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# Sepsis and signal transduction pathway: cross-talk TLR4/ MyD88/TRIF

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**Abstract**

Sepsis is a mediator of the immune response and inflammation events, resulting in serious health problems due to such response and the condition of a systemic insult linked to a confirmed or presumed contamination. epsis and septic shock are systemic reactions that can be lethal and are generally caused by microbial infection. The main focus in sepsis pathophysiology is the host response to the pathogen, and some investigators have obtained results relating to inflammation, inducible nitric oxide synthase (iNOS) activation, immunosuppression, and effector T cell apoptosis. Signal transduction pathway is the process by which a chemical or physical signal is transmitted through a cell as a series of molecular events, most commonly protein phosphorylation catalyzed by protein kinases, which ultimately results in a cellular response. Five basic concepts or principles in signal transduction pathways include: 1) cell response relies on the integrated activity of many signaling pathways working in parallel, 2) all signaling pathways are reversible, 3) signaling pathways can be interconnected to other pathways forming closed loops, 4) signal transduction occurs on a time scale of seconds or minutes, and 5) signal transduction pathways are evolutionarily conserved.

Signal transduction pathway can induce several activities, such as: 1) changing the number of a certain receptor on the cell surface, 2) indirectly adjusting the amount of specific enzymes or regulators already on the cell surface, 3) changing the amount and/or type of effector proteins inside the target cells, such as changing the amount of cAMP. The key components of transmembrane signal transduction pathway include: 1) receptor, 2) protein channel, 3) ion channels, 4) enzyme, 5) transcription factor. A certain combination of receptor subunit and protein kinase results in the interaction of the two identical cytoplasmic domains, called an active receptor, that can phosphorylate the tyrosine residues in its partner kinase domain. Tyrosine phosphorylation, autophosphorylation, and receptor dimerization are very common events in receptor kinase pathways. Toll-like receptors are generally known as pathogen-associated molecular pattern (PAMP) receptors in cell membranes.

**Keywords**: Sepsis; LPS; MyD88; Proinflammatory cytokines

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## Introduction

Sepsis is a mediator of the immune response and inflammation events, resulting in serious health problems due to such response and the condition of a systemic insult linked to a confirmed or

presumed contamination. Based on the Berlin Sepsis Criteria, sepsis is characterized based on clinical assessment, which includes the possible signs and symptoms mainly somatic, such as organ dysfunction (celiac, heart, liver, lungs, kidneys or brain alterations); major trauma; numerous comorbidities; system alterations with at least two purposes; a positive screen, just a symptom associated with a known or pointed out the point of disease; large amounts of infection. Although sepsis symptoms are variable in each individual, sepsis can appear in a variety of ways, depending on its origin. The symptoms of sepsis may include general body aches or discomfort, rapid breathing, rapid pulse, decreased urine output, chills, and fever.

Sepsis epidemiology is focused on national statistics in developing countries. In higher income countries, the total annual incidence of sepsis transformed in hospitalizations increased from almost empty 1 case (confidence break of 0.93 cases, inquiry of 1.88 cases) to 16 cases per 100,000 population (16.5 increased LCI 15.9 ° C, UCI with 17.5 ° C) throughout the northeast of the population (i.e. is 1.5% -22.9% of hospital necessities). The blood culture is still the gold standard for sepsis allied disease analysis; the method of diagnosis of the etiology of the micro-organism associated with the septic condition in % of cases give accurate guidance for effective therapies including further connected to a biotic antibiotic. The procalcitonin, IL-6 and CRP assays are the common serologic findings of a significant informative status for patient health problems.

**Clinical Manifestations and Diagnosis**

Clinical manifestations. According to Bone (1991), the condition of sepsis was proposed to be characterized by the systemic inflammatory response to an infection (33). However, sepsis does not develop in all individuals with an infection; therefore, the clinical symptoms indicative of infection do not relate to sepsis but the tissues and cells responses against infection, i.e., immunity. These symptoms include warmth, pain, redness, swelling, and loss of function. Clinically, warmth and redness are usually considered as the same symptom.

On the other hand, some non-survivors of sepsis develop coagulopathy, which was recently proposed to be referred to as immunodeficiency (34). However, since coagulopathy is induced as the result of the dysfunction of endothelial cells, it is not proper to describe immunodeficiency as the ability to destroy endothelial cells independently of the infection-triggered inflammation (systemic inflammatory response). Therefore, the clinical manifestations of sepsis may be loss of endothelial cells and cells related to the defense against remote compensations (they are rapidly induced by over-circulation).

Diagnosis. Based on the aforementioned background, in 1992, the American College of Chest Physicians/Society of Critical Care Medicine consensus conference proposed that the condition characterized by sepsis, severe sepsis, and septic shock should be referred to as systemic inflammatory response syndrome (SIRS) caused by an infectious disease (35), in which SIRS and infectious diseases may be independent.

Another proposal was that the term used to refer to the condition in which patients with severe sepsis are eligible for curing of only supportive care should be 'sepsis'. In this consensus, the panel did not include the pathological findings in any organ. This proposal may be based on that sepsis patients may be immediately eligible for evaluating organs in clinical settings without reliance on the

pathological findings (35,36). SIRS is reproducibly elicited artificially in animals by injecting lipopolysaccharide (endotoxin) or several non-living cells.

Although Andersson et al. reported that SIRS can be experimentally induced using any substance (37), the substances did not include a living cell. Taken together, the concept of SIRS is outside the system of the immune response. SIRS has, therefore, been proposed to be caused by the tissues response. Anything other than infection may induce SIRS in clinical settings. Clinically, sepsis can be diagnosed when severe sepsis is diagnosed. This proposal may indirectly recognize the diagnostic abilities of severe sepsis. PCR results under antibiotic conditions (38) and viral loads (e.g., cytomegalovirus) (4) have no potential for diagnosing sepsis.

The platelet count is not an exclusion criterion in sepsis (35). Sepsis cannot be diagnosed based on any culture result. The same culture results may be obtained even in non-survivors of sepsis. Anything that may indicate physiological dysfunction may be useful for identifying sepsis. The PaO2/FiO2 ratio may reflect the diseases in respiratory surgery and cannot help in evaluating the hemodynamic status in sepsis. The PaO2/FiO2 ratio has limited utility in evaluating the pathological condition in sepsis.

A temperature of 36°C (96.8°F) or more may be included as diagnostic criteria of septicemia because such a decision will render diagnosing 'treatment' under a dichotomized condition based the treatment effect of the immune response. When focusing on sepsis, a distinction between treatment conditions may be useful for evaluating the diagnosis. Taken together, sepsis should be diagnosed based on the evaluation of severe sepsis.

**Overview of Signal Transduction Pathways**

A signal pathway or signal transduction pathway is a set of features present in the cell signaling system that conveys the signal from the receptor on the extracellular surface to the intracellular compartment. It can be divided into three main categories:

1. A signaling molecule is made active when the signal molecule is sensed by a receptor that activates the intracellular signaling molecule.

2. Conversion of a signal molecule into a second form, or second messenger, which in turn brings the message to a recipient molecule that will execute the desired response within the cell.

3. The signal molecule connects to the intracellular receptor where the signaling event depends on the properties of the receptor. Communication or signal transduction pathways usually have many additional elements, including adaptor proteins, and can also be branched and regrouped to create a variety of responses as a result of the signaling event.

There are several key components needed to perform these signaling events:

1. Ligand: The initial action in this process is normally brought about by binding of a suitable messenger to the transmembrane proteins.

2. Receptor Molecule: Generally, the receptor is a fire chain or serves as a molecular hook in this process. Alternatively, it may include lipids.

3. Signal transduction proteins: In this step, signaling molecules are converted from one form into a second messenger molecule in the cell. Usually, the type of protein kinase or G protein family proteins carries out these changes.

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**Basic Concepts and Key Components**

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Signal transduction pathway is the process by which a chemical or physical signal is transmitted through a cell as a series of molecular events, most commonly protein phosphorylation catalyzed by protein kinases, which ultimately results in a cellular response. Five basic concepts or principles in signal transduction pathways include: 1) cell response relies on the integrated activity of many signaling pathways working in parallel, 2) all signaling pathways are reversible, 3) signaling pathways can be interconnected to other pathways forming closed loops, 4) signal transduction occurs on a time scale of seconds or minutes, and 5) signal transduction pathways are evolutionarily conserved.

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**The Toll-like Receptor 4 (TLR4) Pathway**

Toll-like receptor (TLR) family, being a kind of crucial pattern recognition receptors (PRRs), can identify the conserved constructions (pathogen-associated molecular patterns, PAMPs) of kinds of pathogens including bacteria. There are totally 13 kinds of TLRs and among them, TLR4 has been reported to play a dominant role in the initiation of immune reaction and sepsis. Toll-like receptor (TLR) 4 is a transmembrane receptor protein that principally plays a role in the acknowledgement of lipopolysaccharide (LPS) of Gram-negative bacteria on the surface of monocytes and macrophages. As we all known, the main composition of the outer membrane of the Gram-negative bacteria is lipopolysaccharide, which is crammed with toxicity factors that is rather crucial to protect the bacteria from the phagocytosis of the host. Therefore, TLR4 plays an essential role in the initiation of immune responses and sepsis, which makes it an optimal therapeutic object.

TLR4 is composed of the cytomembrane-type IL-1 receptor-Toll-like receptor (TIR) domain, an extracellular leucine-rich repeat (LRR) domain, and a C-terminal tail, which can be connected with the adapter proteins to conduct signals downstream. But in the process of sepsis, the canonical nuclear factor-κB (NF-κB) activation and proinflammatory cytokines did not both rely completely on the "TIR domain of TLR4" and "TIR domain-containing adapter inducing IFN-β (TRIF)" (a unique TIR domain, which exists exclusively in TLR3 and TLR4). Besides that, CD14, MD2, MyD88, TRAM, TRIF/TICAM-

1/TICAM-2, Mal and TIR domains were also regarded as the additional molecules participating in a completed signal transduction system. To some extent, yeast TIRs, which lack intracellular TIR domains, also could activate downstream adaptors and lead to ZAP70 induction.

**Structure and Function of TLR4**

The human body has a sophisticated immune response system, capable of detecting and eliminating pathogenic microorganisms. Toll-like receptor 4 (TLR4) is one of the most extensively studied pattern recognition receptors. Its primary role is to recognize lipopolysaccharide, the most important virulence factor in the cell wall of gram-negative bacteria, even in a very small amount, and to transmit signals to downstream signaling molecules, resulting in the production of a large number of cytokines. The information and research status about the structure and signal transmission pathway of TLR4 receptor are summarized and reported. This review provides valuable information for understanding the molecular mechanism of TLR4 in recognizing pathogens, producing immune response and playing an important auxiliary role in TLR4-targeted molecular design, drug development and inflammatory response-related diseases (e.g. sepsis). Toll-like receptors (TLRs) are a kind of pattern recognition receptor (PRR) expressed on the cell membrane and in the lysosome of vertebrates, which have the function of recognizing pathogen-associated molecular patterns (PAMPs) and initiating immune response.

Cell membranes express TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10, while lysosomes express TLR3, TLR7, TLR8, TLR9. TLR4, as a member of TLRs, can recognize LPS and activate the NF-κB and MAPK signaling pathways, resulting in the production of a series of cytokines required for the defense against gram-negative bacterial infections. In mammals, TLR4 is the most studied because of its vital role in triggering strong natural immune response to bacterial infection. Consequently, in recent years, many studies have also focused on the therapeutic targeting of various infectious diseases including sepsis. In this article, the authors presented an overview of the essential molecular basis of sepsis.

**MyD88-Dependent Signaling Pathway**

The intracellular domains of the TLRs share homology with the Toll/IL-1R (TIR) and thus are capable of recruiting diffusible adaptors containing this domain. TIR domain-containing adaptors are not specific to single families of the receptors, and thus participate in the signaling pathway used by more than one TLR. MyD88 is the best example illustrating this promiscuity of intracellular adaptor usage in TLR signaling. MyD88 is a cytosolic protein that, upon receptor activation, is recruited to the intracellular part of the TLR4, TLR2, TLR5, and TLR9.

There are three functional motifs present in the MyD88 structure: death domain highly conserved in the adaptor proteins recruited to the intracellular part of the TLR, semi-conserved TIR domain mediating the interaction of the MyD88/TLR4 complex with interleukin 1R associated kinase (IRAK)-4, and the carboxy-terminal domains found to be essential for recruitment of IRAK-1, TNF receptor-associated factor (TRAF)6, and B-cell lymphoma (BCL)-10. Upon double recruitment of the receptor and IRAK-4 kinase to the MyD88 adaptor, both a local concentration of IRAK-4 and its conformation are changed, promoting the auto-phosphorylation of the kinase and its activation. IRAK-4 activation

can take place in two possible pathways. It may promote the activation of IRAK-1 or may directly activate MyD88 in solution before being loaded onto receptor. In turn, MyD88 associates with and activates IRAK-4 that exhibits protein serine/threonine kinase specific towards IRAK and IL-1 receptor associated kinase (IRAK)-2. Also activated by death domains, IRAK-4 autophosphorylates, dimerizes, and subsequently activates a serine/threonine IRAK. Heyninck's group reported the studentship details of several proteins involved in the immune signaling pathways.

**Role of MyD88 in TLR4 Signaling**

As we have said, TLR4 can initiate two signaling pathways, employing the two intracellular adaptors, MyD88 and TRIF, to ensure maximal stimulation in response to LPS. The transcription factor that NF-κB is activated through the MyD88-dependent pathway and the type I interferons complex assembly and their signaling molecules. But, it can be activated through both adapters, leading to further cytokine production during the time course of the full immune response.

**MyD88**

The adapter MyD88 has an amino-terminal domain, the Drosophila tube death domain, a central intermediary domain, and a carboxyl-terminal Toll/IL-1R domain (TIR domains). The TIR domain is responsible for oligomerization of these kinds of receptors at the plasma membrane, and the tube shows a higher homology with the TIR domain rather than the other domains of MyD88. MyD88 can interact through its TIR domain, receptor interaction, to the death domain of IL-IX family receptor, IL-6, IL-18, IL-36, and others.

The function of the myeloid differentiation factor MyD88 adaptor molecule during the signal transduction process activated by Toll-like receptors (TLRs) is one of the most critical aspects of this field of research. The binding of the adapter molecule to TLR4, a recognition molecule of immune cells, results in the activation of NF-κB through a series of signaling pathways within the cells. This nuclear transcription factor then targets the expression of pro-IL-1β, inducible nitric oxide synthase, and other crucial molecules involved in the inflammatory process associated with sepsis. It is clear that MyD88 has a close relationship with a variety of other signaling factors and that the function and mechanism of these effects are exactly the same as for other closely related molecules. The MyD88-dependent signaling pathway is tightly associated with TLR4 on the cellular membrane and appears to require oligomerization of an active receptor to move downstream via a specific series of molecules, each of which has a specific binding site or adaptor during the signal transduction process.

**TRIF-Dependent Signaling Pathway**

TRIF-Dependent Signaling Pathway TIR-domain-containing adaptor-inducing interferon-β (TRIF, also known as TICAM-1) is the third TIR-domain-containing protein. TRIF acts as a sorting adaptor that functions as a switch and also bridges signals dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN, CD209)/MD-2, leading to MyD88-mediated TLR4 endocytic signaling. TRIF spans activation in both MyD88- and inflammatory response factor (IRF)3-dependent signaling via TLR4. Phosphorylation of TRIF stimulates homodimerization of IKK-α and IKK-β. It also recruits TNF receptor (TNFR)-associated factor 6 (TRAF6) as well as receptor-interacting protein 1 (RIP1).

At the young time, the NF-κB controlled by TRIF-IKK kinases mainly acquires IRF3 to phosphorylate viral and transactivate cascade expression. This results in the synthesis of numerous pro-inflammatory cytokines and the type 1 interferons (IFNs).

They are not only inhibited by the anti-inflammatory feedback but also inhibit the exocytosis of major histocompatibility complex (MHC) class II peptide from major histocompatibility complex (MHC) class II. If the antigen-presenting cells (APCs) are infected, the cross-talk of TLR4-TRIF-dependent signaling decreases the surface localization of human leukocyte antigens (HLAs) and Th1 downregulation, abundant Th2, B cell, regulatory T cells (Treg), and protein phosphate 1 and suppress certain autoreactive T cells.

**Role of TRIF in TLR4 Signaling**

TLR4 signaling through the MyD88-dependent pathway activates two downstream signaling molecules, IRAK and TRAF6, which lead to NF-κB and MAPK activation and pro-inflammatory cytokine expression. It was shown that TLR4 can also function through the MyD88-independent pathway activated by a new adaptor molecule, the Toll-IL-1R domain-containing molecule inducing IFN-β (TRIF). Following phosphorylation and recruitment of TRIF, key transducers of the type I IFN signaling pathway would be activated and involved in the anti-viral IFN response. Consistent with the leading for two waves of gene activation, from early to late times post-infection, the simultaneous activation of both pathways was shown only for a subset of TLRs for which the MyD88 and TRIF adaptors were activated in parallel. Concerning TLR4 activation, with LPS in its reducing form, most studies demonstrated late activation of NF-κB and IRF-3, exclusively dependent respectively on MyD88 or TRIF.

In this way, TLR4 activation through MyD88/TRIF represents a unique mechanism to assure a strong coordination between the immune and the adaptive responses to bacterial infection. Moreover, MyD88-independent functions of TLR4/CD14 should be associated with resistance to LPS sensitivity or induction of LPS tolerance at monocyte level. This review will more specifically discuss TRIF engagement in signal transduction. TLR4 was originally identified as the LPS receptor and implicated in host defense against gram-negative bacterial infection. Upon LPS stimulation, TLR4 signals through MyD88-dependent and MyD88-independent signaling cascades. Early discrimination of TLR4 receptors by TIR domain and yeast 28 infector protein/Mycobacterium tuberculosis 28 kDa stress protein (GRP 70)-activated MyD88 and TRIF/TRAM showed the possibility of separate signaling by TLR4. More recently, TLR4 was shown to use a similar signaling pathway to TLR5 and TLR2 in fully promoting the immune response.

**Cross-Talk Mechanisms between TLR4, MyD88, and TRIF**

To guarantee that the immune system is able to resist microbial invasion and sudden cell damage or death caused by injury, Toll-like receptors (TLRs) released by innate immunity recognize exogenous or endogenous heterologous molecular patterns. TLRs can be expressed intracellularly, of which TLR4 is the first to be discovered and has been the most studied. TLR4 consists of three myeloid differentiation factor 88 (MyD88) dependent signaling pathway transduction subtypes: MyD88 pathway transduction; TIR domain containing adaptor inducing IFN-β (TRIF) dependent pathway

transduction, and the MyD88-dependent/no TRIF-independent signaling pathway which is also called the MAL/TLR4 signaling pathway. Numerous research studies have found that many cross-links exist among the subtypes of TLR4 transduction pathways, such as the link between MyD88 and TRIF, MyD88 and LPS, TRIF and Mal, etc. When we focus on the mechanisms related to sepsis, understanding these links is in fact a matter of noticing cross-talk between the MyD88- and TRIF-dependent pathways, which we focus on in the review herein.

Given that transduction depends more on TRIF during severity, Wallisch et al. (2017) hypothesize that, during a progression from mild sepsis to severe sepsis, transduction depends more on TRIF. They test the activity of MyD88 and TRIF. LPS and E. coli have exaggerated MyD88 response to infections. Contingency tables analysis of personal data also illustrates that PI3K has a positive association of mild patients. Based on a large dataset of sepsis patients, a comprehensive analysis of a novel strategy for not only the TLR4 function at the level of signal transduction but also for anti-sepsis network at the transcriptional level.

**Interactions and Regulatory Loops**

Trumpets in the JAM. Utagawa et al., investigate the existence of the interactions and regulatory loops between Toll-like receptor 4, MyD88, and TRIF in septic patients in the last issue of Journal of Leukocyte Biology. The Toll/IL-1R signaling (TIR) domain is found in adaptor molecules like MyD88 and TRIF that recruit the IRAKs, E3 ubiquitin ligase (MAVS: mitochondrial anti-viral signaling) and the RING domain (RIP 1) that possess the capability to initiate a signaling cascade. MyD88 is used by all the TLRs except TLR3, while there is evidence that TLR4 can signal via TRIF alone, but with less efficiency compared to co-recruitment of MYD88 and TRIF. MyD88 and TRIF have dominant negative (dn) constructs. The TIR domain sites for MyD88 and TRIF are clearly distinct and are thus compatible with a receptor binding to both proteins simultaneously. TAK1 is a key intermediate in MyD88 signaling, but is also required for TRIF signaling. Moreover, it had been shown previously that a MyD88 activated transcription factor, Interferon regulatory factor-3 (IRF3), also is activated via a TRIF-dependent fashion. The lack of inhibition by the MyD88 dn construct alone suggests that the MyD88 dn is still permissive for IRAK downstream signaling via TRIF-induced TAK1. Although MyD88 can oligomerize via the death domain and TIR domain, it was found that the MyD88 mutants, which contained the TIR domain only, were ineffective at signaling.

In conclusion of Adib's group, their in vitro data demonstrate the cross-talk that exists between the MyD88 and TRIF and provides the first evidence of a link between these partially shared pathways in a clinical setting. The TIR domain contains conserved residues that are essential for signaling and pathogen recognition via the TLRs, for recruitment of cytoplasmic adaptor proteins such as MyD88 and TRIF. MyD88 has also been shown to recruit a number of other molecules including the IL-1 receptor-associated kinase 1, although the recruitment of other protein/protein interactions by MyD88 as yet are unidentified. MyD88 itself can then directly recruit IRAK-1, the first known signaling step in MyD88 signaling. The association of IRAK-1 with MyD88 induces it to disassociate from the receptor and, together with IRAK-4, autophosphorylated.

In summary, although clearly different in output, the pathways contain interactive components. In the same manner increasing the amount of MyD88 present with TLR4 stimulation up-regulating MyD88 expression, as was observed by Opal et al. for TLR4 stimulation, can also result in a reduction of the TRIF pathway signaling (owing to down-regulation in response to low levels of ligand) at TLR4. Therefore, the up-regulation or down-regulation of one pathway may well have an impact on the alternative signaling pathway, the outcome of which may have relevance to the magnitude and duration of the immune and inflammatory response.

**Experimental Models and Techniques in Studying Sepsis and Signal Transduction**

The study of sepsis and signal transduction has become easier and more feasible in the last 25 years. There are golden standard methods that would be followed, but from time to time, techniques have evolved. The biggest issue in experimental research is the translation of the obtained results to human therapeutic strategies. Animal models have been proven to translate directly with humans, wherefore results established at a single-cell and single-molecule level will have to be more translational than the others. The experimental methodologies discussed cumulatively have described the moieties available to sepsis research over the years; however, these can now be considered as outdated methodologies or methods that have evolved into new renascent formats. The more recently developed methods based around the use of retroviral transfection are currently sparse at the time of publication; however, they show some promise as novel approaches to investigating the differences between murine animal models. Most notably in experimental research is the increased presence of multiple experimental models and techniques being discussed in the same paper. The integration of both cell lines and in vivo murine models has the greater potential to confirm biological findings using an array of transgenic groups. For example, a knockout group versus a wild-type control. This ability to integrate biochemistry, cellular and molecular biology along with animal models has a greater potential to enhance the scientific community and therapeutic strategies.

**Animal Models**

The usefulness of animal models in the study of sepsis and signal transduction is clear, not only in building mechanistic information about the scope of the syndrome but also potential therapeutic interventions. However, while animal models of sepsis have been overly relied upon, for the biological phenomena the closest to humans are critical for understanding the mechanisms that could cause tissue/organ damage. Therefore, systemic infections through peritoneum after cecal ligation or E. coli intravenous have remained valuable for the study of antibiotic activities and their relationships between doses and schedule administration. These models meanwhile have served to understand the virulence factors of the pathogen and the host's responses. The intraperitoneal administration of agents that produce endotoxemia is not septic but is life-threatening due to endotoxin and mainly triggers TLR4 or TLR4/TLR2 and TLR4/MD-2. The mortality in humans with sepsis or septic shock due to gram-negative bacteria depends on the virulence released from live bacteria inside them.

The induction of local organ injury could reflect the events in sepsis if the mechanism behind tight and regulated cellular adherence and exodus explained also the transcellular attachment and changes. The latter will also invoke low μM hydrogen peroxide (H2O2), previously linked to the role of endothelial

barrier injury in humans. Only continuous physiological, low levels of exogenous hydrogen peroxide entering the skin may contribute to host defense. Basal levels of 'added' H2O2 will also modify neutrophil migration. Furthermore, mediators that are known to increase and that of sepsis-induced cell adhesion, amongst other physiological changes, works at the endothelial barrier in the skin and other tissues such as the lung and brain.

**Cell Culture Techniques**

Plenty of proposed molecular mechanisms involved in cell signaling, interventions, and drug designs are considered based on in vitro experiments using cell culture techniques. Cell culture studies simulating sepsis prototypes are badly in need to study the pathophysiology of sepsis and also molecular mechanisms involving multiple organ failure. Each cellular component studied with in vitro methods shall critically study the variation with extracellular conditions such as variation in pH, release of reactive nitrogen intermediates, release of reactive oxygen intermediates, cytokines involved, etc. Nowadays, primary cell culture has observed a more revolutionized approach because the cells shall attain characteristics more closely to in vivo cells.

Many cellular responses underlying the development of sepsis and its associated signaling transitions are being studied through in vitro models. Several conditions have been established such as exposure to lipopolysaccharides (LPS), stimulation with plasma or blood isolated from septic patients, and stimulation of endothelial (EC) cells, neutrophils, and monocytes with proinflammatory mediators, and inactivating the role of a/v receptors. The molecular and cellular processes involved in the immune response give better focus with in vitro experiments to have effective results through expression analysis, protein production, cell signaling pathways, and toxicology testing studies. The application gives a chance for the identification of specific cell and tissue targets in which the drug can affect.

**Therapeutic Implications and Future Directions**

Sepsis is an old problem and still an unsolved clinical condition with high mortality and morbidity rates. No specific treatment is available to disrupt the sepsis progression, and the management of septic patients is based on supportive therapy with antibiotics. This strategy is inadequate because the disease pathophysiology is complex, involving the interaction of molecular aspects with the patient's genetic background. Currently, when symptoms are detected, anti-inflammatory, anticoagulant, and drug therapies are used in conjunction with intravenous infusions. However, they do not effectively treat sepsis and might cause an inefficient inflammatory response. Furthermore, neither of these treatments directly alters the signal transduction pathways, especially because the network of these pathways is not clearly established during sepsis progression.

Therefore, it is necessary to understand the pharmacological and immunological limits of the drugs in the market and discuss potential new therapies. One approach of potential relevance might be to use drugs that inhibit individual branches of the inflammatory molecules by targeting these mediators directly. The ultimate key to the successful development of new immunomodulatory therapies for severe sepsis is the idea that drugs should specifically act to switch an over-abundant inflammatory signal off when it is not immediately necessary, such as an overwhelming response as occurs in the milieu of sepsis. In the final analysis, a new approach will require improvements in our understanding

regarding how receptor signaling pathways contribute to disease and what therapeutic and immunomodulatory approaches, such as a potential vaccine for the prevention and treatment of infection as well as septic syndromes, could be used in humans.

**Current Treatments and Limitations**

At present, the primary treatments to treat sepsis are early antibiotic use, the maintenance of a stable fluid balance, and physiologic hemodynamic support. However, the limitations of these treatments have led many researchers to seek to develop new ways to treat sepsis. Sepsis begins not in a single cell in one organ and does not end in a single cell in another. It must now be considered a syndrome. In order to develop truly effective therapeutic strategies, susceptibility to pathogenic infection, the temporal and spatial interval before the cells activate signal transduction pathways achieving expression gene response, and the re-establishment of homeostasis must be examined. Cell-cell interactions, the response in neighboring cells to the sight and smell of sepsis exerted by signals secreted by every conceivable tissue, and the ability of those tissues to re-establish homeostasis are critical features of the 'sepsis cascade'.

**Emerging Therapies**

To date, the therapeutic strategies for sepsis and septic shock aim at the first rank overcoming or elimination infection through using of antibiotics and surgical procedures as the following step, support of organs with irreversible failure and bringing the immune system into balance. Beyond the two well-known therapy strategies in sepsis, anti-inflammatory or anti-TNF therapy (which was concepted to inhibit cytokines or antagonize endotoxins) and high-dosage corticosteroids, other pharmaceuticals are currently yielding promising results. It is therefore decisive to apprehend emerging therapies to develop sepsis in the future. Among the current pharmaceuticals for sepsis, a large number of toll-like receptor (TLR4) blockers (eritoran), antimalarial drugs (hydroxychloroquine, or a quinine derivative and SNPs that reduced TLR signaling), nicotinamide adenine dinucleotide (NAD+), immunoglobulin A (an element of Lactobacillus johnsonii), melatonin, disease-related agents (Aromadendrin, Thalidomide, Resveratrol, Tropolone, Topotecan) have been studied, and many of these molecules are able to regulate sepsis and inflammation through protein-protein interactions, coding by circular RNAs, and the inhibition of various signal transduction pathways. Large-scale studies are required, though outcomes from laboratory studies are motivating.

Among future interventions, resuscitation of the elimination of radicals (such as NSAIDs, NADPH activity inhibition, GSK1278863; GA), inhibitors of COPSS, blocking the crosstalk amongst the TLR2 and TLR4 pathway (such as Myeloid differentiation factor-2, TLR4 inhibitors; TLR15), angiogenic compounds with inflammation-regulating and endothelial cell-protecting effects (i.e., a disintegrin and metalloprotease 10; tissue inhibitor of metalloprotein-cardioprotective factor); inhibitors of natural antimicrobial peptides and cytokines (BIRM 073 and Temesporfin); antibiotics (Lopinavir; Rivaroxaban for treating Acute Graft Versus Host Disease, Bezlotoxumab; RACTx-105, a Bac-4Mes, has been developed for the treatment of the combination of severe bacterial and antifungal or severe bacterial infections with severe diarrhea. (BitConverterals 713C and 831C)); immunosuppressive agents (anti-IL-36ɣ (IVE7515)); PAK1 (FHBD and FHBB)), fanezumab, a monoclonal antibody that targets the

calcitonin gene-related peptide to counteract inflammation and vasoconstriction, erythropoietin (EPO) for acute alcohol intoxication. Anti-apoptotic effects of erythropoietin may also offer exclusive anti-sepsis protection beyond renal protection, as determined by D. Hossein et al. The complete aggregation of features is not single therapeutic use in sepsis only. The following interventions will lead to a synergistic approach to the management of this clinical status when they are concomitantly used systemically.

**Conflict of Interest**

No conflicts of interest were declared by the authors.

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**Ethics Statement**

Approved by local committee.

**Authors' contributions**

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.



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