Cytokines in Sepsis: Friend or Enemy
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Abstract
Infectious diseases are a major cause of death worldwide. A serious complication of infections is septic shock. Septic shock is a critical syndrome associated with the host response to infection. The severity of infections is related to an activation cascade that results in a magnification of the cytokine production which is termed "cytokine storm". Despite the commonly elucidated etiology of sepsis and its clinical course, the actual progress in therapeutic strategies is still limited. Several studies were carried out on the pathophysiology of sepsis-induced imbalance in the inflammatory/anti-inflammatory response as the main cause of tissue damage, organ failure, and eventually, death. Cytokines are crucial pleiotropic regulators of the immune response, which have an important role in the complicated pathophysiology of sepsis. They possess both pro- and anti-inflammatory properties and are capable of exerting efficacious defense responses towards invading pathogens. On the other hand, cytokines may disturb the immune response and reinforce inflammation. Thus, achieving a balance between these two effects will improve the prognosis of sepsis. Blocking the activities of pro-inflammatory cytokines promotes survival in animal models of sepsis, yet, such a treatment strategy did not enhance the clinical outcome. In this review, we will describe the mechanisms underlying the pathogenesis of the cytokine storm during sepsis and represent in detail the role of cytokines responsible for cell or organ damage. We will compare the various therapeutic approaches investigated to stop or suppress this mischievous process and discuss the reasons for therapeutic failure.

Keywords
TNF-α, Interleukins, immunosuppression, CLP, LPS

1. Introduction
Sepsis is one of the critical global health issues especially in low- to middle-income countries [1]. The consequences of sepsis are especially undesirable in critically ill patients, immunocompromised, and in the elderly [2]. Studies indicated that the incidence of sepsis is surprisingly high beyond that of myocardial infarction [3] or the combination of breast, lung, and prostate cancer [4]. The etiology of the sepsis remains unclear, despite extensive research. The early understanding was that the main source of infection is only the gut microbiota [5]. Later studies indicated that the most commonly associated infection in sepsis was that of the upper respiratory tract caused by colonization of *Pseudomonas sp.* [6]. Moreover, bacteria are not the only causative agents of sepsis, but also parasitic and fungal infections are involved [7-9]. Although the presence of inflammation was remarkable and remained constant until the 2000s, targeting the inflammatory phase of sepsis appeared to have no remarkable potential in patient survival [10]. Recently, researchers started to believe that inflammation is indeed pivotal to combat pathogens causing sepsis [11]. The implementation of such findings and clinical data into genuine therapeutics remains sophisticated and requires an incorporated view of the synchronization of the cytokine storm and its impacts.

2. Sepsis pathophysiology
Exaggerated primary host response associated with sepsis results in an imbalance between pro-inflammatory and anti-inflammatory cytokines [12]. The innate immune response responds immediately to invading pathogens [13]. The main regulators of the innate response are the macrophages / monocytes, basophils, neutrophils, eosinophils, and natural killers [NKs]. A wide variety of molecules originating from the infecting microorganism or necrotic cells activate the innate immunity such as the pathogen-associated molecular patterns [PAMPs] and the damage-associated molecular patterns [DAMPs]. These molecules bind to the pattern recognition receptors [PRRs] such as the Toll-like receptors [TLRs], the nod-like receptors, the retinoic acid-inducible gene receptors, and the C-type lectin receptors [17, 18]. Lipopolysaccharide [LPS], a cell wall component of gram-negative bacteria, was found to act on the TLR4 and is commonly used to study host response following endotoxemia [19]. TLR expression at the cell membrane has a tight control on the regulation of TLR signaling. Higher levels of TLR2 receptors and TLR4 mRNA are detected in septic patients [20, 21]. According to the triggered PRR receptor, certain signaling pathways are activated leading to the upregulation of transcription factors such as Nuclear factor-kappa B [NF-κB] and induction of inflammatory cytokines such as TNF-α, IL-1β, adaptor-protein 1, interferon regulatory factor 3, or IRF7 [22-25]. Production of such molecules results in excessive inflammation and a life-threatening ‘cytokine storm’ [Figure 1]. The term ‘cytokine storm’ refers to the consecutive release of specific cytokines [26]. Sepsis was believed to be associated with an exaggerated release of only proinflammatory cytokines, such as TNF-α, IL-1, IL-6, IL-12, macrophage migration inhibitory factor [MIF] and IFN-γ [27]. Although cytokines are responsible for a variety of inflammatory responses, including the migration of immune cells to the site of infection that restrain a localized
The cytokine storm starts following activation of PRRs by microbial products (PAMPs or DAMPs) leading by activation of immune cells with subsequent release of inflammatory cytokines which lead to multiorgan dysfunction.

PAMPs: Pathogen Associated Molecular Patterns. DAMPs: Damage Associated Molecular Patterns.

**Figure 1:** The cytokine storm starts following activation of PRRs by microbial products (PAMPs or DAMPs) leading by activation of immune cells with subsequent release of inflammatory cytokines which lead to multiorgan dysfunction.

**Table 1:** Most common pro- and anti-inflammatory cytokines released during sepsis

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Source cells</th>
<th>Role in sepsis</th>
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<tbody>
<tr>
<td>TNF-α</td>
<td>Monocytes/macrophages, T and B lymphocytes, natural killer cells, mast cells, fibroblasts, neutrophils and osteoclasts [in smaller quantities] [37]</td>
<td>Induction downstream inflammatory pathways&lt;br&gt;Induction of thrombotic and fibrinolytic pathways&lt;br&gt;Release of potent vasodilators [38-41]</td>
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<tr>
<td>IL-1β</td>
<td>Blood monocytes, tissue macrophages and dendritic cells [42]</td>
<td>Stimulates a cascade of inflammatory mediators [43]&lt;br&gt;Recruitment of leukocytes to the site, activation of endothelial cells, provoking of fever and other systemic symptoms [44]</td>
</tr>
<tr>
<td>IL-6</td>
<td>Macrophages, lymphocytes, endothelial cells, dendritic cells, fibroblasts, and smooth muscle cells [45]</td>
<td>Increased migration of activated T cells&lt;br&gt;Induces the production of C-reactive protein [46]</td>
</tr>
<tr>
<td>IL-12</td>
<td>Monocytes/macrophages and neutrophils] and dendritic cells [47-49]</td>
<td>Activates NK, CD4+, and CD8+ T cells and induce TH1 differentiation and IFN-γ production [50]</td>
</tr>
<tr>
<td>MIF</td>
<td>from DCs, macrophages, monocytes, neutrophils, eosinophils, basophils, lymphocytes, mast cells [51, 52]</td>
<td>Controls the production of IL-6 through regulation of NF-κB [53]&lt;br&gt;Enhances the recognition of LPS by TLR4 so exacerbates of the symptoms of endotoxemia [54, 55]&lt;br&gt;Production of several proinflammatory cytokines, chemokines and induction of iNOS following exposure to LPS [58, 59]</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>NK cells and T lymphocytes [56, 57]</td>
<td></td>
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<tr>
<td>IL-10</td>
<td>Activated Th2 subset of CD4+ T cells, monocytes/macrophages, epithelial cell, dendritic cells, keratinocytes, and bronchial epithelial cells [60]</td>
<td>Inhibition of proinflammatory cytokines production [61-63]&lt;br&gt;Inhibits NK cell function [64] and weakens the neutrophil oxidative burst [65]</td>
</tr>
<tr>
<td>TGF-β</td>
<td>By many cell types, including macrophages [66, 67]</td>
<td>Decreases the proliferation and differentiation of T cells and B cells&lt;br&gt;Reduces of the percentage of CD4+CD25+Foxp3+ regulatory T-cells [68]</td>
</tr>
<tr>
<td>IL-4</td>
<td>T cells, mast cells, and basophils [69-72]</td>
<td>Inhibit the release of inflammatory cytokines [73]&lt;br&gt;Downregulates the human alveolar macrophages and peripheral blood monocytes [74]&lt;br&gt;Inhibits macrophage activity by blocking their cytotoxic activity and nitric oxide production [75, 76]</td>
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**Figure 2:** A diagram showing the hyperdynamic and hypodynamic phases evoked during sepsis and the released cytokines during these phases.

TNF-α, Tumor Necrosis Factor-α, IL-1β, Interleukin-1 Beta, IL-6, Interleukin-6, IL-12, Interleukin-12, MIF, Macrophage Migration Inhibitory Growth Factor, IFN-γ, Interferon-Gamma, IL-10, Interleukin-10, TGF-B, Transforming Growth Factor-beta, IL-4, Interleukin-4.
infection from being systemic, uncontrolled cytokine release leads to endothelial dysfunction, recognized by vasodilation and increased capillary permeability, hypotension, hemoconcentration, macromolecular extravasation, and edema, which are frequent findings in septic patients and correlate to the resulting leakage syndrome [28]. The dysfunctional epithelial barriers allow pathogens and their products to further infest the host organism, hinder regulatory mechanisms, and cause distant organ dysfunctions [29]. Additionally, growing evidence has indicated that immune and inflammatory responses are tightly intermingled with different physiologic processes within the human host such as metabolism [30, 31] neuroendocrine activation [32, 33], and coagulation pathways [34]. However, certain anti-inflammatory cytokines are pivotal for restraining extreme further inflammation induced-tissue damage trying to retrieve immunological equilibrium. These include IL-10, transforming growth factor [TGF]-β, IL-4, and soluble inhibitors of proinflammatory cytokines, such as soluble TNF receptors (sTNFRs), IL-1 receptor antagonist [IL-1Ra], and soluble IL-1 receptor type II [IL-1R2] [12, 35, 36] (Figure 2 and Table 1).

A highly inflammatory form of programmed cell death, pyroptosis results from the activation of innate response activator B cells which results in IL-3 production and subsequent myelopoiesis in addition to cytokolic immunosomasomes, which induce the generation of IL-1β and IL-18 [77, 78]. When those responses are caused by sepsis, these mechanisms can become unregulated and cause a life-threatening inflammatory cascade. This case is different under physiological conditions when these processes allow the innate immune system to eliminate pathogens and damaged cells.

3. Proinflammatory Cytokines

Tumor Necrosis Factor-alpha

Tumor necrosis factor-α [TNF-α] was first identified in 1975. It was found to induce cell death in tumor cells and to have inflammatory effects [79]. It was then regarded as a pivotal target in the management of various inflammatory diseases such as Crohn’s disease [80], rheumatoid arthritis [81] and ankylosing spondylitis [82]. TNF-α have been implicated in a large number of infectious diseases as well as in the pathophysiology of sepsis [83]. TNF-α binds to two transmembrane receptors; TNF receptor 1 [TNFR1], and TNF receptor 2 [TNFR2], also known as p55 or p60 and p75 or p80, respectively [84]. Soluble cytokine receptors termed sTNFRs modify the actions of this cytokine and were also found to be correlated with the severity of the disease and mortality in septic patients [85]. Lipopolysaccharide and peptidoglycan- bacterial products are potent stimulators of TNF release in the pathogenesis of sepsis [86, 87].

Upon investigating different TNF inhibitors [e.g., anti-TNF immune serum, anti-TNF antibodies, sTNFRs, TNFR fusion proteins, TNF siRNA small interfering RNA], the survival increased in different septic models [e.g., LPS, bacterial, fungal, cecal ligation and puncture [CLP] and pneumonia] [88-90]. But, blocking TNF resulted in worsened microbial clearance and outcome in multiple animal models of infections [91, 92]. Also, an important role of TNF for the host defense was supported in TNF knockout models [93]. Such conflict can be explained by the beneficial role of TNF for host defense during sepsis.

Interleukin-1

Interleukin-1 was recognized as “human leukocytic pyrogen” in 1977 [94]. IL-1 family is a pivotal mediator of immune response to sepsis including two agonists IL-1α and IL-1β both bind to the same cell surface receptor [IL-1RI] and one antagonist [IL-1 receptor antagonist: IL-1ra] [43, 95, 96]. Blocking IL-1 showed great efficacy in a broad spectrum of inflammatory diseases [97]. Development of shock, multi-organ system failure, and death in septic patients are directly linked to excessive IL-1 production [43].

Unlike the IL-1α precursor which is active and constitutively present in the cells of healthy individuals [42], the IL-1β precursor becomes activated after cleavage by caspase-1 which has to be previously activated by inflammasome [98]. IL-1β participates in the cytokine storm and the production of IL-6 [99]. High levels of IL-1β and TNF-α released from macrophages in sepsis and septic shock were found to cause significant cardiac contractility depression [100].

In a study to investigate whether IL-1β contributes to LPS-induced dysfunction of multidrug resistance-associated protein 2 [Mrp2], diminished Mrp2 protein expression and activity, as well as its internalization to intracellular domains following exposure to LPS, was partially diminished after in vivo immunoneutralization of IL-1β, which hypothesize that it may contribute to the decreased of expression of Mrp2 at the brush border membrane during experimental endotoxemia [101]. The effect of IL-1α on the cognitive impairment associated with sepsis was investigated using the CLP model. Such treatment decreased the levels of IL-1β, IL-1α and TNF-α, decreased blood brain barrier permeability and oxidative parameters in the prefrontal cortex, hippocampus and striatum [102]. Recently, the effect of IL-1β on reversing the immune paralysis in late-phase sepsis and increasing survivability was investigated where it was reported to increase survival, numbers of BMCs and liver immune cells [103].

Interleukin-6

Interleukin-6 [also known as interferon-β2] is a pleiotropic interleukin. [104]. It is excessively produced during chronic inflammatory diseases, such as rheumatoid arthritis [RA], and hyperinflammation, such as cytokine storms [105]. IL-6 receptors consist of two subunits: CD126 and CD130 [gp130] [104]. IL-6 receptor is expressed by several cell types, such as B and T lymphocytes, monocytes/macrophages, and in turn, boosts their functionality [106]. The concentration of IL-6 increases following exposure to bacterial products in the early stages of infection [107].

High levels of IL-6 in septic patients were found to be correlated with increased mortality [108, 109]. Anti-inflammatory effects of IL-6 also were reported which include inhibition of the release of TNF-α and IL-1 [110] and increasing the levels of anti-inflammatory mediators in circulation such as IL-1Ra, sTNFRs, IL-10, TGF-β, and cortisol [111-113]. The advantage of being elevated for a longer period of time than TNF or IL-1β made IL-6 the most extensively studied as a potential biomarker [114].

In the CLP model in mice, prophylactic administration of anti-interleukin-6 antibodies effectively reversed the gastrointestinal motility disturbances and disturbed colonic barrier function that occurs during sepsis. Serum and colonic proinflammatory cytokines levels were lowered with the anti-interleukin-6 antibodies [115]. Tocilizumab, a humanized anti-IL-6 receptor monoclonal antibody has been proved successfully against rheumatoid arthritis, juvenile idiopathic arthritis and Castleman disease [116]. In a recent study using the CLP model of sepsis, treatment with tocilizumab alleviated acute lung and kidney injury associated with sepsis and improved survival of septic rats. Such effects were correlated with up-regulated P-glycoprotein [P-gp] expression in pulmonary and renal tissues, inhibition of
NF-κB activation and attenuation of JNK signaling pathway [117].

**Interleukin-12**

The heterodimeric cytokine; interleukin-12 [IL-12] is structurally related to the IL-6 cytokine family and IL-12 [IL-12p70], consisting of a p35 and a p40 subunit [47-49].

IL-12 links innate and adaptive immune responses either indirectly by NK cell activation or directly through activating CD4+ and CD8+ T cells [118]. IL-12 was found to be higher in survivors of sepsis and vice versa unlike, IL-6, IL-10 and TGFβ1 levels that were higher than IL-12 in the non-survivor group [119].

Administration of a polyclonal IL-12 Ab has been reported to increase survival of mice after CLP or i.p. injection of live E. coli [120, 121]. But, the administration of anti-IL-12 antiserum was reported to increase the bacterial load following CLP or i.p. administration of E. coli, indicating that IL-12 has an effective antibacterial role [122, 123]. Also, in another study to investigate the role of IL-12 on the host defense during polymicrobial sepsis using the CLP model, IL-12 knockout mice showed higher susceptibility to sepsis manifested as higher serum TNF-α and lung neutrophil infiltration which was similar to that of the wild type mice. Such effects were correlated with diminishing the systemic IFN-γ synthesis, decrease of microbial activities of their neutrophils, phagocytosis and NO production [124].

Cardiac dysfunction is a well-known serious complication of sepsis [125]. A recent study suggested that IL-12p35 [II12a] may be considered an important target during the management of such complications as its deletion worsened CLP-induced cardiac dysfunction [126].

**Figure 3:** MIF release and action

MIF release from macrophages is triggered by microbial products such as LPS and by glucocorticoids. MIF then acts on immune cells to produce antibodies and upregulate the expression of TLR.

**MIF:** Macrophage Migration inhibitory factor, LPS: Lipopolysaccharide, TNF-α: Tumor Necrosis Factor- alpha, IL-1β: Interleukin-1 beta, IL-6: Interleukin -6, TLR-4: Toll Like Receptor-4, NF-κB: Nuclear Factor Kappa- b. INF-γ: Interferon-gamma

**Macrophage Migration Inhibitory Factor**

Macrophage migration inhibitory factor [MIF] is a pleiotropic cytokine that contributes to the pathogenesis of severe sepsis, ARDS, and autoimmune diseases owing to its pro-inflammatory and immunomodulatory properties. The poor prognosis and severity of such diseases are correlated with the high levels of MIF [32, 127]. MIF regulates the initial immune response which initiates and exaggerates acute respiratory distress syndrome and sepsis [127-129].

Upon induction of its secretion by glucocorticoids during stress, MIF acts as a pro-inflammatory cytokine and a stress response mediator. It acts to impair the eradication of activated monocytes/macrophages by apoptosis, so the inflammatory response remains sustained and so the production of prostaglandins, matrix metalloproteinases and nitric oxide [127, 130]. Proinflammatory mediators such as TNF, IFN-γ and C5a, bacterial endotoxin and exotoxin induce neutrophils to secrete MIF [131] (Figure 3).

MIF-induced autophagy had a crucial role in thrombin-induced endothelial hyperpermeability. Inhibition of MIF or blocking autophagy was effective to reduce mortality in septic mice through attenuation of vascular permeability and leakage [132]. MIF levels were found to be higher in sepsis than in noninfectious systemic inflammation [133, 134]. MIF can predict the prognosis and severity of the disease as it was found to be higher in groups with poor prognosis [135].

In models of polymicrobial peritonitis and mice infected with E. coli, serum and peritoneal fluid MIF levels were found to be high and mice survival was enhanced when treated with anti-MIF antibodies. Same results were obtained using the CLP model even if the anti-MIF antibodies were administered 8 h following the induction of sepsis [136]. Anti-MIF antibodies attenuated TNF-α production and neutralized MIF activity and protected mice from endotoxic shock. Likewise, MIF -knockout mice when compared with wild-type mice, showed less plasma levels of TNFα and were protected against lethal doses of staphylococcal enterotoxin B and LPS [137].

MIF was identified to have tautomerase activity [138]. Such activity was suggested to have potential in the management of the multi-organ dysfunction syndrome following trauma and/or hemorrhage [139]. Treatment with ISO-1, a small molecular weight inhibitor that inhibits of MIF’s tautomerase activity, following the CLP model by 24 h resulted in a higher survival in rats. Specific inhibition of MIF tautomerase activity also showed less NF-κB activation and TNF production in LPS-treated macrophages [140]. Reduction of serum MIF was used as a strategy to reduce sepsis-associated mortality. Continuous renal replacement therapy was found to be an effective strategy to reduce the high serum level of MIF [141].

**Interferon-gamma**

Interferon gamma [IFN-γ], is a homodimeric protein [56, 57]. Mice deficient of IFN-γ or IFN-γR are more resistant to LPS-induced responses [142, 143]. Systemic administration of IFN-γ decreased survival and aggravates systemic inflammation following induction of sepsis using the CLP model [144]. Also, blockade of IFN-γ improved survival after induction of sepsis using the LPS or CLP model [145, 146]. Several studies have demonstrated that NKT cells promote LPS- or CLP-induced sepsis in mice through the production of IFN-γ [147, 148].

Much higher mortality in patients with sepsis is associated with monocyte deactivation, which is characterized by evident reduced HLA-DR expression of monocytes, a marked reduction of their capacity to produce LPS-induced TNF-α in vitro, and loss of antigen-presenting capacity; the well-known “immunoparalysis” phenomenon [149, 150]. Upon application of IFN-γ to septic patients, their monocytes restored the deficient HLA-DR expression and in vitro LPS-induced TNF-α secretion. Restoration of the function of monocytes resulted in clearance of sepsis in eight of nine patients [149].

Natural killer cell-derived IFN-γ facilitates the activation of myeloid cells to augment phagocytosis, respiratory burst,
microbial killing. IFN-γ-induced cytokines secretion results in a positive feedback loop that amplifies infection-induced activation of NK cells and myeloid cells. Although these interactions facilitate beneficial antimicrobial functions among macrophages and neutrophils, excessive cytokines amplify inflammation during systemic infection [151].

4. Anti-Inflammatory Cytokines

Interleukin-10

Interleukin [IL]-10 is a potent immunoregulatory molecule and was originally known as cytokine synthesis inhibitory factor produced by T-helper type 2 cells that inhibit T-helper 1 function [63]. Also, IL-10 is associated with the down-regulation of key signaling receptors on antigen-presenting cells such as CD40, CD80, CD86, and MHC II, decreased Mac-1 expression [152], the maintenance of FoxP3 expression in regulatory T cells. The proinflammatory effects of IL-10 were previously documented in many clinical trials for the treatment of patients with rheumatoid arthritis [153] and volunteers with experimental endotoxemia [154]. Increased IL-10 levels were correlated with sepsis score and may be an indicator of poor patient recovery and high risk for organ dysfunction and fatal outcome [155-157]. A later study conducted by Frencken et al. compromised 708 patients in the ICU with severe sepsis or septic shock showed that mortality is associated with higher IL-10 levels [158].

In a CLP model of sepsis in rodents, systemic administration of high-dose of IL-10 did not affect outcome [159] but also suppressed the immune response to Pseudomonas pneumonia in another study using CLP model in mice [160]. Systemic administration of recombinant human IL-10 caused decreased inflammatory response in human endotoxemia and showed no hemodynamic benefits [154].

AS101, an inhibitor of IL-10 expression administered 12 h following sepsis induction increased MHC II expression on APCs, T cell IFN-γ production, and bacterial clearance also decreased tissue damage and so increased the survival [161]. The neutralization of IL-10 resulted in a decrease in the percentages of regulatory T [Tregs] cells in CD4⁺ T cells, restored the percentages of CD4⁺ T cells in spleen MNCs, and improved survival rates in septic mice [68]. On the other hand, neutralizing antibodies that block IL-10 resulted in increased neutrophil accumulation and decreased survival [64], which may indicate that the timing for IL-10 neutralization is critical to enhance the host response during sepsis.

Interleukin-10/lymphocyte ratio [IL10LCR] may be a beneficial biomarker for sepsis-induced immunosuppression as its level is significantly associated with the severity and outcome [162]. A recent study conducted by Jensen, Mconagill et al. found that NK cells promote survival by limiting the scope and duration of the cytokine storm. Specifically, NK cell-derived IL-10 which is pivotal for survival during sepsis [163].

Transforming Growth Factor-beta

Transforming growth factor-beta [TGF-β], a cytokine with broad immunosuppressive functions that is pivotal for the resolution of inflammation during tissue injury [164]. Significant induction of TGF-β in mice is evident during the septic response, and TGF-β has been shown to contribute to endotoxin desensitization of monocytes [66, 67]. Specifically, TGF-β inhibits IL-1β and TNF from monocytes [165, 166]. Furthermore, the TGF-β1 signal was found to be partly involved in the apoptosis of CD4⁺CD25⁺ T cells promoted by CD4⁺CD25⁺ Tregs [167].

Another study conducted by Nullens et al. demonstrated that lymphocyte depletion occurred in all tissues examined [spleen, mesenteric lymph node, ileum, colon] at day seven, correlating with increased levels of IL-10 and TGF-β in a murine sepsis model [168]. Anti-TGF-β blocking antibody causes a decrease in Treg cell numbers in the lung tissues which suggests that accumulation of Tregs in the lung tissues- which contributes to immunosuppression and increased susceptibility to secondary infection- is associated with TGF-β [169].

Interleukin-4

A highly pleiotropic cytokine was initially identified as a B-cell differentiation factor, as well as a B-cell stimulatory factor [170]. IL-4 is produced by T cells, mast cells, and basophils and can induce the expression of other anti-inflammatory mediators, including IL-1ra and TNF soluble receptors from monocytes [69-72]. Protective, as well as deleterious effects of IL-4, were reported in Staphylococcus aureus-induced murine sepsis, which were shown to be dependent on the host’s genetic map [171]. Interleukin-4 suppresses the polarizing and differentiating of Th1 cells. It also has a crucial role in B cell differentiation, thus promoting a Th2 mediated response [172].

In animal models of sepsis, IL-4 suppressed cell-mediated immunity and so death through activation of the Stat 6 pathway [173]. In 56 cases of patients with severe trauma who developed sepsis, showed no correlation between il-4 and severity neither outcome of sepsis [174].

5. Trials targeting cytokines

Targeting Tumor Necrosis Factor-alpha, Interleukin-1, Interleukin-17 and Interleukin-18

In experimental studies of sepsis, blocking TNF-α effects showed promising results as it effectively reduced both morbidity and mortality [175]. Other experimental studies also showed the same results [176-178]. That is why the anti-cytokine therapy was assumed to have some efficacy against sepsis. However, such results could not be replicated in the clinical trials, but such therapy showed deleterious effects. In a randomized, double-blind study, when septic patients were dosed with recombinant soluble TNF-α receptor, it did not enhance survivability but high lead to high mortality rates [179]. Additionally, the use of anti-TNF-α therapy has been associated with an elevated risk of infections [180].

In a phase III, randomized, double-blinded, placebo-controlled, multicenter trial, rIL-1ra failed to reduce mortality when compared with standard therapy, suggesting that overproduction of either pro-inflammatory mediators [IL-1ra and IL-1β] or anti-inflammatory cytokines [IL-1ra] might lead to organ dysfunction and even death [181].

Treatment of septic mice with anti-IL-17A increased chemokines and HMGB-1 and reduced serum levels of proinflammatory mediators and increased survival rates when administered 12hr following CLP [182]. However, A prospective cohort study that included 60 patients with severe sepsis revealed that IL-17 was not detected [183]. Multiple studies have demonstrated that high plasma levels of IL-18 are correlated with poor clinical outcomes in severe septic and inflammatory cases [184, 185]. Experimental data hypothesize such biological neutralization may have a promising therapeutic efficacy in the treatment of sepsis [186].

Immune-augmentation strategies:

Although multiple immunomodulators have been tried to reduce levels of targeted cytokines and showed promising results in experimental trials but they failed to show the same effects in
clinical trials but also increased mortality. Such failure may be due to the fact that septic patients die later in the course of sepsis when the phase of immunosuppression. During such phase, the innate and adaptive immune responses are impaired. In addition, enhanced apoptosis and dysfunction of lymphocytes, impaired phagocyte functions, monocytic deactivation with diminished HLA class II surface expression, and altered ex vivo cytokine production occur [12, 187]. So, the term “immunoparalysis” emerged to describe the inability of the host to defend himself against infections [188].

Granulocyte-Macrophage Colony-Stimulating Factor
Granulocyte-macrophage colony-stimulating factor [GMCSF] is a potent cytokine with immunostimulatory effects through potentiation of antimicrobial host defense as it improves survival, proliferation, differentiation, phagocytosis of neutrophils and monocytes/macrophages. And also, through enhancing migration and adhesion of neutrophils [189]. This effect has been illustrated in a randomized, un-blinded, placebo-controlled prospective study where GM-CSF group showed a significantly higher increase in total leukocyte counts which indicated increased rates of infection clearance in addition to the clinical improvement. But, still no difference in mortality [190]. Reduced survival in sepsis was found to be associated with prolonged downregulation of low levels of monocytic HLA-DR [mHLA-DR] [187]. In a prospective, randomized, double-blind, placebo-controlled, multicenter trial; GM-CSF was a safe and effective measure to restore mHLA-DR expression and to increase the numbers of neutrophils and monocytes, also the time of mechanical ventilation and hospital/ intensive care unit stay was shortened [191]. Regardless of these desirable effects in many clinical outcomes, 28-day mortality was not lowered.

Interleukin 7 and interleukin 15
Cells from septic patients treated ex vivo with rhIL-7 significantly showed improved lymphocyte functionality which was manifested as CD4+ and CD8+ lymphocyte proliferation, B cell lymphoma 2 induction, IFN-γ production, and STAT5 phosphorylation. Such results supported its ability to restore normal lymphocyte functions during sepsis [192]. IL-7 improved survival significantly in CLP-induced sepsis followed by P. aeruginosa pneumonia. Regarding the high mortality associated with secondary P. aeruginosa pneumonia, IL-7 increased the number of immune effector cells in the lung and spleen, increased IL-17, IFN-γ, and TNF-α-producing ILCs and CD8 T cells in lung tissues which are essential for the host defense against sepsis and P. aeruginosa pneumonia. In addition, NF-κB and STAT3 pathways were induced in the lungs [193]. In a phase 2 trial, administration of recombinant human IL-7 [CYT107] in patients with septic shock and severe lypmphopenia improved the marked loss of CD4+ and CD8+ immune effector cells, which is considered the key mechanism in morbidity and mortality during sepsis [194].

Serum levels of IL-15 were found to be an indicator of the prognosis of septic patients following emergent abdominal surgery as it correlated with the duration of SIRS and organ dysfunction. It correlated positively with creatinine levels and negatively with the PaO2/FiO2 [195].

On the other hand, in a rat model of CLP-induced sepsis, recombinant IL-15 raised the Levels of IL-15 and IFN-γ in peripheral blood of septic rats, in addition to the numbers of peripheral T cells and natural killer [NK] cells, which resulted in enhanced survival [196].

The severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2] outbreak, which was first appeared in Wuhan, China, in December 2019, has had a tremendous influence on China and the whole world [197]. Viral sepsis was hypothesized to be pivotal to the disease mechanism of COVID-19 [198]. Management of the cytokine storm was suggested to be critical for rescuing patients with severe COVID-19. Immunomodulators and cytokine antagonists for early control of the cytokine storm are crucial to increase the survival rate of patients with COVID-19 [199-201].

In a retrospective cohort study of patients with consecutively COVID-19, moderate-to-severe ARDS, and hyperinflammation, the high-dose intravenous anakinra; an IL-1 receptor antagonist [IL-1ra] treated group showed a higher survival compared to the standard treated group, reduction in serum C-reactive protein and progressive improvements in respiratory function [202]. Despite the limitations of this study; the relatively small size of the cohorts and the need for more follow-up to investigate the long-term outcomes, but such results still seem to be promising.

In a retrospective observational study conducted by Capra, De Rossi et al., 85 consecutive patients were administered the IL-6 blocker; tocilizumab to treat COVID-19, showed significantly greater survival rate when compared to control patients and improved respiratory function [203]. McElvaney, Curley et al. reported that groups with a high CRP might get more benefit of the anti-IL-6 therapy regarding the role of IL-6 as a CRP inducer [204].

The risk of patient’s requirement to ICU in COVID-19 patients was found to increase by 8.8-fold when it was accompanied by elevated MIF levels [205]. Also, MIF is considered an early biomarker to predict the response to the early initiated ICU treatment in critically ill patients with COVID-19 disease [206].

Conclusion
Sepsis remains to be one of the global serious health problems. Many factors contributed to the lack of such effective strategies in clinical settings. Until now, the use of irrelevant models in sepsis research resulted in the emergence of non-targeted and incapable drugs for managing septic cases. This could be explained in the light of the fact that immune response varies greatly between species, also during such experiments; drugs are dosed at a certain time before or after induction of sepsis which is considered impossible to be clinically determined exactly. Heterogeneity in septic patients is another factor contributing to such detach between experimental trials and clinical cases which may be correlated to the difference in sepsis sources, different levels of inflammation, and yet different responses to treatment. Although all anti-cytokine treatments were found to be promising in murine models, they did not reveal any change neither in the overall survival nor in the patient outcome. Moreover, they increased mortality in some cases. So lately, the chronic immunoparalytic phase became the focus of interest as the cause of most sepsis-associated deaths which is characterized by a decrease in pro-inflammatory cytokine levels and leukopenia, increased risk of infection by opportunistic pathogens, and subsequently death. During such phase, immunostimulatory adjuvant therapies reverse the immunoparalysis that takes place aiming to increase the number of functional leukocytes and target diminished apoptosis. In other promising approaches, blocking the already self-sustaining inflammatory cascade is not the target but limiting what is ultimately killing patients; like vascular-induced tissue damage. Targeting Slit-Robo4 seemed to be effective in the endotoxemia model to reduce the levels of...
circulating inflammatory cytokines e levels of circulating inflammatory cytokines. Uprogulation of PD-1 on T lymphocytes and particularly PD-L1 on APC’s is another contributing pathway in immune tolerance. Blocking PD-1 and PD-L1 antagonizes the interaction between the two molecules which in turn retrieves T cell function which is of significant importance in countering such infectious diseases.

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