hormones, corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH). The main effect of this pathway activation is to increase the availability of energy reserves in crisis. In sepsis patients, the stress response mediated by the HPA axis is variable between individual patients. In particular, plasma cortisol levels may be low, normal, or high, yet inadequate to ensure the body’s metabolic requirements and to control the anti-inflammatory response. This situation is referred to as relative adrenal insufficiency (RAI) or critical illness-related corticosteroid insufficiency (CIRCI). According to data from observational studies, RAI

has a deleterious effect on mortality rates [27]. Based on these findings, interventional studies using corticoid replacement have been performed, but have led to inconsistent and contradictory results [28].

Endothelium

The endothelium plays a key role in the modulation of the inflammatory response in sepsis, its total weight is approx. 1 kg and it covers an estimated 4000–7000 m². The endothelium covers the vessel surface from the heart to the tiniest capillaries, where it forms not only a mechanical barrier but, also, a dynamic communication and transportation interface between blood and cells of individual tissues. Of key importance is its role in the regulation of hemostasis [29]. Sepsis is associated with endothelial activation, which manifests most frequently as a combination of decreased barrier function, increased leukocyte adhesion, and alterations in the endothelial surface as it becomes markedly procoagulative.

An accompanying sign of sepsis is widespread endothelial apoptosis or only individual cells may be affected unless the strength of the inflammatory insult has overcome a threshold value. Whatever the case, unprotected gaps in the endothelial lining develop, with blood directly communicating with the extracellular matrix and subendothelial cells, which express tissue factor (TF) on their surface. Consequently, coagulation activation and platelet aggregation follows. Some of the activated endothelia can be released from the endothelial layer/lining and thus increase the levels of circulating endothelial cells. This also occurs under physiological conditions, but in small quantities. Sepsis is associated with the production of large amounts of cytokines, chemokines, and other mediators, with endothelial cells being a major source. Thus the endothelium is at the center of the regulation and communication between the compartments (intravascular and extravascular) and different cells. While communicating with all main blood cell types on the luminal side, the endothelium interacts with pericytes, parenchymal cells, and, in particular, with smooth muscle cells on the subendothelial side. There is clear evidence that this communication is markedly impaired in septic individuals.

Coagulation Abnormalities

The marked procoagulant state in MODS is due particularly related to tissue factor (TF) expression, which subsequently activates numerous pathways in the coagulation cascade. Under physiological circumstances, TF is expressed constitutively and predominantly extravascularly, and only after vessel injury does activation of clotting occur following TF/factor VII/VIIa complex formation (extrinsic pathway). Severe sepsis is associated with TF expression on intravascularly localized cells, particularly monocytes and endothelial cells. This expression is the consequence of activation of TLRs recognizing components of the cell wall of pathogens. Tissue factor reacts with factor VIIa with subsequent activation of factor X, either directly or indirectly, via factor IX. Activated factor X converts prothrombin to thrombin, which eventually cleaves fibrinogen to fibrin. Recently, it has been shown that TF has another origin within the intravascular compartment.

The latest reports suggest the presence of microparticles in blood composed of TF and P-selectin glycoprotein ligand-1 (PSGL-1). These microparticles are fragments of activated or apoptotic cells possibly originating from monocytes, thrombocytes, or endothelial cells [30].

In severe sepsis, the clotting balance is shifted toward a procoagulant state by inhibition of natural anticoagulation mechanisms, including in particular, three major representatives of the anticoagulation pathways: tissue factor pathway inhibitor (TFPI), antithrombin, and protein C. The mechanisms linking inflammation and coagulation have been identified. Proinflammatory cytokines, such as TNF- α , IL-1 and IL-6, activate the coagulation system. In contrast, coagulation factors, which are themselves proteases, activate the inflammatory process through protease-activated cell receptors (PARs). Their receptor function is unique as they act both as the receptor and the ligand. Receptor cleavage by proteases, e.g., thrombin, factor VIIa, and activated protein C, results in the exposure of one of the receptor ends (neo-amino terminus), which itself activates its own receptor and triggers transmembrane signaling. The PAR family is composed of four subtypes named PAR-1 to PAR-4. These receptors are localized on the surface of the endothelium, monocytes, fibroblasts, thrombocyte, and smooth muscle cells. The consequence of PAR activation is a predominantly increased proinflammatory response in sepsis.

Coagulation is activated in almost all patients with sepsis, and patients with sepsis accompanied by disseminated intravascular coagulation (DIC) have a worse prognosis. Likewise, it has been suggested that sepsis-induced DIC leads to deterioration of MOF. In summary, modulation of coagulation in sepsis should, in theory, affect the natural course of sepsis in a given patient and various strategies for modulating coagulation in sepsis have been tested.

ALTERATIONS AT THE CELLULAR LEVEL

Oxidative Stress

The term “oxidative stress” refers to an imbalance between excess production of free radicals and the body’s antioxidant capacity. Oxidative stress caused by the increased production of free radicals overcoming the antioxidant capacity has been identified as one of the factors in the development and propagation of tissue injury in critically ill patients with MOF. The main sources of reactive oxygen species (ROS) *in vivo* are the enzymes xanthine oxidase, myeloperoxidase, NADPH-oxidase, and the mitochondrial respiratory chain complexes. The body’s main antioxidants are the enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase, vitamins (C, E, beta-carotene), and glutathione [31].

Sepsis has been shown to be associated with excess free radical production through activation of leukocyte NADPH-oxidase by proinflammatory cytokines. At the same time, high levels of nitric oxide (NO) in sepsis inhibit the respiratory chain and induce a high reduction potential with escape of electrons and reduction of molecular oxygen to a superoxide radical. Superoxide is subsequently the source of other ROS hydroxyl radicals (OH \cdot) and hydrogen peroxide (H $_2$ O $_2$) In combination with high NO levels, superoxide leads to the formation of reactive nitrogen

species (RNS), in particular, peroxynitrite. The decreased antioxidant capacity, together with decreased levels of tocopherol, retinol, and carotenoids, potentiates the oxidative stress in sepsis [32]. Oxidative stress causes cellular dysfunction in several ways. It acts cytotoxically and mutagenically by affecting the biological properties of nucleotides, causing structural DNA damage and, through NAD⁺ dimerization, it causes depletion of NAD⁺ reserves. Free radicals cause dysfunction of enzymatic systems via oxidation of thiol groups, covalent binding to proteins, or by nitrosylation. Furthermore, oxidative stress triggers the intrinsic mitochondrial pathway of apoptosis activation.

In the body, free radicals have many physiological regulatory functions. They are a major post-translational modifier of the TLR cascade, thus significantly regulating secretion of proinflammatory molecules. Organisms with defective genes encoding proteins regulating antioxidant levels show overexpression of proinflammatory cytokines. It has recently been suggested that the regulatory effects of ROS are wide and pleiotropic, having both proinflammatory actions and, on the other hand, causing increased production of anti-inflammatory molecules such as IL-10 and soluble TNF receptors [33].

Nitric oxide (NO) is a highly diffusible lipophilic gas with a short half-life of 6–10 seconds. The interest in NO in connection with sepsis and septic shock dates back to the 1980s based on reports of its vasodilatation properties and its identification as a hypothetical endothelium-derived relaxing factor (EDRF).

Nitric oxide is formed in tissue from L-arginine by the family of NO-synthase (NOS) enzymes. To date, three NOS types have been identified, and named either in the order of their identification or, alternatively, by the tissue they predominantly occur in: NOS-1 or neuronal NOS (nNOS), NOS-2 or inducible NOS (iNOS), and NOS-3 or endothelial NOS (eNOS). Recently, the existence of a mitochondrial isoform (mtNOS) has been confirmed. Nitric oxide is believed to play a major and pleiotropic role in the pathogenesis of sepsis. From a pathophysiological point of view, the action of NO can be divided into three categories.

First, it has hemodynamic effects that involve activation of guanylate cyclase leading via cyclic guanosine monophosphate (cGMP) to a decrease in intracellular Ca levels in smooth muscle cells, and, hence, hyporeactivity of the systemic vasculature toward catecholamine effects. Increased iNOS expression on cardiomyocytes in sepsis suggests the role of NO in the development of myocardial dysfunction in sepsis.

Second, the metabolic action of NO is mediated primarily by its effects on the mitochondrial respiratory chain. At a physiological concentration, NO reversibly competitively inhibits complex IV (cytochrome C oxidase). By stopping the flow of electrons through the respiratory chain, NO increases respiratory chain reduction potential and hence its ability to reduce molecular oxygen to the superoxide anion. Reaction of superoxide with NO produces peroxynitrite, which irreversibly inhibits complexes I and III. Nitric oxide is thus capable of inducing mitochondrial dysfunction

and cellular hypoxia even under conditions of adequate partial oxygen pressure in tissues. In this way, NO contributes to the development and potentiation of organ dysfunction in sepsis [34].

The third major role of NO in the pathogenesis of multiple organ dysfunction is the induction of apoptosis.

Impaired Microcirculation

The main characteristics of severe sepsis and septic shock include tissue hypoperfusion and cellular hypoxia. This is explained by impaired microcirculation and the redistribution of tissue perfusion, which are other major mechanisms in the development of organ dysfunction [35]. Microcirculatory dysfunction has also been proposed to explain impaired oxygen extraction in sepsis, i.e., an inability to raise O_2 consumption (VO_2) in the presence of its adequate supply. However, the normal or increased tissue partial O_2 pressure (ptO_2) in sepsis indicates that impaired microcirculation may not play a crucial role in the development of MOF. Moreover, lower ATP levels suggest impaired cellular utilization of O_2 rather than its inadequate supply, at least in the advanced stage of sepsis [36].

Mitochondrial Dysfunction

It has been suggested that the reason for decreased systemic extraction of O_2 in advanced sepsis was an acquired defect of oxidative phosphorylation referred to as cytopathic hypoxia [37]. Research into cellular oxygen consumption and mitochondrial function in sepsis has provided a large body of data, and mitochondrial dysfunction is today a well-documented fact. The activities of respiratory chain complexes I, II, and IV are significantly low in animal models of sepsis, with the decrease correlating with the magnitude of the initial insult and sepsis severity. In addition, the activity of cytochrome oxidase, which—as the final oxidase in the oxidative chain—is directly responsible, for O_2 consumption, has been repeatedly shown to be low [38]. Similar findings were reported from clinical research in patients with severe sepsis. Decreased levels of ATP, phosphocreatine, and decreased complex I activity in the skeletal muscle of patients with sepsis correlated with increased mortality rates.

The role of mitochondrial dysfunction has not been fully clarified. Fever, protein catabolism, negative nitrogen balance, hyperglycemia, and insulin resistance suggest hypermetabolism rather than metabolic down-regulation, which can be explained by overexpression of uncoupling proteins (UCP), whose thermogenic effect is well recognized. At the same time, a hypermetabolic state is typical for the early stage of sepsis. Later on, in the stage of organ dysfunction, reduced O_2 consumption has been documented. Hence, there are only limited data suggestive of a disorder in oxygen tissue extraction and energetic metabolism of the different cell populations in sepsis (39). The theory of mitochondrial shut-down suggests that bioenergetic cell failure, i.e., mitochondrial dysfunction, is the primary defense mechanism whereby the body responds to the complex changes in sepsis in an effort to reduce basal metabolism through hibernation, thus allowing the body to cope with the primary septic insult and its sequelae.

SUMMARY

Oxidative stress with subsequent cellular dysfunction is a major factor in the development of MODS. This process is caused by excess production of free reactive oxygen species interacting with NO and subsequent peroxynitrite formation leading to mitochondrial dysfunction, apoptosis induction, and eventually progression of organ dysfunction.

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