Molecular basics of sepsis development

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Abstract

Bacterial infections and sepsis remain major causes of morbidity and mortality in intensive care units. The normal host response to infection is a complex process that serves to localise and control the invasion of microbes and to repair injured tissue. Local inflammatory processes are regulated through the production of cytokines by macrophages. In some cases, mediator release exceeds the boundaries of the local environment and results in the development of sepsis. It is well known that the innate immune system plays a crucial role in preventing microbial invasion. The human innate immune system consists of genetically programmed defence mechanisms that are directed against molecular components found only in microorganisms. Understanding the complexity of early response to infection with respect to innate immune response is required for the future development of therapeutic drugs that will effectively control infectious diseases.

Key words: inflammation, innate immune response; inflammation, innate immune response, sepsis

The body's normal response to infection is to recognise an infectious agent and control its spread, to limit the inflammation and to repair the tissues. Both cell-mediated and humoral components of the immune system are involved in these processes. The immune activation leads to increased production and release of pro- and anti-inflammatory factors at the site of injury. However, for not fully clear reasons, in some proportion of infections the sequence of events triggered results in massive systemic release of mediators, development of systemic inflammatory response syndrome (SIRS) and sepsis. Sepsis, severe sepsis and septic shock are the causes of hospitalizations of about 34% of patients treated in intensive care units (ICUs) in Poland. The annual incidence regarding all forms of sepsis in 2004–2005 was 91/100,000 citizens, and mortality ranged from 39.4% to 54.5% [1, 2]. According to the Sepsis Occurrence in Acutely Ill Patients (SOAP) study, the sepsis incidence in ICUs in Europe ranged from 18% to 73%, depending on the country whereas the mean mortality was 27% in the entire study group, 32.2% in severe sepsis and 54.1% in septic shock [3]. Despite substantial advances in medical sciences and new drugs introduced, the mortality in sepsis is still high. Therefore, research is being continued to find some novel methods of treatment involving pharmacological interventions during the early inflammatory response. Detailed knowledge about the structural components of bacteria and molecular mechanisms responsible for the host response to their presence is a prerequisite of the effectiveness of such methods.

Identification of microorganisms

One of the key elements of the body reaction to infection is the immune response activation, which involves specific and non-specific mechanisms. The non-specific mechanisms, defined as the innate immune response, develop earlier in the phylogenetic development, are less precise but react quickly. The specific mechanisms, which belong to the acquired immune response, are highly specialised, molecularly sophisticated and phylogenetically younger yet occur with delay [4]. The infectious agent stimulates both the innate and acquired immune response; however, it is the former, which is the first-line defence and is most likely responsible for the survival of Homo sapience until present days, despite the delicacy of mechanical barriers protecting against injuries.

The innate immune system includes the physical barriers, enzymes of epithelial and phagocytic cells, phagocytes, cells releasing inflammatory mediators, cell surface proteins, cell receptors and soluble components of plasma,
including acute phase proteins, the alternative complement pathway, and cytokines [4, 5]. The majority of microorganisms are eliminated due to innate mechanisms. These mechanisms are incapable of recognizing each possible antigen but can recognize several pathogen-associated molecular patterns (PAMPs) present in most microorganisms. PAMPs include components of the bacterial cell wall, such as lipopolysaccharide (LPS), peptidoglycan, lipoteichoic acid, yeast mannan, sequences of bacterial DNA, viral RNA, glucans, polysaccharides or proteins of microorganisms. The characteristic feature of PAMPs is the stability of their structure, which cannot mutate and overcome the host defence mechanisms [6, 7]. In cases of SIRS without infection (NSIRS), the endogenous damage-associated molecular patterns (DAMPs) play a similar role to PAMPs; these include heat shock proteins (HSPs), fibrinogen, fibronectin, hyaluronates, high-mobility group protein box-1 (HMGB-1), DNA fragments and adenosine triphosphate (ATP). PAMPs and DAMPs are recognised by pattern recognition receptors (PRRs), which belong to the innate immune system [5, 6]. PRRs include blood circulating proteins as well as transmembrane and intracellular receptors of signal transduction (Table 1).

**ACTIVATION OF INFLAMMATORY AND IMMUNE RESPONSE**

The activation of inflammatory and immune responses in sepsis and NSIRS is mediated by PRRs, of which the toll-like receptors (TLRs) are best known. They occur on the surface of the immune system cells, i.e. lymphocytes, monocytes, neutrophils, dendritic cells, macrophages, and on the epithelial cells of the gastrointestinal and respiratory tract, endothelial cells, fibroblasts, fatty cells as well as the cells of the myocardium, spleen, lungs, thymus and microglia [7]. TLRs are glycoproteins consisting of the extracellular part, formed by the domains with leucine-rich repeats that recognise PAMPs, the transmembrane and endocyttoplasmic part, homologous to the IL-1 receptor, which are called TIR (Toll-IL-1R). Four adaptor proteins are involved in signal transduction from TIR: myeloid differentiation factor 88 (MyD88), MyD-adaptor-like/TIR-associated protein (MAL/TIRAP), toll-receptor-associated activator of interferon (TRIF) and toll-receptor-associated molecule (TRAM). The above proteins bind IL-1R-activating kinases (IRAK 1,2,7), activating the TNF receptor-associated factor 6 (TRAF 6), which leads to activation of the nuclear factor kB (NF-kB) and ultimately activates the cytokine promoter genes [6]. Within 60-90 minutes, the TLR4 – activated macrophages initiate the production of pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6, IL-8 and IL-12. The experimental and clinical studies confirm a relevant role of TLR2 and TLR4 in the development of SIRS after injury and during infection [8, 9]. The ongoing studies focus on the use of TLR4 antagonist for the treatment of severe sepsis in humans [10].

The activation of inflammatory response in sepsis and NSIRS is mediated not only by TLRs but also by two other pattern recognition receptors [6]. TLRs are expressed on the cell surface while cytosol PRRs serve to identify intracellular pathogens. The latter include the family of nucleotide-binding domain, leucine-rich repeat containing (NLR) proteins known as NOD-like receptors consisting of NLRC and NLRP subfamilies as well as other proteins. NLRC1 and NLRP2 are present in the myeloid, epithelial and phagocytic cells. They show the ability to bind fragments of bacterial peptidoglycan and to transmit the signal, irrespective of TLRs [11]. Moreover, they activate NF-kB and mitogen-activated protein kinase (MAPK) via receptor-interacting serine/threonine-protein kinase 2 (RIPK 2). In response to PAMPs, NLRPs induce the release of IL-1 inflammatory cytokines, such as IL-1beta, IL-18 and IL-33. The process of transformation of inactive forms of these cytokines into active ones takes place within the molecular platforms called inflammasomes, in which caspase-1, indispensable for this process, is activated by the apoptosis-associated speck-like protein (ASC) containing caspase activation and

### Table 1. Examples of pattern recognition receptors and their role in the development of innate immune response. According to [5, 6, 7]

<table>
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PRRs — pattern recognition receptors; TLRs — Toll-like receptors; PAMPs — pathogen associated molecular patterns; DAMPs — damage-associated molecular patterns; RLHs — RIG-I-like helicases
recruiting domain. Depending on the type of NLR protein, three major kinds of inflammasomes are distinguished: NLRP1, NLRP3 and NLRC4, corresponding to former NALP1, NALP3 and IPAF [7, 11]. Inflammasomes are activated by both infection-associated factors and stress-associated factors, including the effects of K+, reactive oxygen species (ROS) and cathepsins. They are likely to be responsible for detection of intracellular stress and the initial response to this stress.

The third group of PRRs is the family of retinoic-acid-inducible gene-I (RIG-I)-like receptors (RLRs). Their role is to detect the nucleic acids of viruses and to activate the anti-viral immune response [6, 7]. It should be stressed that all PRRs activate the innate and regulate the acquired immune response to infections and non-infectious tissue injury, leading to the development of SIRS.

**SIGNAL ENHANCEMENT AND TRANSDUCTION**

After the initial interaction between the host receptors and structural components of a pathogen, the early activation of inflammatory response takes place, the key element of which is NFκB. This nuclear factor is an inactive protein complex suspended in the cytoplasm consisting of five subunits RelA, RelB, cRel, p50 and p52, which contain the Rel homology domain (RHD). The monomers mentioned above can form 15 different homodimers or heterodimers. The NFκB signalling system involves also inhibitory proteins — IκBα, IκBβ and IκBε. The NFκB activation is the crucial element of cell-mediated response to any injury; however, depending on the type of cell and stimulus, different complexes of dimers are expressed. Cytokines, PAMs and DAMs activate IκB kinase (IKK) via PRRs, which induces degradation of the inhibitor, as well as release and activation of NF-κB. The active form is transferred into the cell nucleus; the process of transcription and translation is activated with increased production of pro-inflammatory cytokines and activation of immune responses [12]. This mechanism of activation of the nuclear factor, called the canonical pathway, is characteristic of acute, reversible injury or inflammation.

This is the way NF-κB regulates the synthesis of cytokines and other proteins, including caspase inhibitors, affecting apoptosis and immune response. The process of maturation and activation of NF-κB occurs within the protein complex called a signalosome [13]. Increased expression of NF-κB was demonstrated in animal lung tissues on day 1, 3 and 7 of sepsis [14], in alveolar macrophages, peripheral blood mononuclear cells (PBMCs) and neutrophils (NUs) of humans with sepsis [15, 16]. Considering large numbers of NFκB dimers, IκB proteins, IKK and connections between the canonical and non-canonical pathways of signalling activation, it seems impossible to translate the experimental results into clinical management [12].

The inflammatory process during infection is additionally enhanced by activation of molecular platforms called inflammasomes, which are protein complexes suspended in the cytoplasm. Inflammasomes are involved in the development of inflammation by activation of caspase -1, which transforms inactive forms of IL-1β, IL-18 and IL-33 into their active forms [17]. With active participation of inflammasomes, these cytokines are released from the cell to the extracellular space and regulate the immune response. The activation of inflammasomes and caspase-1 can be triggered by bacteria and their toxin, viruses, bacterial RNA, TLRs (directly or indirectly), and DAMPs. The study findings demonstrate that the NALP3 inflammasome activated by TLRs or bacterial RNA is essential for the development of sepsis. Some bacteria and viruses as well as LPS can activate caspase-1 through the Ipaf inflammasome or ASC [17]. Inflammasomes and activation of caspase-1 play a major role in the development of SIRS in the course of infection. Reduced expression of inflammasome mRNA was observed in monocytes of patients with sepsis, which was accompanied by substantially elevated concentrations of cytokines in blood serum [17]. Blocked caspase-1 activation reduces the synthesis and secretion of inflammatory cytokines in the animal model [18]. On the other hand, caspase-1 is necessary for efficient action of some protective mechanisms, such as the generation of ROS and reactive nitrogen forms, which enable the killing of microorganisms in the human body.

**Figure 1. Development of systemic inflammatory response syndrome**
NEW MEDIATORS OF INFLAMMATION IN SEPSIS

In recent years, additional pathways were detected used by the host cells to recognize bacterial components. The triggering receptor expressed on myeloid cells-1 (TREM-1) glycoprotein, belonging to the superfamily of immunoglobulins, expressed in the late stages of myeloid cell maturation, is involved in the activation of monocytes and NUs during the inflammatory process. The expression of TREM-1 increases in bacterial and fungal infections and the elevated serum level of the soluble receptor form might be indicative of infection [19, 20]. In response to the infection, activated macrophages and monocytes release many cytokines, including HMGB-1, responsible for maintaining proper structure of the cell nucleus and for gene transcription processes. By binding with TLR4 and the soluble receptor for advanced glycation endproducts (sRAGE), HMGB-1 activates NF-κB and affects the synthesis of cytokines. Clinical studies in patients with injuries show that serum HMGB-1 concentration is a late mediator of sepsis and an early indicator of non-infectious SIRS [21]. Moreover, the involvement of sRAGE in the development of inflammation has been increasingly stressed; sRAGE belongs to immunoglobulins and binds advanced glycation endproducts (AGEs). AGEs are formed due to non-enzymatic glycation of proteins, lipids and other molecules and are harmful for the human organism as by interacting with RAGE receptors they modify the function of cells. The AGE/RAGE interaction activates NFκB and MAPK inducing oxidative stress and formation of AGE in the cell, which again activates RAGE, thus the inflammation persists. In the experimental sepsis, blockage of information transfer by RAGE leads to increased survival [22]. Increased levels of the soluble receptor form were observed in patients with sepsis, which suggests possible usefulness of sRAGE in the development of new methods of treatment of severe infections in humans [22].

ROLE OF APOPTOSIS

Apoptosis is the programmed cell death without damage to the cell membrane, release of cell content and development of the inflammatory response. During this process, specific biochemical reactions lead to condensation of nuclear chromatin and cytoplasm, activation of cell endonucleases and DNA degradation. The factors inducing apoptosis include glucocorticoids, cytokines, e.g. TNFα, TNFβ, IL-1β and IL-6, Fas ligand (FasL), heat shock, ROS, nitrogen oxide and products of cytotoxic lymphocytes T with FasL expression on their surface [23]. Three main pathways are involved in apoptosis: extrinsic, called membranous connected with death receptors, intrinsic, called mitochondrial and endoplasmic reticulum [23].

In the early response to infection, a relevant role is played by apoptosis of NUs, which control the local multiplica-

tion of bacteria preventing the spread of infection and, on the other hand, induce the activation of endothelial cells, development of systemic inflammation and organ damage. The experimental and clinical findings demonstrate inhibited NU apoptosis in patients with sepsis yet the mechanisms responsible for these processes have not been fully elucidated [14]. The activation of NF-κB, leading to inhibition of caspase-3 activity and maintenance of transmembrane mitochondrial potential, seems to be decisive [12, 23]. It has been shown that bacterial lipoproteins mediated by TLR2 and CD14 on the surface of NUs inhibit depolarisation of the mitochondrial membrane, which reduces the level of active caspase-3 [24]. Delayed apoptosis may also result from accelerated breakdown of active caspase-3 caused by the endotoxin or from induction of antiapoptotic proteins. Prolonged half-life of NUs due to inhibition of apoptosis is associated with increased severity of sepsis, accumulation of NUs in the lung tissues and development of acute lung injury (ALI) [14]. The activation of inflammation in septic shock is also characterised by enhanced expression of caspase-3,-8 and -9 of lymphocytes and apoptosis of PBMCs via the extrinsic and intrinsic pathway [25]. Increased apoptosis of lymphocytes is suggested to impair the innate and acquired immune responses resulting in the development of opportunistic infections [26]. Inhibited lymphocyte apoptosis in animal models of sepsis by MyD88 knockout increases mortality [27], whereas the knockout of Bid limits apoptosis and inflammation improving the survival rates [28]. Thus, apoptosis of inflammatory cells is a relevant element of response to infection; however, the explanation of this process requires further comprehensive research.

SUMMARY

Sepsis is a life-threatening condition associated with the generalised reaction of the body to infection, which is accompanied by impaired mechanisms of non-specific immunity. Despite numerous studies on its pathogenesis, sepsis remains one of the major causes of morbidity and mortality of patients treated in ICUs. Its development and course depend not only on the virulence of pathogens but also on the presence and activity of receptors localized on the surface of immune cells. The complex inflammatory response that is observed in sepsis involves provision of dynamic equilibrium amongst a number of opposite molecular mechanisms to maintain the immune balance. The developing pro-inflammatory activity triggers the anti-inflammatory mechanisms that block it in order to restore homeostasis. To understand better the development of sepsis and design new therapeutic options, it is essential to widen our knowledge on structural components of microorganisms and mechanisms responsible for the initiation of inflammatory responses.
References:


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