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REVIEW

Sepsis: in search of cure

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Abstract
Introduction Sepsis is a complex inflammatory disorder believed to originate from an infection by any types of microbes and/or their products. It is the leading cause of death in intensive care units (ICUs) throughout the globe. The mortality rates depend both on the severity of infection and the host's response to infection.
Methods Literature survey on pathobiology of sepsis in general and failure of more than hundred clinical trials conducted so far in search of a possible cure for sepsis resulted in the preparation of this manuscript.
Findings Sepsis lacks a suitable animal model that mimics human sepsis. However, based on the results obtained in animal models of sepsis, clinical trials conducted so far have been disappointing. Although involvement of multiple mediators and pathways in sepsis has been recognized, only few components are being targeted and this could be the major reason behind the failure of clinical trials.
Conclusion Inability to recognize a single critical mediator of sepsis may be the underlying cause for the poor therapeutic intervention of sepsis. Therefore, sepsis is still considered as a disease—in search of cure.

Keywords Sepsis · Lipopolysaccharide (LPS) · Toll-like receptors · Cytokines · Activated protein C

Introduction

"Small creatures, invisible to the eye, fill the atmosphere and breathed through the nose cause dangerous diseases"—a definition for Sepsis given by an ancient Roman scholar and writer—Marcus Terentius Varro (116 BC–27 BC) [1]. Today, the world's human population has crossed seven billion and the technology in the field of healthcare to predict/manage diseases is also developed to a great extent. Despite such great advancements in science, human population is still susceptible to diseases caused by pathogenic microorganisms and one such disease/syndrome that has become a nightmare is "SEPSIS"—a condition of overwhelming systemic inflammation initiated in response to an infection by microbes and/or their released endotoxins leading to multiple organ dysfunction syndrome (MODS) and death [2, 3].

Sepsis accounts for high mortality rates standing at more severe than breast cancer, prostate cancer and HIV/AIDS combined [4], or Hepatitis [5] and continues to be a major life threatening condition, taking more and more lives. The causative agents/organisms behind this devastating syndrome are believed to be microbes [6, 7] and/or their products [8].

Hyperactivation of the inflammatory response is a remarkable feature of sepsis, which can be initiated at any site vastly by bacteria and/or its prime product—Lipopolysaccharide (LPS) [9]. However, targeting either whole bacteria [10] or LPS [11], their receptors [12] and the downstream pro-inflammatory mediators [11] involved in exaggerating the inflammation never lead to a fruitful

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outcome of reducing mortality. This indicates the involvement of other bacterial components such as membrane lipoproteins in exaggerating the inflammatory process, which are previously unappreciated.

Quantification of pro-inflammatory cytokines is an extensively employed method to determine the extent of inflammatory insult and this holds good in case of sepsis too [13]. Besides, few molecules are identified as the early mortality predictors, where their levels predict the early mortality of patients with severe sepsis [14–17]. Although these early mortality predictors are proven to be the best markers of severe sepsis, they need extensive validation for their use in clinical practice [18, 19].

With the failure of more than 100 clinical trials [20] and early-goal directed therapy (EGDT) formulated by the "Surviving Sepsis Campaign" (SSC) committee [21] in reducing the mortality of patients with severe sepsis and septic shock, researchers are now concentrating on several new strategies so as to come up with an efficient drug to overcome this devastating disease.

Although proven to be successful in animal models of sepsis, none of the drugs reached the market, clearing the hurdles of clinical trials. These unsatisfactory results obtained in translating the treatment options to bedside may be attributed to the disadvantages of each animal model employed in the studies [22]. Besides, development of new strategies employing modern technology for the identification of cause and disease progression, use of combination of drugs and identification of new markers which can predict the mortality of a patient have led to the better understanding of pathophysiology associated with the diseased state.

Definitions for sepsis

Although, the word 'sepsis' is under use for more than 2700 years [23], a perfect and universally agreeable definition was lacking to define sepsis and related disorders. To solve this issue, and to provide a conceptual and practical framework to define the systemic inflammatory response to an infection, the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) hosted a "Consensus Conference" in August 1991 [24] at Chicago, USA and designed various criteria for defining sepsis and related disorders (Fig. 1). These definitions served as the basis for designing inclusion criteria for various clinical trials and also helped in better understanding of the pathophysiology associated with sepsis and related disorders. Based on the subsequent understandings in the pathophysiology of sepsis, experts in the field decided to revisit and modify the earlier definition of sepsis so as to reflect the recent understandings in the

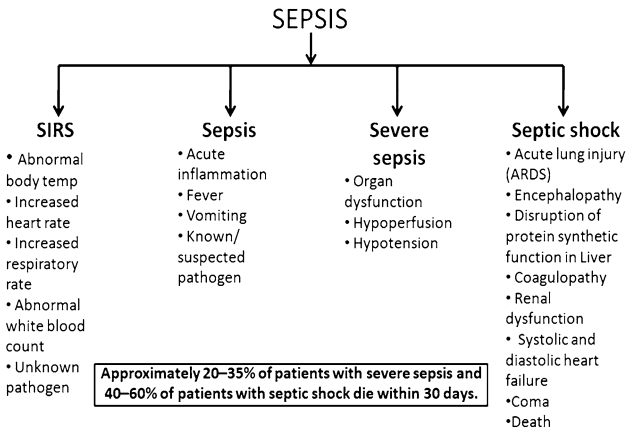

Fig. 1 Classification of sepsis based on the severity of infection and criteria for defining each group

pathophysiology of sepsis and related disorders. The "International Sepsis Definitions Conference—2001", jointly organized by SCCM, The European Society of Intensive Care Medicine (ESICM), ACCP, the American Thoracic Society (ATS), and the Surgical Infection Society (SIS) in Washington D.C. USA [25], resulted in no new or better definitions, as there was no evidence in support of replacing the existing definitions for sepsis and related disorders and hence, the earlier definitions were retained (Fig. 1).

The SCCM in association with ESICM and the International Sepsis Forum (ISF) initiated "Surviving Sepsis Campaign" (SSC) in the year 2002. The goal was to understand the pathophysiology of sepsis and related disorders, designing appropriate definitions, improving diagnosis, and treatment options thereby to reduce the mortality rate due to severe sepsis and septic shock. To date the SSC committee has met four times and has successfully put forth the international guidelines for management of severe sepsis and septic shock in the SSC-2012 meeting [26]. A 7.5 year study as per SSC-2004 guidelines by Levy et al. [27] to determine the association of compliance with the SSC performance bundles and mortality indicates a 25 % relative risk reduction in mortality rate.

Epidemiology of sepsis

Sepsis and related disorders are the leading cause of death throughout the world accounting for 19 million cases each year [28] and 1,400 deaths each day [29]. In a developed country like United States alone, the incidence of sepsis is estimated to be 1,655,000 [30] resulting in more than 250,000 deaths each year [31]. This has become a major economic burden to United States that accounts for a total of $16.7 billion towards healthcare [29].

The result of a multicentre, prospective observational study of 5478 patients admitted at various Intensive Care Units (ICUs) in India shows 25 % of the patient admissions are due to SIRS and organ dysfunction. Out of all admissions the incidence of severe sepsis was 16.45 % and the mortality rate was 12.08 %, of which 59.26 % of patients died of severe sepsis [32].

What are the causes of sepsis?

Sepsis is a result of an infection from any microorganisms (Bacteria/Virus/Fungi) [33] with bacteria being the commonest (Table 1). It is estimated that there are ten bacteria for every human cell and human evolution occurred in parallel with bacteria [34], allowing them to symbiotically inhabit many vital organs such as buccal cavity, gastrointestinal tract, skin and nasal linings of human body. However, many times, when our microbial neighbors penetrate previously negotiated boundaries, complications arise.

The infection, being the primary cause, can be initiated anywhere in the body, especially in the urinary tract [35], abdomen [36], blood stream [37] or lungs [38]. The microbial invasion activates the host immune system leading to the recruitment of first line of cells of innate immune system. These immune cells abrogate the further invasion and multiplication of microbes by phagocytosis and killing [39]. However, the results of two popular clinical trials [40, 41] using anti-microbial components as a possible cure for sepsis syndrome was in vain. This indicates whole bacteria are not a prerequisite for initiating sepsis. Even the fragmentation products produced after bacterial killing by phagocytic cells are probably more than sufficient to elicit a strong inflammatory response leading to sepsis, as evident from the negative blood cultures obtained in severe sepsis patients [42]. One such component of gram-negative bacterial membrane, widely employed as a surrogate endotoxin in research is the Lipopolysaccharide (LPS).

Table 1 List of Bacteria and their common site of infection

Site of infection	Bacteria
Skin and soft tissue infections	*Streptococcus pyogenes*
	Staphylococcus aureus
Lungs	*Streptococcus pneumoniae*
	Hemophilus influenzae
	Klebsiella pneumoniae
	Legionella pneumophila
	Mycoplasma pneumoniae
Abdomen	*Escherichia coli*
	Klebsiella pneumoniae
	Enterobacter cloacae
Urinary tract	*Escherichia coli*
	Klebsiella pneumoniae
	Enterobacter cloacae
	Pseudomonas aeruginosa
	Proteus spp.

Lipopolysaccharide (LPS): the bacterial endotoxin

LPS is the major component of gram-negative bacterial membrane. It is under experimental research since its discovery in 1894 by a German Physician, Richard Friedrich Johannes Pfeiffer who coined the term "Endotoxin" [43]. It is estimated that each bacterial cell contains two-million LPS molecules [44], covering approximately 75 % of the membrane surface [45].

LPS is a complex molecule, comprised of an O-specific chain (O-antigen), core oligosaccharides and a covalently bound Lipid A moiety [46]. LPS is the most studied pathogen associated molecular pattern (PAMP) well known to elicit its actions through Toll-like receptor-4 (TLR4); a type of pattern recognizing receptor (PRR) expressed on the cells of innate immune system [47].

Activation of TLR4 by LPS is not a simple mechanism which involves many crucial molecules that carry LPS to TLR4. An acute phase protein produced in the liver, Lipopolysaccharide binding protein (LBP), mediates the decisive step of LPS recognition by binding to Lipid A moiety and forming a LPS-LBP complex [48]. This complex is then recognized by the Cluster of Differentiation 14 (CD14) receptor, a surface molecule that is also known to form a tertiary complex [49] helping in presenting LPS to TLR4 [50]. Once bound, the TLR4 undergoes dimerization with myeloid differentiation factor 2 (MD2) bringing their intracellular Toll/interleukin-1 receptor (TIR) domains together and allowing the binding of other adaptor proteins [51]. Recruitment and binding of adaptor proteins initiate the TLR4 signaling cascade ending up in the activation and nuclear translocation of NF-κB [51], which further leads to the upregulation of a battery of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, TNF-α (Fig. 2) along with other molecules such as, COX-2, E-Selectin, MCP-1 and iNOS [52–55].

However, strategies of using monoclonal antibodies and receptor antagonists against LPS and TLR4 to block the deleterious effects initiated by LPS have been in vain, suggesting that LPS is not the only component of bacterial membrane responsible for inducing all the lethal symptoms in patients with severe sepsis and septic shock. This hypothesis is also evident from the study conducted by Freudenberg et al. [56] where the mice strains having

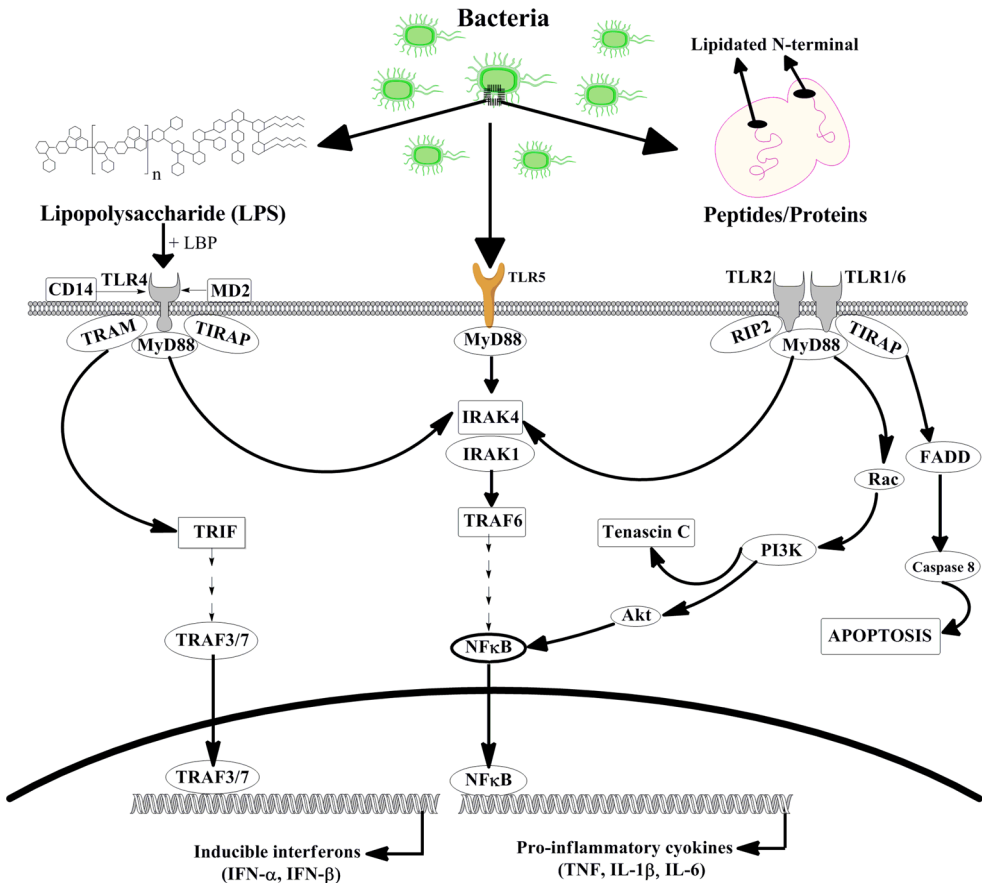

Fig. 2 Schematic representation of Toll-like receptor signaling pathway. TLRs specifically recognize bacteria and/or their products and their activation is mostly dependent on MyD88. Although activation of TLR4 & TLR2 initiates the pathway in a similar manner leading to the activation of NFκB, there are also notable differences in both the pathways and this schematic diagram represents their major differences. Interferon (α/β) genes are predominantly upregulated by the activation of TLR4, while Casapase 8 dependent apoptotic machinery is activated predominantly by the activation of TLR2. Tenascin C, a glycoprotein with a myriad of functions is recently known to be upregulated in response to TLR2 activation in in vitro model [59]. (The pathway was designed using ChemBioDraw Ultra 12.0 software). *LBP* lipopolysaccharide binding protein, *TLR* toll-like receptor, *MD2* lymphocyte antigen 96, *CD-14* cluster of Differentiation 14, *TRAM* toll-like receptor 4 adaptor protein, *TIRAP* toll-interleukin 1 receptor (TIR) domain containing adaptor protein, *MyD88* myeloid differentiation factor 88, *TRIF* TIR-domain-containing adapter-inducing interferon-β, *TRAF* tumor necrosis factor receptor-associated factor, *IFN* interferon, *IRAK* interleukin-1 receptor-associated kinase, *RIP2* receptor-interacting protein 2, *FADD* fas-associated death domain protein, *PI3K* phosphoinositide 3-kinase, *NF-κB* nuclear factor κB

mutation in their *Tlr*4 gene (C3H/HeJ and C57BL/10ScCr) were resistant to LPS action, yet highly susceptible to gram-negative infection, suggesting the involvement of other components of bacterial membrane in eliciting inflammation.

Bacterial lipoproteins and peptides are equipotent as LPS

The probable reason behind the failure of vast number of clinical trials targeting LPS, its receptor TLR4 or downstream signaling molecules can be ascribed to the involvement of other components of bacteria. The lipoproteins and peptides of microbial origin possessing N-terminal lipid modifications are also shown to be potent inducers of inflammation like LPS [57–59]. However, wide use of LPS in sepsis research has made these components unappreciated. Bacterial proteins are known to elicit their inflammatory properties through TLR2 [60], a different but related receptor dedicated to recognizing PAMPs of microbial origin.

Braun lipoprotein (BLP) is the next most abundant component of bacterial membrane after LPS in *E. coli*. It is a low molecular weight lipoprotein with its N-terminal cysteine residue bearing three palmitoyl residues making the structure a triacylcysteinyl-modified peptide [61]. It is estimated that each bacterial cell is composed of 10^5 molecules of BLP [62]. Although purified and characterized as a structural component of bacterial membrane over

40 years ago [63], BLP has not gained much attention as an inflammatory component except for few reports describing its potent inflammatory roles in in vitro systems. BLP has been shown to activate macrophages [64], lymphocytes [65] and endothelial cells [57] as efficiently as LPS. The experiments conducted by us clearly indicate the potential pro-inflammatory roles of purified BLP in Swiss albino mice when administered intraperitoneally. The results show endotoxemia-like pathology, along with the upregulation of pro-inflammatory cytokines, while these effects were not observed in BLP-injected TLR2 knockout mice (Lakshmikanth et al., unpublished data). Researchers often use Pam3CSK4, a synthetic structural analogue of BLP while targeting various drugs against TLR2. However, Pam3CSK4 has been shown to be less potent when compared to intact BLP [57] and hence this aspect also needs to be considered while targeting TLR2.

Although activation of TLR4 and TLR2 leads to similar outcomes such as overproduction of pro-inflammatory mediators, there are quite a few differences. This is evident from the study conducted by Neilsen et al. [57] where, IFN-γ-inducible protein-10, a CC-chemokine was specifically upregulated by LPS (Fig. 2), but not by BLP. Similarly, a report by Barrenschee et al., [59] indicates Macrophage-activating lipopeptide-2 (MALP-2), a TLR2 agonist from *Mycoplasma fermentas,* uniquely upregulated by inflammatory marker, tenascin C but not by LPS (Fig. 2). Hence, despite targeting TLR4, TLR2 pathway should also be considered while attenuating sepsis and related inflammatory disorders.

Early mortality predictors of severe sepsis

Besides traditional inflammatory markers implicated in sepsis and related disorders [52–55], a variety of molecules have been identified as the predictors of early mortality. Macrophage migration inhibitory factor (MIF) released from white blood cells in response to their activation by bacterial components are known to exaggerate the inflammatory mechanisms [66]. An increase in serum levels of MIF is associated with an increased risk towards early mortality during severe sepsis [67]. Similarly, procalcitonin (PCT)—a peptide precursor of calcitonin is produced from the neuroendocrine cells of lungs and intestine in addition to its production from the thyroid gland. PCT levels increase in response to infection and is more specific for bacterial infections when compared with infections by other microbes [68]. The higher level of PCT is often observed in sepsis patients and the level of PCT depicts the severity of the diseased state [69]. Presepsin, a glycoprotein expressed on monocytes and macrophages has been shown to be superior over PCT in terms of specificity and its increased levels correlate with the in-hospital mortality rates of patients with sepsis [70]. Yet another useful component of serum which can be employed as a risk marker to predict mortality is lactate, as its levels are shown to be at the higher end in patients with sepsis and related disorders [71]. Although, serum lactate levels correlate with organ dysfunction [72], a study by Mikkelsen et al. [73] indicates the ability of initial serum lactate levels to predict mortality irrespective of organ dysfunction. Likewise, higher serum levels of soluble urokinase plasminogen activator receptor (suPAR), a marker of immune activation has also been found to be a marker of mortality risk prediction [74].

Strategies that failed in attenuating the severity of sepsis

Despite enormous advancements in understanding the pathophysiology of sepsis, the mortality rate has failed to show any major decrease as evident from the failure of more than 100 clinical trials [20]. The only drug which made an attempt to reach the market after FDA approval was 'XIGRIS' (activated Drotrecogin alfa), a recombinant form of activated protein C (rhAPC). Unfortunately, this was also pulled down from the market by Eli Lilly, Indianapolis, USA [75, 76] and that has left the critical care specialists with no choice of a single medication for the treatment of sepsis and related disorders. Out of all the molecules employed in clinical trials with an aim to reduce the severe mortality rates due to sepsis and related disorders, very few of them gained a lot of importance as they were successful in reducing the mortality in homogeneous population. They include corticosteroids [77], antibodies against LPS [78–80], antibodies against key cytokine mediator TNF-α [81–85], receptor antagonists for TLR4 [86, 87], interleukin-1 receptor (IL-1R) [88] and bradykinin receptor [89], blood coagulation pathway inhibitors [90, 91] and the agents that block platelet activating factor (PAF) mediated effects [92, 93]. However, none of them were successful in clearing the phase III clinical trials (Table 2).

Never ending failure of clinical trials made the SSC committee employ early-goal directed therapy (EGDT) to simplify complex processes while treating the patients with severe sepsis [26]. EGDT include bundles of tasks to be employed in the first 6 h of admission of patients at the hospital, some of which include: (1) obtaining blood cultures before treating with antibiotics and administering with broad spectrum antibiotics, (2) imaging studies to confirm the potential source of infection and their control

Table 2 Year-wise list of most important failed clinical trials in search of a cure for sepsis

Year(s)	Strategy employed	Target	Patient group size	Reference(s)
1987 and 1989	Methylprednisolone	HPA axis	651	[182–184]
1988	Human IgG antibody to *Escherichia coli* J5 and a standard IgG preparation	Core region of endotoxin (LPS)	100	[78]
1991	Human monoclonal IgM antibody (HA-1A)	Lipid A domain of endotoxin LPS	543	[79]
1994	Human monoclonal IgM antibody (HA-1A)	Lipid A domain of endotoxin LPS	621	[185]
1994	Human monoclonal IgM antibody (HA-1A)	Lipid A domain of endotoxin LPS	600	[186]
1994	BN 52021 (Ginkgolide B)	Platelet Activating Factor Receptor (PAF-R) antagonist	262	[187]
1995	Murine monoclonal antibody (E5)	Gram-negative bacterial endotoxin LPS	847	[80]
1995	Anti-tumor necrosis factor alpha monoclonal antibody (TNF-alpha MAb)	Cytokine pathway	994	[81]
1995	MAB-T88 Human monoclonal IgM antibody directed against the enterobacterial common antigen (ECA)	Gram-negative bacterial endotoxin LPS	826	[188]
1995	Taurolidine	Anti bacterial agent	100	[40]
1995	Bradycor(TM) (CP-0127)	Bradykinin receptor antagonist	251	[89]
1996	Dimeric form of the type II TNF receptor linked with the Fc portion of human IgG1 (TNFR:Fc)	Neutralization of TNF-alpha	141	[83]
1996	BAY × 1351, a murine monoclonal antibody to recombinant human tumor necrosis factor alpha (TNF-α)	Cytokine pathway	420	[82]
1997	Lenercept (p55 tumor necrosis factor receptor fusion protein)	Neutralization of TNF-α	498	[189]
1997	Human recombinant interleukin-1 receptor antagonist (rhIL-1ra)	Cytokine pathway	696	[88]
1997	Bradycor (Deltibant, CP-0127)	Bradykinin receptor antagonist	504	[190]
1997	Ibuprofen	Inhibitor of prostaglandin synthesis (COX inhibitor)	455	[191]
1998	BAY × 1351, a murine monoclonal antibody to recombinant human tumor necrosis factor alpha (TNF-α)	Cytokine pathway	1879	[85]
1998	BN 52021 (Ginkgolide B)	Platelet activating factor receptor (PAF-R) antagonist	609	[92]
2000	Murine monoclonal antibody (E5)	Gram-negative bacterial endotoxin LPS	1090	[192]
2000	rBPI21	Bactericidal/permeability-increasing protein	393	[41]
2000	Lexipafant	PAF receptor antagonist	131	[193]
2000	BB-882	PAF receptor antagonist	152	[194]
2001	Lenercept (p55 tumor necrosis factor receptor fusion protein)	Neutralization of TNF-alpha	1342	[84]
2003	LY315920Na/S-5920	Inhibition of phospholipase A2 type IIA	586	[195]
2003	Tifacogin (recombinant tissue factor pathway inhibitor)	Blood coagulation pathway	1754	[196]
2004	Recombinant human platelet activating factor acetylhydrolase (rPAF-AH)	Inactivation of platelet activating factor (PAF)	1261	[93]
2004	NG-methyl-L-arginine hydrochloride (546C88)	Inhibition of nitric OXIDE synthase	312	[197]
2005	Drotrecogin alfa activated (Activated form of Protein C)	Blood coagulation pathway	2613	[198]
2005	LY315920Na/S-5920	Inhibition of phospholipase A2 type IIA	373	[199]
2006	Hydrocortisone and 9-alpha-fludrocortisone	HPA axis	354	[200]
2007	Drotrecogin alfa activated (Activated form of Protein C)	Blood coagulation pathway	477	[201]

Table 2 continued

Year(s)	Strategy employed	Target	Patient group size	Reference(s)
2008	Hydrocortisone	HPA axis	499	[202]
2009	Phospholipid emulsion (GR270773)	Endotoxin neutralization	1379	[203]
2010	TAK-242, a small-molecule inhibitor of Toll-like receptor-4-mediated signaling	Toll-like receptor-4 signaling pathway	274	[87]
2010	Eritoran tetrasodium (E5564)	Toll-like receptor-4 signaling pathway inhibitor	300	[204]
2012	Drotrecogin alfa activated (Activated form of Protein C)	Blood coagulation pathway	1697	[205]
2013	Eritoran tetrasodium (E5564)	Toll-like receptor-4 signaling pathway inhibitor	1961	[86]
2013	Recombinant thrombomodulin (ART-123)	Blood coagulation pathway	741	[91]
2014	Pyridoxalated hemoglobin polyoxyethylene (PHP)	Hemoglobin-based nitric oxide scavenger	377	[206]

with attention to the balance of risks and benefits, (3) measuring lactate levels as it is known to be increased (>4 mM/L) in severe sepsis cases due to various factors [71], (4) fluid resuscitation by administering crystalloids (30 ml/kg) for patients with hypotension and supplementing albumin to patients who continue to require substantial amounts of crystalloid to maintain adequate mean arterial pressure, and (5) applying vasopressors for persistent hypotension [26]. However, large, multicentre, randomized clinical trials conducted by protocol-based care for early septic shock (ProCESS) group [94], the Australasian Resuscitation in Sepsis Evaluation (ARISE) group [95] and Protocolised Management In Sepsis (ProMISe) group [96] employing EGDT protocols also resulted in no better outcomes. These failures have bound clinician's hands when treating patients arriving at the emergency departments.

Animal models employed in sepsis research

Animal models play an important role in drug development by creating a replica of the diseased condition, and an ideal animal model should mimic and translate all the relevant information concerned with the progressive pathophysiology associated with the diseased state. Wide varieties of animal models of sepsis have been developed [22] and are categorized as surgical models and non-surgical models [97].

Despite being expensive and involving animal welfare issues, surgical models are the most relevant models of sepsis [97], since a significant amount of sepsis cases are a result of an acquired infection during invasive surgery [98, 99]. Based on the surgical procedure involved, the surgical models of sepsis are of two types [22], one being cecal ligation and puncture (CLP) model of sepsis—which involves ligation below the ileocecal valve followed by puncturing the cecum using a needle so as to leak the fecal matter into the peritoneal cavity [100] and the other being colon ascendens stent peritonitis (CASP) model of sepsis—which involves inserting a stent into the ascending colon so as to leak abdominal content [101]. Although, both CLP and CASP models are efficient in bringing up polymicrobial sepsis conditions mimicking clinical course of intra-abdominal sepsis, there are significant differences in bacterial load, generation of cytokines and the survival time [102]. CLP and CASP models also suffer from inconsistent results as the ligation distance, size of the needle used to puncture the cecum in CLP model and diameter of the stent used in CASP model are the major determining factors of mortality [22, 102, 103].

To overcome the disadvantages of CLP and CASP models of sepsis, a reliable and reproducible rodent model of sepsis—'polymicrobial peritoneal contamination and infection (PCI) model' has been developed [104, 105]. PCI involves the administration of human fecal matter into the peritoneal cavity of rodents to develop the classical symptoms of sepsis [104, 105]. In contrast to CLP and CASP models, the major advantage of PCI model is its simplicity in terms of sample preparation, injection and the ease of controlling the severity of infection by manipulating the amount of fecal matter to be injected [105]. With these advantages, PCI is now being employed in sepsis research [104, 106–109].

Yet another surgical model of sepsis is the 'implantation model' which involves implanting a fibrin clot impregnated with live bacteria into the peritoneal cavity through a major surgery [110]. Fibrin clots aid in the slow release of bacteria into the bloodstream. This model is an elegant one

over others due to slow mortality and choice of organism based on the field of interest [97], thus allowing the researcher to design specific drugs based on the organism being used.

The non-surgical model is the widely used animal model of sepsis for mimicking pathophysiological conditions associated with sepsis syndrome as it is more advantageous in terms of cost and animal welfare issues [97]. It involves the administration of animals with pathogenic organisms either in live or heat-killed form or administration of endotoxins through various routes (i.p/i.v) depending on the experimental needs. Based on the component of injections the non-surgical model of sepsis has been categorized as infection model, which involves injecting live bacterium and intoxication model, which involves injecting non-infectious components such as heat-killed bacteria or bacterial endotoxin LPS [111].

The infection model involving administration of a live pathogenic organism of interest would induce sepsis [112, 113], however, the spreading of infection, the generation of inflammatory cytokines and the mortality rates are all determined by the route of administration [97] and the bacterial strains used in the experiments determine the hemodynamic responses [112].

The intoxication model known also as endotoxemia model of sepsis is the widely used model, since the administration of LPS mimics almost all the pathological consequences that occur during sepsis [114]. However, there are many disadvantages of using endotoxemia model, since the systemic clinical signs are initiated immediately after the administration of LPS. For example, the TNF-α level increases within an hour of LPS administration and the levels decline after 4 h. But, this is more unlikely to occur in human system and the inflammatory cytokine levels may raise based on the severity of infection [114]. The other major disadvantage of using endotoxemia model is that the concentration of LPS required to elicit strong inflammatory response is much higher in mice when compared to humans [115], indicating a variation in the sensitivity to LPS in different animal species [116]. In addition to differences in sensitivity among various animal models of different species, Yang et al. showed variation in sensitivity to LPS in murine models of sepsis. In this, the most widely used strains of mice, C57BL/6J strain, shows less sensitivity to LPS while BALB/c is more sensitive [117].

All animal models, irrespective of whether surgery is involved or not, possess disadvantages in addition to various advantages [118]. So, the researchers should consider all the disadvantages before opting for a particular animal model. Unfortunately, an ideal animal model of sepsis is yet to be developed.

Inclusion of antibiotics along with novel candidate drugs while treating sepsis

Strategies of targeting a single entity in clinical sepsis never lead to fruitful outcomes as evident from the failure of a vast number of clinical trials. Yet another strategy that could be employed while treating patients with severe sepsis is the use of antibiotics in combination with candidate drug molecule. Although very few reports suggests the beneficial effects of inclusion of antibiotics along with various drugs, the results of experiments using animal models of sepsis clearly demonstrate the potentially beneficial roles of combination therapy in reducing the mortality rates. Reports by Bauhofer et al. [104] and Aydin et al. [119] describe the positive effects of combination of antibiotics with Granulocyte colony stimulating factor (G-CSF)—a stimulator of bactericidal activity of granulocytes, in reducing the mortality of animals. Similarly, use of antibiotics in combination with Tumor Necrosis Factor Inhibitor and an endotoxin antagonist, E5531, significantly protected mice from lethality as reported by Fei et al. [120] and Christ et al. [121], respectively. With these beneficial effects, including antibiotics along with various drugs would improve the survival of sepsis patients.

Recent advancements in search of cure for sepsis

Technologies employed in delineating the cause of sepsis

Decades of struggle in sepsis research has lead to the better understanding of disease progression. Researchers are now involved in identifying the exact cause by employing various advanced technologies so as to treat the patients immediately in a more specific way. The particular pathogen behind the microbial infection is being analyzed by Polymerase Chain Reaction (PCR)-based assays, replacing the standard microbial culture methods [122, 123]. The disseminated intravascular coagulation is one of the major problems often observed in patients with severe sepsis and septic shock [124] and this defect can be quantified in vitro by modified thromboelastometry [125], a technique that measures the fibrinolytic activity in whole blood samples. Quantification of metabolic biomarkers is of great importance in diagnosing the worsened diseased state to initiate timely treatment options. For this purpose Garcia-Simon et al., have developed a 1H NMR based analysis protocol to quantify the metabolic markers in urine [126]. However, implementing the use of these techniques in clinical labs is only after their extensive validation and most importantly, depends on their cost effectiveness.

Strategies employed to attenuate cardiac dysfunction

With the failure of more than 100 randomized clinical trials [20], researchers are finding new avenues to block the severity of sepsis and related disorders. Of the organs most affected during severe sepsis and septic shock, heart plays a major role [127], as low cardiac output is often the major problem seen in cases of severe sepsis resulting in hypoperfusion and end-organ damage [72]. To circumvent the problems associated with low cardiac output, the ionotropic drug—Levosimendan, a calcium sensitizer, earlier shown to be effective in animal models [128, 129] and also to be superior over widely employed ionotropic drug Dobutamine [130], is now being employed in clinical trial to test its efficacy in improving the organ dysfunctions in septic shock patients [121]. Resuscitation using selepressin, a selective vasopressin type-1a receptor agonist involved in vasodilation has been shown to be effective in animal models of sepsis and is also shown to be more effective than vasopressin in blocking the vascular leakage [131, 132]. Selepressin is now under phase 2b/3 initiated by Ferring Pharmaceuticals for the treatment of septic shock. Trimetazidine (TMZ), an inhibitor of β-oxidation pathway, has been shown to possess anti-ischemic activities through extensive utilization of myocardial glucose and maintaining proper energy metabolism [133]. TMZ has recently shown to have protective roles by blocking LPS-induced myocardial dysfunction and apoptosis in experimental sepsis model [134, 135]. Hence, TMZ can also be a candidate drug molecule to alleviate the problems associated with heart.

Strategies to circumvent the problems associated with acute kidney injury

Acute kidney injury (AKI) following cardiac dysfunction is the frequent condition often observed in sepsis patients [136]. The severity of kidney dysfunction can be determined by quantifying the serum troponin I level as reported recently by Thiengo Dda et al. [137]. Administering the dephosphorylating enzyme, alkaline phosphatase to sepsis associated AKI patients has shown beneficial effects by reducing the urinary excretion of tubular injury biomarkers and plasma markers of inflammation [138]. Recent prospective, two-center, open-labeled randomized, controlled trials conducted by Abdul-Aziz et al. have shown the beneficial effects of continuous administration of β-lactam antibiotics in patients not undergoing renal replacement therapy [139].

Nuclear proteins as the marker of mortality due to sepsis

Histones, the component of eukaryotic cell nuclei involved in folding the DNA into nucleosomes [140] are now shown to have a role in inflammatory condition, where these nuclear components are released to the exterior by damaged and activated cells. They possess cytotoxic and pro-inflammatory effects which depict the severity of inflammation, thereby joining the list of damage associated molecular patterns (DAMPs) [141]. These released histones act through TLR2 and TLR4 and are responsible for cytokine release, endothelial dysfunction, end-organ damage and mortality in animal models of sepsis [142, 143]. Infusion of histones in mice has been shown to cause pulmonary vascular obstruction, induced right ventricular pressure increase and dilatation leading to cardiac injury [144]. These deleterious effects are shown to be attenuated by the use of heparin [145]. Extracellular histone levels are also shown to predict mortality in sepsis patients [146] and hence, targeting extracellular histones is of great importance so as to come up with an efficient drug to treat sepsis and related disorders.

High-mobility group protein B1 (HMGB1) are the abundant and ubiquitous chromatin-associated nuclear proteins [147, 148] belonging to the superfamily of high-mobility group (HMG) proteins [149]. Its presence is restricted to nucleus in naive cells because of the presence of two lysine-rich nuclear localization sequences [150]. However, it is released to the extracellular milieu by various cell types in response to their activation by various factors [151–157]. The released HMGB1 may act through TLR4 [158, 159] or through the receptor for advanced glycation endproducts (RAGE) [160, 161] leading to the activation of NF-κB resulting in the production of pro-inflammatory cytokines [162]. Recent report by Zheng et al. [163] shows the loss of vascular endothelial monolayer integrity in vitro induced by HMGB1 present in the sera of patients with sepsis. Use of Dabrafenib, a B-Raf inhibitor, ameliorated HMGB1-induced vascular permeability in addition to blocking HMGB1 release as reported by Jung et al. [164]. Anti-HMGB1 antibodies are shown to reduce the LPS-induced lethality in mouse model of endotoxemia [151] and are also shown to be beneficial in protecting the mice against endotoxin-induced acute lung inflammation [165]. Release of HMGB1 (late mediator) and its inflammatory properties might be the reason behind the failure of monoclonal antibody therapies directed against TNF-α or IL-1β (early mediators). Developing an efficient drug targeting HMGB1 and using it in conjugation with other drugs would be a wise idea to bring down the mortality rates associated with sepsis and related disorders.

Combination therapy as a new strategy to treat sepsis

Sepsis-induced organ damage is mediated by the involvement of multiple pathways [166] and hence, designing a single drug to block all the pathways simultaneously is not feasible. In this regard, researchers are now employing the use of a combination of drugs to attenuate the septic challenge in animal models of sepsis. A recent study by Kwon et al. [167] show the beneficial effect of combination of niacin and selenium in attenuating the severity of lung injury and mortality in endotoxemia and CLP models of sepsis. Besides, treating animals either with niacin or selenium individually failed to mount the similar response [167]. In a similar study, Lima et al. [168] have used monoclonal antibodies against the two major receptors TLR2 and TLR4 in combination with antibiotics to circumvent the adverse effects of polymicrobial sepsis. The results have shown a remarkable improvement in survival rate in addition to reduced neutrophil infiltration and cytokine production [168]. Although proven to be beneficial in experimental models of sepsis, the strategies of using combination of drugs have to undergo validation to reach clinical trials.

New cell-based therapies against sepsis

Mesenchymal stem cells (MSCs) are shown to be promising therapeutic options against various tissue injuries and immune disorders [169]. MSCs are shown to be protective in models of acute lung injury [170], cardiac dysfunction [171], renal failure [172] and hepatic injury [173]. Apart from this, MSCs therapeutic roles have been proved even in animal models of endotoxemia [174, 175] and polymicrobial sepsis [176, 177]. With all these beneficial roles, MSCs have not yet reached the phases of clinical trials for the treatment of sepsis [178].

Future perspectives in search of cure for sepsis

Since sepsis is a complex disease involving multimediators, targeting a single entity is unattainable as evident from the failure of clinical trials against LPS which throws light on the involvement of other possible components of bacterial membrane in the pathogenesis of sepsis. As observed by us (Unpublished data) and others [57], purified BLP is as potent as LPS in inducing severe inflammatory response both in vitro and in vivo. Hence, targeting several other components of bacteria like BLP along with LPS would be a good strategy in attenuating the severity of sepsis. In addition, researchers need to focus on blocking the deleterious effects mediated by the released nuclear proteins as they are shown to be the late mediators of sepsis. Another strategy would be to employ combination of drugs as they are proven to be beneficial in regulating the process of initiation and disease progression [168]. Similarly, inclusion of antibiotics in combination with other standard drugs would improve the survival rates of sepsis patients as shown in various animal models of sepsis. However, the strategy of employing a combination of drugs needs to be validated both in animal models of sepsis and clinical trials. Although very few reports suggest the beneficial roles of adjunct therapy using high-dose of IgM, enriched intravenous Ig, (IVIG) [179, 180], considering this option would be a good strategy for the treatment of patients with sepsis. As the use of MSCs have been proven to be beneficial in modulating the immune response and converting the macrophages and neutrophils to anti-inflammatory phenotype in animal models of sepsis [181], clinical trials may be carried out to bring MSCs to market as one of the therapeutic options.

Conclusion

Although, many of the molecular events occurring during sepsis are dissected, an appropriate single molecular entity that needs to be targeted is still a mystery. One can argue that, in a disease involving multimediators it is unlikely to have a single entity that can be targeted. Moreover, more than 100 clinical trials have been undertaken and have failed to identify a critical mediator for sepsis and related disorders [20]. Furthermore, this disease/syndrome also lacks a suitable and appropriate animal model as evident from the disadvantages of each model [118] and this may also be the reason behind the failure of so many clinical trials. Therefore, sepsis is still considered as a disease, in search of a cure. Hopefully, modern biology will come up with a suitable animal model or a molecular target so as to develop a magic bullet to treat this deadly disease soon.

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