Impact of Cytokines on Sepsis

Subjects: Biology

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Sepsis is an overwhelming inflammatory response to infection, resulting in multiple-organ injury. Neutrophils are crucial immune cells involved in innate response to pathogens and their migration and effector functions, such as phagocytosis and neutrophil extracellular trap (NET) formation, are dependent on cytokine presence and their concentration. In the course of sepsis, recruitment and migration of neutrophils to infectious foci gradually becomes impaired, thus leading to loss of a crucial arm of the innate immune response to infection.

Keywords: neutrophil ; sepsis ; cytokine

1. Cytokines

Cytokines are molecules that regulate various processes, such as proliferation, differentiation, and cell mobility. By affecting a broad spectrum of cells, cytokines mediate inflammatory and immune reactions and participate in the regulation of hemopoiesis. Over one hundred and several dozen cytokines have already been identified. Their number is constantly increasing. There is no single cytokine classification scheme. Due to structural similarities, there are: type 1 cytokines (hematopoiesis), type 2 cytokines (interferons and IL-10 families), chemokines, and the TNF-α superfamily [1][2].

Some cytokines are often referred to as interleukins to reflect their effects between different leukocyte populations. With time, however, it turned out that many interleukins can be secreted by other types of cells (keratinocytes, fibroblasts) and that they can influence not only leukocytes, but almost any other type of cell. Although the cytokines initially secreted by monocytes were referred to as monokines and those that produced lymphocytes were called lymphokines, these terms are not currently used as none of the cytokines are secreted exclusively by a single cell type [1][3].

The characteristic features of cytokines are (1) pleiotropy, i.e., the ability of a given cytokine to affect many different cells and induce different effects, and (2) redundancy, i.e., the ability of different cytokines to cause the same effect. Some cytokines can antagonize each other, blocking the biological effects of each other. Other cytokines acting simultaneously on the same cells achieve a synergistic effect. Another property of cytokines is the ability to induce positive and negative feedbacks [1][2].

When analyzing the participation of cytokines in the activation, proliferation, and differentiation of cells, it should be remembered that these processes are also regulated by direct intercellular interactions [4][5]. Some cytokines are produced initially as membrane molecules involved in the direct activation of target cells. An example of this type of interaction is TNFs, which initially occurs in the form of a membrane protein but is subsequently secreted into the environment by a suitable metalloproteinase [6]. Cytokines can act on the same cells that secrete them (autocrine effect), cells in the immediate vicinity (paracrine effect), or on cells in other organs (endocrine effect) [7].

It may seem that due to such a large number of cytokines and their intricate interactions, the immune response should be a chaotic rather than an organized phenomenon. However, the sensitivity of cells to cytokines depends on the prior recognition of the antigen. Thus, when produced at high concentrations, cytokines will not act on all lymphocytes, but only on those cells that specifically recognize the antigen and are ready to perform effector functions. Moreover, the action of many cytokines is related to their local secretion, i.e., to the immune synapse, thanks to which they achieve local high and effective concentration.

1.1. Receptors for Cytokines

Cytokines can exert their functions only due to the presence of receptors on target cells. Research into the structure of these receptors and their signaling pathways has answered many questions about the role of cytokines in the functioning of the immune system and the other systems they act on [8].

A characteristic feature of most known cytokine receptors is that their extracellular fragments contain characteristic domains. These domains are responsible for the ligand binding specificity, but also influence the way the signal will be transduced after cytokine binding. The second characteristic feature of cytokine receptors is the presence of intracellular domains, which are directly responsible for initiating signals in the cell. The extracellular part is connected to the cytoplasmic section by transmembrane fragments of the receptor. Despite the distinct differences in the structure of cytokine receptors, these receptors can be divided into five different types: Ig-like receptors, class 1 cytokine receptors (hematopoietin receptors), class 2 cytokine receptors (receptors for interferons and the IL-10 family), receptors for the TNF superfamily, and G-protein coupled receptors (chemokine receptors) [1][9].

Receptors that cross the cell membrane only once must be di- or trimerized-processes necessary for signaling. In this case, the cytokine acts as a molecule that creates a binder that allows the horizontal shift of the receptor subunits in the plane of the cell membrane. Only then, as the cytoplasmic sections of the receptors come closer to each other, signal transmission is initiated due to the cross phosphorylation of these sections or proteins associated with them.

The binding of cytokines to receptors on the cell membrane leads to the activation of signal transduction pathways in the cell. The pathways of GTPases and MAP kinases, tyrosine kinases from the Src- and Tec-like families, and phosphatidylinositol-3-kinases (PI-3K) are involved here [10]. However, most cytokines activate the signal transduction pathway by JAK (Janus kinases) tyrosine kinases and STAT (signal transducers and activators of transcription) proteins.

1.2. Sepsis and Cytokines

Current theories suggest that the sepsis may be associated with an early overwhelming innate immune response, characterized by dysregulation of protein mediators: activation and release of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8), and their receptors (IL-1RA, TNF-R1/2) and dysregulation of crucial molecules, which modulate immune response (e.g., MCP-1, HMGB-1, PD-1, CTLA-4, NGAL, MMP-9, TIMP2, PAI-1). Importantly, the recent studies provide convincing data that mitochondrial DNA (mtDNA) can influence the immune system through toll-like receptor 9 and inflammasomes. Clinical trials provide evidence that mtDNA is elevated in critically ill patients and is associated with mortality [11].

Recently drugs targeting cytokines signaling are extensively studied in COVID-19, as in acute respiratory distress syndrome (ARDS) phase symptoms in large part results from cytokine storm and collateral organ damage. Corticosteroids were some of the first investigated drugs and indeed, RECOVERY trial show favorable influence of dexamethasone [12]. According to National Institutes of Health guidelines (last update on 24 February 2022), IL-6 or Janus Kinase inhibitors (i.e., tocilizumab, sarilumab, or baricitinib, tofacitinib, respectively) may be added as a second immunomodulating drug. Evidence supporting use of tocilizumab mainly comes from REMAP-CAP trial [13]; nevertheless, other studies also favor the drug, as reviewed in [14]. Evidence speaking for baricitinib and tofacitinib are based on randomized controlled trials (COV-BARRIER [15], and STOP-COVID [16], respectively). Anakinra, IL-1β inhibitor, also seems to reduce mortality in the late phase of COVID-19, as reviewed in [14].

The presence of cytokines is normally restricted to an area of injury. However, when a local infection spreads, a strong systemic reaction occurs, and signs of sepsis are apparent. Under such circumstances, mediators can be detected systemically, and may lead to septic shock. On the other hand, innate deficiency of cytokine release during acute severe infections leads to a rapid multiplication of the invading microorganisms, which results in reactions of the host consisting of pro-inflammatory and anti-inflammatory reactions (term SIRS is still used in this context, and term compensatory anti-inflammatory response syndrome (CARS) is used, respectively), which could ultimately lead to shock and death. An inadequate systemic inflammatory response is partially counterbalanced by sustained expression of potent anti-inflammatory mediator IL-10 [17][18].

Cytokines are small (8–26 kD), highly active molecules, which are synthesized primarily by the cells of the immune system. Concentrations of circulating pro-inflammatory cytokines are low or undetectable in healthy individuals but their production is stimulated during host invasion by pathogenic microorganisms. Four cytokines, TNF-α, IL-1β, IL-6, and IL-8 have been most strongly associated with sepsis. In human and experimental animal models of sepsis, cytokines are released in a sequential manner resulting in a “cytokine cascade” [19]. It is initiated when a stimulus, such as a Gram-negative bacterial endotoxin (e.g., lipopolysaccharides released by E. coli), induces production of the “early inflammatory cytokines”, such as TNF-α and IL-1β. TNF-α is regarded as a central mediator of immune regulation and of the pathophysiological changes associated with bacteremia and sepsis syndrome [17]. Plasma TNF-α concentrations are increased in patients with both Gram-negative and Gram-positive infectious diseases [18]. Overproduction of TNF-α correlates with enhanced properties of phagocytes. In contrast, IL-1β serum levels are only slightly increased during sepsis. The release of “early inflammatory cytokines” intensifies the production of the “late inflammatory cytokines”—IL-6

and IL-8. The mortality rate is significantly higher in patients who present with a high IL-6 serum level (above 1000 pg/mL). IL-6 concentration is recognized as a marker of sepsis with high specificity [20]. Redl and colleagues [21] showed that treatment with anti-TNF-α antibodies results in significantly decreased IL-8 concentrations in the bloodstream. The increased plasma IL-8 concentration in adult sepsis-occurring patients may correlate with mortality [22], however, not enough observations clearly confirm this hypothesis.

Proinflammatory cytokines play a crucial role in the activation of the host defense. However, various experimental studies have shown that an overwhelming production of these mediators can lead to vasodilation, increased vascular permeability, hypotension, multiple organ failure, dysregulation of other protein mediators and ultimately shock and death [23].

Although various pro-inflammatory cytokines contribute to the inflammatory cascade, other cytokines also display anti-inflammatory properties, serving to counterbalance a potentially inadequate proinflammatory state. IL-10 in particular has been implicated as the primary endogenous modulator of inflammatory response during sepsis. The importance of IL-10 production during sepsis has been well established in various sepsis models [24][25]. Gerard and colleagues [26] showed that treatment of mice with IL-10 before endotoxin administration could prevent endotoxin-induced mortality and diminish plasma TNF-α release. On the other hand, inhibition of IL-10 during course of sepsis may also be beneficial [27]. This may seem like a contradiction, resulting for example from inconsistent experimental models, but different roles of a single cytokine may result from interactions with other cytokines at different stages of the disease. Future studies are needed, as indicated by Mazer et al. [28].

2. The Impact of Cytokines on Phagocytosis Performed by Neutrophils

Cytokine-like 1 (CYTL1) increased phagocytosis of activated neutrophils both in in vivo and in vitro models [29]. Researchers hypothesized that CYTL1-enhanced phagocytosis of *Escherichia coli* by activated neutrophils is dependent on phosphorylation of protein kinase B (Akt). Additionally, CYTL1 also increased the release of ROS in LPS-stimulated neutrophils. ROS are powerful antimicrobial agents produced in phagosomes and phagolysosomes; thus, their concentration directly affects the efficiency of the whole phagocytosis process.

Onogawa et al. tested whether IL-6 affects phagocytosis efficiency during sepsis. Using mice model infected with *Staphylococcus aureus*, they proved that the augmentation of the IL-6 signal by recombinant IL-6 receptors (rIL6R) allows the functional recovery of phagocytes in a peritonitis murine model, and consequently improves their phagocytic functions. The researchers noticed an increased uptake of *S. aureus* and phagosomal acidification, which favors bacteria killing and phagolysosomes' formation.

The direct effect of IL-6 on phagocytosis and ROS production was also evaluated in vitro on neutrophils isolated from healthy volunteers [30]. IL-6 treatment resulted in a significantly increased bacterial uptake, as well as stimulation of ROS generation. Interestingly, co-treatment with IL-6 and TNF-α intensified ROS generation, but did not affect phagocytosis [30]. These findings underline that not just a single cytokine's concentration matters, but rather concentrations of numerous cytokines, which together constitute cytokines' profile, exert certain functions. Gaber et al. showed that inhibition of IL-6 signaling by tocilizumab affects phagocytosis in an oxygen-dependent manner—in normoxia tocilizumab stimulates, whereas in hypoxia it impairs phagocytosis [31]. Considering that in sepsis and especially in septic shock oxygen supply is reduced, rather the former result may be expected.

IL-10 was classified by Mittal et al. as a cytokine, which stimulates *E. coli* clearance [32]. It is observed that administration of IL-10 during a high-grade bacteremia clears antibiotic-sensitive and -resistant *E. coli* from blood of infected mice. The suggested underlying mechanism was an increased expression of CR3 in phagocytes, which was caused by suppression of prostaglandin E-2 release. It may suggest that IL-10 mediates *E. coli* phagocytosis by precisely guiding bacteria via complement-dependent pathway.

Moreno et al. tested the role of IL-12 and IL-18 on neutrophil phagocytic functions in sepsis induced by cecal ligation and puncture (CLP) in a murine model [33]. Wild-type mice, as well as IL-18(−/−) mice were resistant to sepsis. On the contrary, IL-12(−/−) mice were susceptible to SL-CLP sepsis with high bacteria concentration, similarly to IFN-γ (−/−) mice. However, stimulating IL-12-deficient neutrophils with IFN-γ restored their phagocytic functions, stimulated NO production and more effective clearance of pathogens.

Increased level of IL-17 stimulates pathogen clearance but does not have a major impact on the inflammation pathology [34]. Another study was performed by van de Veerdonk et al. in two fungal-induced septic models. Intravenous infection

with live *Candida albicans* and zymosan injection showed a protective role of IL-10 during *C. albicans* clearance. Although IL-10 did not protect against zymosan-induced organ failure, the role of IL-10 was classified as important but not crucial.

The next cytokine that affects phagocytosis during sepsis is IL-34 [35]. It was tested in two models: wild-type C57BL/6 mice were used for in vivo studies, and septic human patients and healthy volunteers were recruited to obtain blood for in vitro studies. IL-34 concentration was significantly elevated in human sepsis and puncture-induced experimental sepsis. Additionally, administration of IL-34 successfully increased chemotaxis of neutrophils and strengthened their phagocytic functions. Decreased IL-34 concentration increased mortality in mice model and weakened pathogen clearance.

Flores-Mehia et al. demonstrated that in SIRS, compared to healthy volunteers, higher levels of both pro-inflammatory (TNF-α, IL-1β, IL-6, and IL-8) and anti-inflammatory cytokines (IL-1Ra and IL-10) do not affect bacteria uptake performed by neutrophils, however phagosome maturation is decreased [36].

Tofacitinib may impair immune response to *Candida albicans*, among others, by impairing phagocytic capacity of neutrophils [37]. Drugs, which modulate neutrophils phagocytosis without targeting cytokine signaling, were reviewed elsewhere [38].

3. The Impact of Cytokines on NET Formation

IL-1β is one of the “early” proinflammatory cytokines, also associated with increased mortality during sepsis. Time- and dose-dependent immunosuppressive agent—anakinra, shows positive correlation between blocking IL-1β receptor, NET formation, and IL-1β cytokine production. Suppressed expression of IL-1β receptor significantly reduced NET formation [39].

Yaqinuddin et al. showed that IL-1β/neutrophil extracellular traps feedback loop is present during SARS-CoV-2-induced acute lung injury. The researchers noticed that both SARS-CoV-2 and sepsis are accompanied by IL-1β overproduction. They concluded overproduced IL-1β stimulates NET formation via activating NLRP3 inflammasome complex [40]. IL-1β produced by the NLRP3 inflammasome is a key inducer of NETs [41].

According to Tomar et al., SARS-CoV-2 infections may be accompanied by cytokine storm and sepsis [42], as mentioned above. Although the knowledge about triggers of the cytokine storm during SARS-CoV-2-induced sepsis is still not complete, researchers hypothesized that the crucial role is played by neutrophils and their ability to form NETs [42]. They correlated appearance of IL-1β, and TNF-α with increased NET formation but the direct influence was not explored.

Multiple inducers of NETs have been reported; however, IL-8 seems to be the most effective stimulator of NET formation among investigated cytokines. Abrams et al. tested the influence of IL-8, IL-6, TNF-α, IL-1β, and selected histones on NET formation. A significant increase of NET formation was observed only for IL-8. When added to neutrophils, harvested from healthy volunteers, IL-8 induced NET formation. Conversely, incubation of healthy neutrophils with plasma obtained from septic patients attenuated NET formation by a functional anti–IL-8 blocking. It is showed that IL-8-induced NET formation is dependent on Ras/Raf/MAPK pathways as ERK inhibition attenuates the effect and anti-IL-8 mAb diminishes ERK phosphorylation [43]. This confirmed the role of IL-8 and MAP kinases in NET formation, but whether there are other molecules/pathways affected by IL-8 remains of interest.

A similar question was asked by Alsabani et al. [44]. It is tested the influence of plasma obtained from septic patients and septic mice on NET formation by neutrophils isolated from healthy donors or mice, respectively. The treatment of healthy cells by septic plasma resulted in an increase of NET formation in both experimental models. Inhibition of CXCR1/2 (receptors of IL-8) using reparixin in septic mice reduced NET formation, which points to CXCR1/2 signaling-induced NET formation dependence [44].

Huang et al. compared wild-type mice group with wild-type mice sepsis group and detected a significant increase in TNF-α and IL-6 concentration comparing in the control. Increased NET formation was detected in the lung tissues in the sepsis group, which was significantly higher than in the control group, but researchers did not correlate both observations [45]. Thus, convergence of both mechanisms remains unexplained.

Contrary to above-mentioned findings, Kaufman et al. did not observe any correlation between cytokines and nucleosomes or HNE-DNA [46]. Although the researchers tested only IL-6 and TNF-α, they did not exclude influence of other cytokines.

Chrysanthopoulou et al. observed in the murine model of ferric chloride-induced thrombosis that IL-29 activates NETosis via mTOR inhibition [47].

Chemokine PF4 (CXCL4) can play a role in regulating in vitro human NETosis [48], and its recombinant form may also directly stimulate neutrophils [48]. In vivo, MKEY (a peptide inhibitor of CXCL4/CCL5 heterodimer formation) reduces NETosis in a model of acute lung injury [49]. Gollomp et al. showed that PF4 increased NET-mediated bacterial uptake and improved outcome in murine models of sepsis [50]. Little data exist on the distinction between neutrophils' dysregulation in SIRS and CARS. During the former, as well as in sepsis, the apoptosis of neutrophils is inhibited, thus leading to increased tissue damage by the release of ROS and elastase [51][52][53]. The half-life of these cells, usually not exceeding 6 h, is markedly prolonged under pro-inflammatory conditions. Delayed apoptosis may be attributed to activated anti-apoptotic factors and NF-κB and further suppression of caspases 3 and 9 [54]. Accumulation of activated neutrophils is also associated with increased NETosis [55]. Under acute inflammatory circumstances, ICAM-1+ neutrophils and low-density neutrophils produce increased amounts of NETs [56][57].

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