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# Innate Immune System in Sepsis Immunopathogenesis and Its Modulation as a Future Therapeutic Approach

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## 1. Introduction

Immune system plays an important role in the development of systemic as well as compartmentalized inflammation though it may arise due to the various causes i.e. infection, trauma, burns, hemorrhagic pancreatitis and immune-mediated tissue injury. Pathogenic as well as commensal microorganisms evoke an immune response if they, or their constituents, pass the barrier between the external and internal environment. After recognition of the bacteria or their products, body launches an attack, kills the bacteria, and repairs putative damage. This sequence of events is highly regulated, enabling the body to combat infection by a tailor-made mechanism that is potent enough to eradicate the pathogen but not so potent as to cause unnecessary damage to the body. But, when this regulated immune mediated defense mechanism against the invading pathogenic bacteria gets deregulated then it causes harm to the body's own organs and leads to the development of the particular organ specific damage (compartmentalized inflammation) or the development of systemic inflammatory response syndrome (SIRS) or the sepsis.

Accordingly, acute inflammation is a self resolving property of immune mediated reaction and is a highly regulated cascade of events (Khatami, 2011). These events were recently described as 'Yin' (i.e. apoptosis, pro-inflammatory molecules etc.) and 'Yang' (i.e. wound healing, anti-inflammatory, resolution phase etc.) phenomenon with an intimate involvement of vascular components (Khatami, 2008; Khatami, 2009). For example, in severe acute inflammatory conditions like sepsis, which is mediated by cytokine storm or pneumonia the causative agents bypass normal host immune response activated in the form of acute inflammation by first damaging the blood vascular system integrity and then gain access to different compartments of the body and induce excess production of pro-apoptotic as well as tissue damaging molecules (i.e.  $\text{TNF-}\alpha$ , various interleukins, and free radicals) (Khatami, 2011). These molecules are potent enough for damaging and shutting down the immune-tissue interaction leading to enhanced tissue damage and in case of sepsis, development of multi organ failure, septic shock and ultimately death of the patient (Khatami, 2011).

According to the nomenclature, SIRS associated with a documented infection is sepsis. To date, most studies of the etiology and outcome of SIRS have focused on severely ill patients treated at intensive care units (Davis and Wenzel, 1995; Rixen et al., 1996; Headley et al., 1997; and Bonten et al., 1997). Sepsis/SIRS and septic shock originated due to gram negative or gram positive bacterial infection or caused by other pathogens like fungi, parasites or

viruses have become increasingly important over the past decades due to increased incidence of their occurrence as well as increased mortality and morbidity associated with sepsis in both developing as well as developed world (Glauser et al., 1991). For example, in the United States alone, the rate of septicemia got more than doubled between 1979 and 1987 causing up to 250,000 deaths annually (Perillo, 1993; Opal and Cohen, 1999). However, more than 18 million people are affected by sepsis worldwide and has an expected 1% increase annually in intensive care units (ICUs) (Ulloa and Tracey, 2005). It accounts for about 9.3% of overall deaths occurring annually in USA (Chen et al., 2005).

For example, it affects 600,000 people annually in United States with a mortality rate varying from 20-60% despite extensive use of antibiotics and other advanced supportive therapies (i.e. Use of ventilator assisted respiration, drugs for reversing high blood pressure and continuous cardiac monitoring) (Ward, 2004). The management of septic patients and their treatment, costs approximately \$ 17 billion annually only in United States (Angus et al., 2001). This data makes the sepsis 3<sup>rd</sup> leading cause of death in developed and industrialized world and deaths associated with sepsis equals the death associated with myocardial infarction in these countries (Martin et al., 2003). Sepsis is characterized by overwhelming stimulation of innate immune cells (i.e. Macrophages, Neutrophils, Mast cells and Dendritic (DCs) cells etc.) in response to pathogens and their products [i.e. Lipopolysaccharide (LPS), Lipoteichoic acid (LTA) or Peptidoglycan (PGN)], which leads to exaggerated release of pro-inflammatory mediators [i.e. cytokines (TNF- $\alpha$ , IL-1, IL-6, IL-18, MIP, IL-12 etc.)] responsible for the development sepsis and SIRS. Initially these mediators are released by these innate immune cells to contain the infection and to warn the body against invading pathogen or danger signal. But increased synthesis and release of these mediators in an uncontrolled manner due to overstimulation of the innate immune system instead of protecting the host leads to the development of SIRS. This SIRS, in turn becomes detrimental to host and causes capillary leakage, tissue injury, multiple organ failure, disseminated intravascular coagulation (DSIC), leading to development of septic shock and ultimate death of the patient (Cohen, 2002).

Earlier immunotherapeutic approaches like use of monoclonal antibodies against TNF- $\alpha$ , IL-receptor antagonists and TNF receptor perfusion proteins proved effective in combating various other inflammatory disorders like Crohn's diseases (CD), Inflammatory Bowel Disease (IBD), Rheumatoid Arthritis (RA) and showed modest effect in clinical trials and failed to receive US FDA approval for their use as therapeutic agents in sepsis management (Kumar and Sharma, 2008). Thus, the agents effective in treating other inflammatory disorders failed to treat sepsis or SIRS like conditions. This failure compelled us to understand the role of innate immune system in the immunopathogenesis of sepsis or SIRS associated with gram negative or gram positive bacterial infections, for designing better immunomodulatory therapeutic agents, which will be able to only modify the pathogenic immune response leaving the protective immune response intact.

In the present review (Chapter) an in depth attempt has been made to understand the role of innate immune system in the pathogenesis of sepsis or SIRS and exploration of various innate immune system targets to be used as future immunomodulatory strategies for sepsis management.

## 2. Sepsis in its clinical presentation

The clinical symptoms of sepsis were already known to Hippocrates (460-377 BC), and he introduced the term 'wound putrefication'. Additionally, the Persian 'father of modern

medicine,' Ibna Sina (AD 980-1037) observed that septicemia was usually accompanied by fever (Rittirsch et al., 2008). However, the modern concept of sepsis or severe inflammatory response syndrome entered into daily practice of medicine by Roger Bone and colleagues, who defined the sepsis as SIRS that can occur during infection (Bone et al., 1992). Sepsis or its more severe form called systemic inflammatory response syndrome (SIRS) or septic shock can be described as a very complex clinical presentation of array of pathological symptoms occurring as a result of exaggerated activation of host's innate immune system against infectious agents or to other stimuli like trauma, burns, hemorrhagic pancreatitis as well as immune mediated tissue injury (Bone et al., 1992). However, clinically onset of sepsis in a patient can be determined by the presence of bacteria in blood, hypothermia ( $<36^{\circ}\text{C}$ ) or hyperthermia ( $>38^{\circ}\text{C}$ ) tachycardia ( $>90$  beats/min), tachypnea ( $>20$  breaths per minute or  $P<32\text{mm Hg}$ ) and leukocytopenia ( $<4 \times 10^9$  cells/L) or leukocytosis ( $>12 \times 10^9$  cells/L) (Kumar and Sharma, 2008). SIRS can be diagnosed in patients when they do not have bacteria in their blood or their blood culture reports are negative for bacteria but are showing two or more above mentioned symptoms. This observation is true for about 50% patients with the above mentioned signs and symptoms. However, SIRS is accompanied by signs of damage to vital organs (i.e. lungs, kidneys, liver, heart and brain etc.) like development of hypoxia, oliguria, lactic acidosis, elevated levels of hepatic enzymes (i.e. Aminotransferases i.e. Aspartate aminotransferase (AST or SGOT) or Alanine aminotransferase (ALT or SGPT) and altered cerebral function along with bacterial growth (Matot and Sprung, 2001; Angus et al., 2001). While, severe sepsis is sepsis associated with hypotension (a systolic blood pressure  $<90$  mmHg or a reduction of  $\geq 40$  mmHg from baseline in the absence of other causes of hypotension), hypoperfusion or organ dysfunction. Septic shock is hypotension despite adequate fluid resuscitation with the presence of organ perfusion abnormalities (Takala et al., 1999).

The cost of treatment and management of sepsis in the USA alone is around \$ 16.7 Billion per year (Abraham et al., 2000). More than 500,000 patients develop sepsis in the USA alone with an incidence rising  $\sim 1.5\%$  per year (Abraham et al., 2000) and more than 210,000 people in USA alone die with sepsis (Deans et al., 2005). Therefore, it has become very important for researchers involved in sepsis research to correctly understand the immunopathogenesis of sepsis or SIRS so that better therapeutic targets for sepsis can be revealed.

### 3. Immunopathogenesis of sepsis

The molecular bacterial motifs which are recognized by host's innate immune system are described as pathogen-associated molecular patterns (PAMPs) or more accurately microorganism-associated molecular patterns as it is not clear how the innate immune system distinguishes signals from pathogenic organisms and commensal microbes. PAMPs include components of the cell wall i.e. lipopolysaccharide (LPS) from Gram-negative bacteria, and lipoteichoic acid (LTA) from Gram-positive bacteria as well as CpG DNA (bacterial DNA rich in cytosine-phosphate diester guanosine), bacterial flagellins and double-stranded RNAs (dsRNA) from viruses (Alexopoulou et al., 2001). On the other hand, immunological recognition of damaged tissue is mediated by intracellular proteins or mediators which are released from dying cells. These proteins are known as 'alarmins' (Box 1) and, together with PAMPs, are referred to as damage-associated molecular patterns (DAMPs) (Bianchi et al., 2007; Yang et al., 2009)

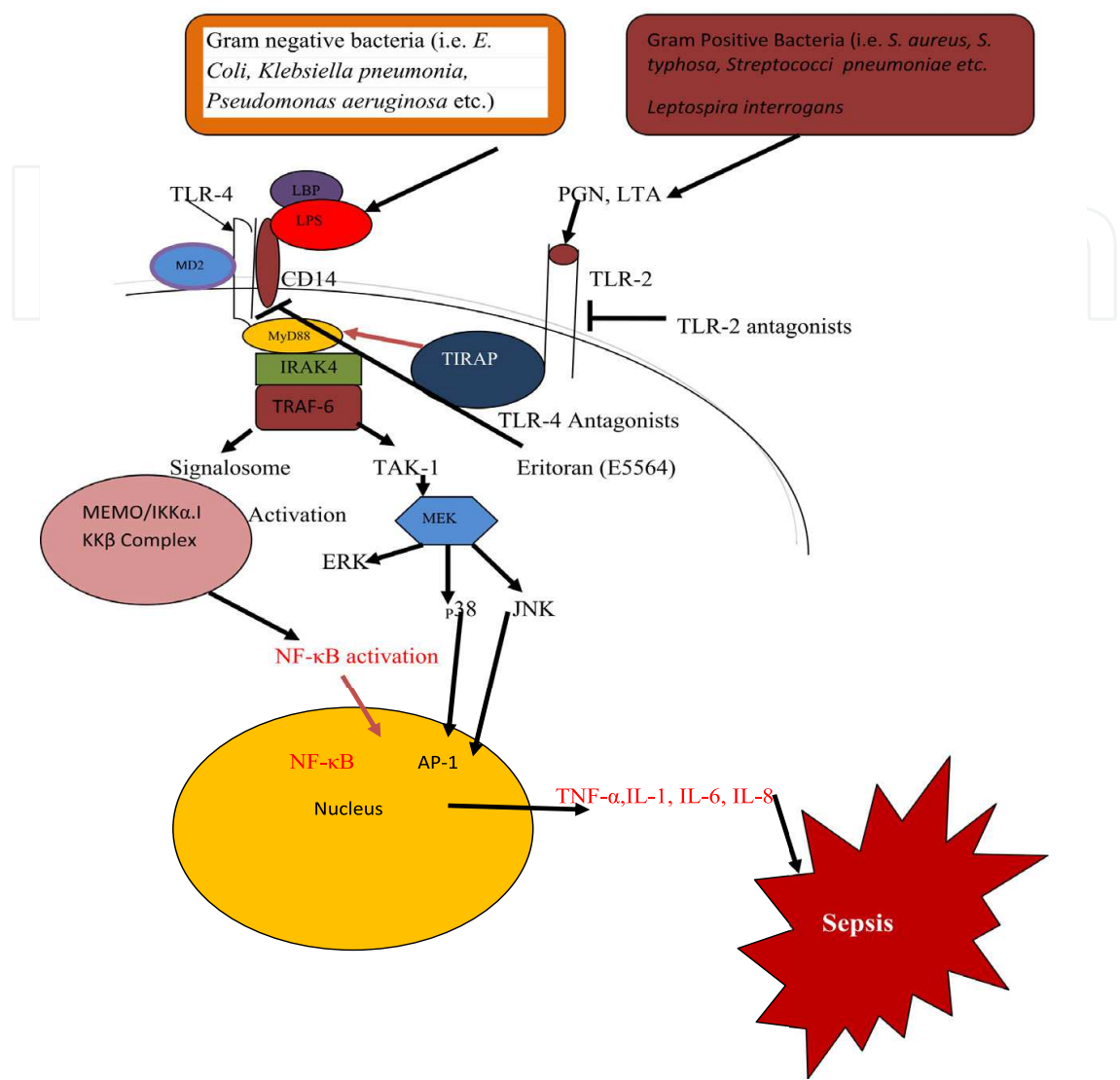


Fig. 1. Role of TLRs in sepsis pathogenesis and their inhibition of its management.

In Gram-negative bacteria (i.e. *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* etc.), LPS plays a major role by acting as a microbial associated molecular pattern. Having withstanding or by tolerating the host's local immune defense these gram negative bacteria enter the blood stream. Once the LPS gets released from the bacteria into the blood it binds to LPS-binding protein (LPB), which then delivers it to CD14 (a 55kDa cell-surface molecule) present on cells of myeloid origin i.e. monocytes and macrophages (Wright et al., 1990). CD14 is linked to glycosylphosphatidylinositol (GPI) on the cell surface and is not bound by transmembrane domains. Therefore, it requires formation of trimolecular receptor cluster with Toll-like receptor 4 (TLR-4) and the accessory protein MD-2 (Shimazu et al., 1999), for transmitting signal to innate immune cells leading to hyperactivation of innate immune response (Akashi et al., 2000) (**Fig. 1**). But several studies have also shown the CD14 independent activation of TLR4 receptors on innate immune cells by LPS (Lynn et al., 1993; Triantafilou et al., 2000) and that the monoclonal antibodies blocking CD14 do not inhibit LPS-induced TNF-α secretion, which confirms the existence of some alternative pathways of



LPS recognition by TLR4 (Gessani et al., 1993). Recently, a study by Brown et al (2011) has shown that Platelet cells (do not express CD14 but express TLR-4 receptors) are activated by LPS stimulation and release pro-inflammatory IL-1 $\beta$  rich microparticles, which also contributes to exaggerated immune response observed during sepsis and promotes endothelial cells activation. This accumulating evidence suggests that the CD14-MD2-TLR4 model of LPS recognition is an oversimplified presentation of LPS recognition by innate immune cells. Thus, various pattern recognition receptors (PRRs) are involved in LPS recognition and in activation of overwhelming innate immune response. The various PRRs involved in pathogenesis of sepsis are listed in Table 1.

Receptors	Immune cells
CD14	Monocytes and macrophages (Wright et al., 1990)
TLR4	Monocytes, macrophages, mast cells, neutrophils, endothelial cells, regulatory cells, platelets (Horenf et al., 2003; Giardin et al., 2003)
TLR2	Myeloid cells, epithelial cell, surface membranes and phagolysomes, mast cells, NK cells, mature DCs and T cells (Giardin et al., 2003; McCurdy et al., 2003; Kumai-Koma et al., 2004)
CD11b/CD18	Monocytes, macrophages, NK cells and neutrophils (Wright et al., 1986)
CD55	Leukocytes (Heine et al., 2001)
TREM-1	Neutrophils, monocytes (Nathan and Ding, 2001))
RP105	B cells (Ogata et al., 2000)
CXCR4	Monocytes, macrophages, neutrophils, T, B, NK, and dendritic cells (DCs), astrocytes and endothelial cells (Triantafilou et al., 2001)
MDL-1	Neutrophils, monocytes and macrophages (Liu et al., 2001; methe et al., 2005)
NOD1 and NOD2	All immune cells (Bouchan et al., 2001; Giardin et al., 2003)
CCR4	Dendritic cells (DCs), macrophages, NK cells, Platelets, Basophils, T cells (Giardin et al., 2003)

Table 1. Pattern recognition receptors involved in sepsis development.

3.1 Role of Toll like receptors in sepsis pathogenesis

Toll-like receptors are evolutionary conserved proteins expressed by innate immune cells in vertebrate immune system. Toll is a transmembrane receptor, which was first identified as an essential component in dorsal-ventral embryonic development in Drosophila (Gobert et al., 2003). Until now, 11 TLRs have been identified in mammals (Zhang et al., 2004) but only 10 are found in humans and have highly conserved intracellular TIR domain playing an important role in protein-protein interaction and signaling activation. The extracellular domains of TLRs which are involved in recognition of PAMPs contain leucine-rich repeats (LRRs). Although LRRs vary, and how they recognize differences between different PAMPs is not clear.

TLR-4 plays a key role in the onset of sepsis syndrome. Initial studies in C3H/HeJ and C57BL/10ScCr mice have shown that these strains are resistant to sepsis development as they have mutated TLR4 genes. This is further confirmed clinically where individuals exhibiting the missense mutations (Asp299Gly and Thr399Ile) affecting extracellular domains of TLR4 are resistant to sepsis development (Arbour et al., 2000; Lorenz et al., 2001; Schwartz et al., 2002). Thus, these studies prove importance of TLR-4 activation in sepsis development. Once the CD14-MD2 TLR-4 complex is formed, the stimulatory signals are transmitted from cell membrane to the cell's internal environment through MyD88 (O'Neil, 2000). This MyD88 pathway leads to recruitment of IL-receptor (IL-R) associated kinase (IRAK) isoforms i.e. IRAK4 (Suzuki et al., 2002), tumor necrosis factor (TNF) receptor-associated factor-6 (TRAF-6) and transforming growth factor-activated kinase-1 (TAK-1) (Zhang et al., 1999; Lomaga et al., 1999; Lee et al., 2000). These events activate signalosome (MEMO/IKK $\alpha$ /IKK $\beta$  complex) and subsequently allow the entry of nuclear factor- $\kappa$ B (NF- $\kappa$ B) into the nucleus and transcription of various pro-inflammatory cytokine genes (i.e. TNF- $\alpha$ , IL-1, IL-6 and IL-8 etc.) (**Fig.1**). But ligation of TLR-4 also recruits an additional adaptor molecule called TIR domain-containing adapter-inducing interferon- $\beta$  (IFN- $\beta$ ; TRIF) (Yamamoto et al., 2003; Hoebe et al., 2003). Thus, this pathway further synergizes the earlier pathway and leads to the release of pro-inflammatory cytokines (i.e. TNF- $\alpha$ , IL-1, IL-6 and IL-8 etc.) along with interferon- $\beta$  (IFN- $\beta$ ) and up regulates IFN- $\beta$  dependent genes i.e. IFN-inducible protein 10 (IP-10) and inducible nitric oxide synthase (iNOS).

Yamamoto et al (2003) showed that TLR-4 recruits another adaptor molecule called TRIF-related adaptor molecule (TRAM) which is involved in MyD88 independent pathway. Thus, involvement of specific adaptor molecules in the TLR4 pathway made this innate immune response more specific to particular PAMP. Together with Gram-negative sepsis, the incidence of Gram-positive bacterial sepsis (i.e. *Staphylococcus aureus*) has also been increased. The PAMPs associated with these bacteria are lipoproteins, lipoteichoic acid (LA), and peptidoglycan (PGN) which, act as a ligand for TLR2. The binding of LA or PGN to TLR2 leads to activation of TIRAP and subsequently MyD88 which follows the downstream pathway of pro-inflammatory cytokine release similar to TLR4 (**Fig.1**). Werts et al (2001) have shown that LPS from *Leptospira interrogans* stimulates innate immune cells and hence the release of pro-inflammatory cytokines via binding to TLR2. However, TLRs act as essential innate immune receptors which sense the presence of foreign invading bodies and send signals to the immune system about the presence of dangers but their increased and uncontrolled activation leads to development of sepsis syndrome (**Fig. 1**).

#### 4. Host factors beyond TLRs responsible for recognizing and responding to bacterial components

Why the pathogenesis of sepsis is so complicated can be understood by the observation that TLRs are not the only mediators of this overwhelming immune response but some other host factors are also involved in its pathogenesis which make sepsis development pathway more complex and devastating to the host. For example, peptidoglycan-recognition proteins (PGRPs) were first discovered in moths and this led to their subsequent discovery in *Drosophila* (Werner et al., 2000) and humans (Liu et al., 2001). Triggering receptors expressed on myeloid cells (TREM-1) and myeloid DAP-12 associated lectin (MDL-1) are newly recognized and are expressed on human neutrophils and monocytes. TREM-1 shows enhanced expression in the presence of different microorganisms and upon LPS exposure (Bouchan et al., 2000). Thus, it plays an important role in inflammatory response to LPS

during sepsis development. A study by Bouchan et al (2001) has shown that TREM-1 Ig Fc fusion protein competes for TREM-1 ligand and results in lowering of serum TNF- $\alpha$  and IL-1, protecting LPS-exposed mice from death. Hence, this blocking of TREM-1 stimulated pro-inflammatory cytokine release is an important immunomodulatory therapeutic approach if these findings can be reproduced in clinical settings.

Nod-like receptors (NLRs) are intracellular microbial sensing proteins and the first NLRs discovered for their role in recognizing pathogens intracellularly were NOD1 and NOD2. NOD1 and NOD2 are cytosolic receptors, which recognize D- $\gamma$ glutamyl-meso-DAP (mDAP) and muramyl dipeptide (MDP), both are the subcomponents of peptidoglycan (PGN) as well as LPS of gram negative bacteria (Giardin et al., 2003). More specifically, NOD2 recognizes a minimal motif of muramyl dipeptide (MDP) called GlcNAc-Mur-NAc-dipeptide that is found in all PGNs, while NOD1 recognizes muropeptides (iE-DAPs) or unique diaminopimelate-containing N-acetylglucosamine-N-acetylmuramic acid (GlcNAc-MurNAc) which are found in the PGN of gram negative bacteria and only some gram positive bacteria (Inohara et al., 2001; Giardin et al., 2003; Elinav et al., 2011). N-glycosyl muramyl dipeptide from mycobacteria and viral ssRNA also act as additional ligands for NOD2 (Coulombe et al., 2009; Sabbah et al., 2009.)

Structurally, NOD1 and NOD2 are tripartite domain containing molecules which comprised of: (1) N-terminal pyrin domain (PYD) or caspase recruitment domain (CARD) and regulate homotypic or heterotypic binding. (2) the nucleotide-binding domain (NBD) which follows the effector domain (3) the c-terminus, comprising of a series of leucine-rich repeats (LRR) and binds to bacterial LPS or PGN in a similar manner to CD14 and TLRs (Tschopp et al., 2003), thus playing an important role in ligand binding and autoregulation (Chamillard et al., 2003). NOD1 and NOD2 activate NF- $\kappa$ B through the recruitment and oligomerization of receptor-interacting protein (RIP) 2 or RIP-like interacting CLARP kinase (RICK) and CARD-containing ICE-associated kinase (CARDIAK), which results in activation of I $\kappa$ B kinase complex (Bertin et al., 1999; Ogura et al., 2001). Recently, Cartwright et al (2007) have also shown that NOD1 agonist FK 565 causes shock and organ dysfunction even in TLR4<sup>-/-</sup>, 154TLR2<sup>-/-</sup>, or MyD88<sup>-/-</sup> mice, emphasizing the importance of NOD1 in sepsis development. Hence, NLRs, especially NOD1 and NOD2, are emerging as intracellular PRRs which sense bacteria and bacterial products intracellularly and synergize TLRs in an overwhelming and uncontrolled innate immune response which leads to the development of sepsis.

Many studies have shown that ligands of NOD1 and NOD2 synergize with many TLR ligands, which also include TLR2 ligands for the release of TNF- $\alpha$  and IL-12 p40 42-44. However, analysis of IL-12 production by human DCs revealed that NOD and TLR can also act in an antagonistic manner since combined stimulation of NOD2 and TLR2 resulted in decreased production of IL12p70, whereas NOD2 activation increased IL12p70 production along with stimulation of other TLRs i.e. TLR7 and TLR 8. Watanabe et al (2004) have also shown an increased production of cytokines by TLR2 ligands, whereas other TLR ligands failed to produce inflammatory cytokines in mice deficient in NOD2 as compared to wild-type mice. Thus, much work is required to elucidate proper molecular signaling pathways involved in TLR2 and NOD2 interaction leading to development of exaggerated systemic inflammatory immune response during sepsis.

## 5. Complement system and sepsis

The complement system is another part of the innate immune system which acts as a potent protective factor against invading pathogens leading to increased production of C5a, which



can actually cause an impaired immune response. The complement system was first discovered or recognized by famous microbiologists and Immunologists namely, Paul Ehrlich, Jules Bordet and George Nuttall, when they found the bactericidal function of blood component against Anthrax bacilli (Nuttall 1888; Bordet, 1895; Bordet, 1898; Ehrlich and Morgenroth, 1899). These workers found that bactericidal function of that component of blood was inhibited when blood was heated up to 55°C or kept at room temperature, and they called that component “alexin”. However, in 1899 Paul Ehrlich renamed alexin as complement and pronounced it as the heat-stable substance, amboceptor (Ehrlich and Morgenroth, 1899). The complement system has three different amplification pathways through which it acts: 1) classical, 2) alternative, and 3) lectin-binding pathway. All three pathways converge at the level of complement factor called C3 and lead to synthesis of cleavage products i.e. C3a, C3b, C5a, C5b and C5b-C9 or membrane attack complex (MAC). The complement system plays an important role in sepsis development and multiorgan dysfunction syndrome (MODS) associated with sepsis (Bangston and Heidman, 1988; de Boer et al., 1993; Nakae et al., 1994; Fierl et al., 2006). The classical pathway is activated by antigen-antibody complexes (Reid and Porter, 1988; Muller-Eberhard, 1988), but it is also observed that C-reactive protein (CRP), viral proteins, beta amyloid proteins, polyanions (bacterial lipopolysaccharides, DNA and RNA) as well as mitochondrial fragments, necrotic/apoptotic cells and amyloid P are also able to activate classical pathway (Gewurz et al., 1993; Barrington et al., 2001; Gasque, 2004; Thurman and Holers, 2006). While, the alternative pathway comes in action by surface sugars and endotoxin molecules of bacteria along with protein A, C-reactive protein (CRP), cobra venom factor and damaged tissue (Reid and Porter, 1988; Muller-Eberhard, 1988; Gasque, 2004; Ganter et al., 2007). The “mannan binding lectin” pathway also recognizes Gram-negative bacterial oligosaccharides or lipopolysaccharides (LPS) and activates the complement pathway (Fujita, 2002). Zhao et al (2002) have shown that the O-antigen region of LPS activates the complement pathway via the lectin pathway and contributes to sepsis. However, Dahlke et al (2011) have shown an important role of alternative complement pathway in the contribution of host's innate immune response during sepsis when it is compared to classical complement pathway. This is because they found that despite normal bacterial clearance capacity early during the onset of sepsis, alternative complement knockout (*fd<sup>-/-</sup>*) mice showed increased inflammatory cytokine levels and neutrophil recruitment into the lungs and blood when compared with wild type (WT) control of classical (*C1q<sup>-/-</sup>*) mice. Thus, alternative complement pathway also plays an important role in sepsis pathogenesis.

## 6. C5A in sepsis immunopathogenesis

The increased levels of C5a are now considered as “too much of a good thing” (Gerard, 2003) and “the dark side in sepsis” (Ward, 2004). The higher concentration of C5a is found both in experimentally induced sepsis in animals as well as in humans suffering from sepsis (Bangston and Heidman, 1988; Smedegard et al., 1989; de Boer et al., 1993; Nakae et al., 1994; Huber-Lang et al., 2001; Ward, 2010). C5a is not only generated from systemic activation of the complement system but may also be produced by serine proteases produced by activated macrophages and neutrophils, which directly cleaves the C5 into C5a (Sacks et al., 1978; Huber-Lang et al., 2002). Upon its release into circulation, C5a binds to its corresponding receptors, i.e. C5aR (CD88) and decoy receptor (C5L2), and exerts its pro-inflammatory and tissue damaging effects (Shin et al., 1968; Goldstein et al., 1974). C5a

receptor expression on neutrophils and in lungs, liver, kidneys and heart increases during sepsis and contributes to multiple organ failure during sepsis (Hoesel et al., 2007; Ward, 2008). However, activated C5a may also lead to immune paralysis along with thymocyte apoptosis (Guo, 2000; Riedemann et al., 2002) (Fig.2). Recent findings have suggested that the decoy receptor C5L2 can also mediate the biological action of C5a and C3a via mitogen activated protein kinase (MAPKs) activation (Chen et al., 2007) and the loss of C5L2 in blood neutrophils mediates sepsis-induced lethality (Rittirsch et al., 2008) (Fig.2).

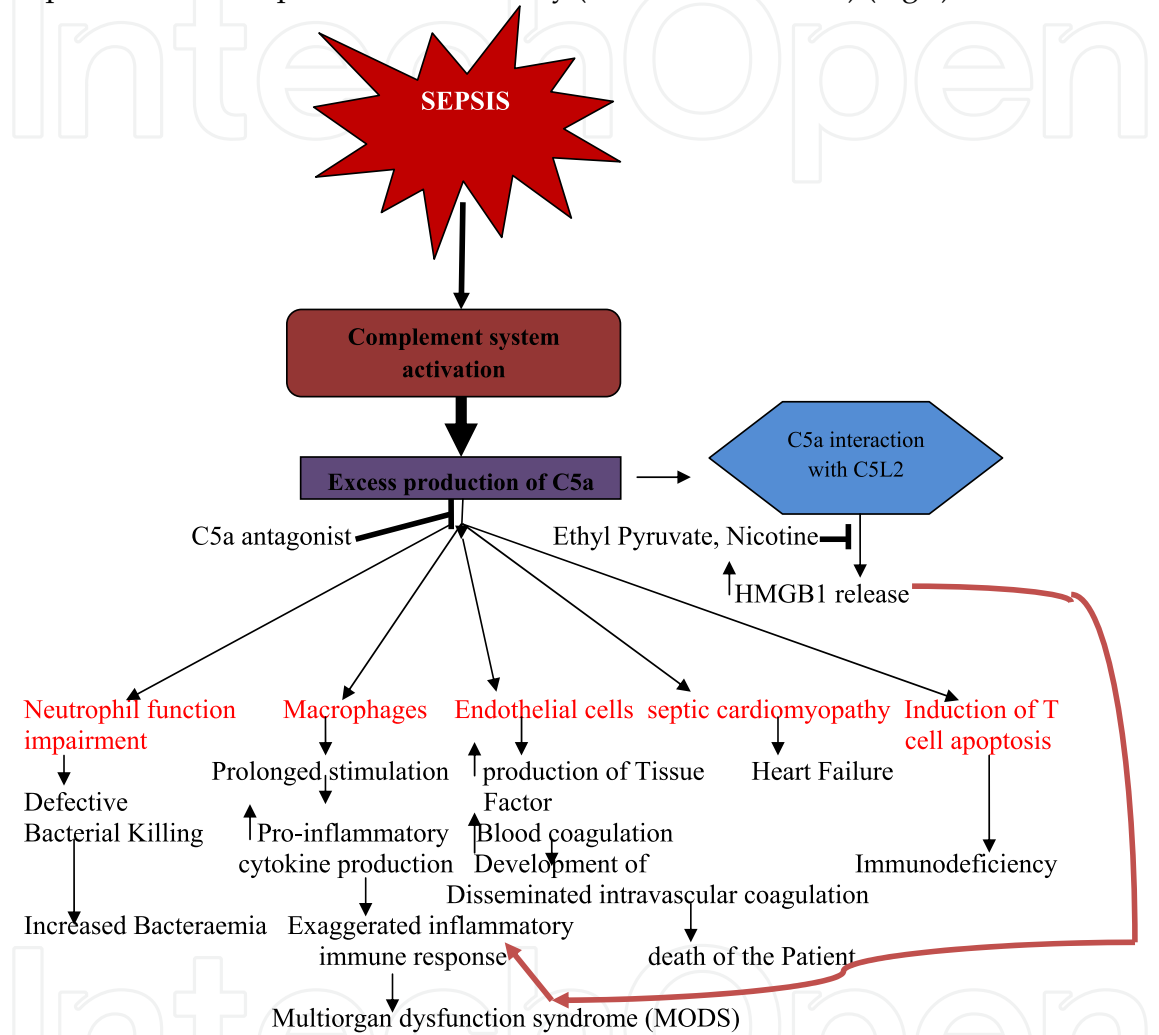


Fig. 2. Role of complement factor C5a in sepsis pathogenesis and its inhibition. HMGB1 is a late stage inflammatory mediator of sepsis pathogenesis and its inhibition (i.e. Ethyl Puruvate and Nicotine) helps in decreasing the sepsis associated mortality during experimental sepsis.

Rittirsch et al (2008) showed that C5aR and C5L2 in cooperation with each other provide an important role in the pathogenesis of sepsis. This is because they have found a finding that C5aR and C5L2 gene knockout mice have an enhanced survival rate compared to wild type mice challenged with cecal ligation and puncture (CLP)-induced sepsis (Rittirsch et al., 2008). They have also shown a link between C5L2 and high-mobility group box-1 protein (HMG-B1) and proved that the release of HMGB1 from dying cells during sepsis requires the active participation of C5L2 (Fig.2). While, binding of C5A to C5AR leads to release of macrophage migration inhibition factor (MIF) from phagocytes (i.e. Neutrophils)

(Riedemann et al., 2004). C5a activates endothelial cells and induces the expression of adhesion molecules (i.e. ICAM, VCAM) causing vasodilation (Schumacher et al., 1991). In addition C5a leads to increased production of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 (Strieter et al., 1992; Hopken et al., 1996) from human leukocytes and in synergy with LPS it also stimulates production of macrophage inflammatory protein-2 (MIP-2), cytokine induced neutrophil chemoattractant-1 (CINC-1), in addition to other pro-inflammatory cytokines (Guo et al., 2004). C5a also increases the coagulation cascade by increasing the tissue factor expression on endothelial cells and monocytes, thus contributing the induction of disseminated intravascular coagulation during sepsis pathogenesis (Muhlfelder et al., 1979; Carson et al., 1990; Ikeda et al., 1997). C5a also plays a major role in the septic cardiomyopathy (Niederbichler et al., 2006) (**Fig.2**).

Inhibition of C5a activity by anti-C5a antibody in the rat model of sepsis ameliorated the coagulation or fibrinolytic protein changes as well as disseminated intravascular coagulation (Laudes et al., 2002). Also Flierl et al (2008) found that in the absence of either C3 or C5 very low levels of pro-inflammatory mediators were observed in experimental animals challenged with sepsis. This data suggests that complement system activation plays an important role in the pathogenesis of sepsis and sepsis related induction of MODS. However, no clinical data is available for the use of C5a antagonistic antibody in clinical trials for the management of sepsis in septic patient. But the blockade of C5a activity in experimental set up has provided beneficiary effect to septic animals and increased their survival (Guo and Ward, 2006), so more studies are required for designing better molecules for targeting exaggerated tissue damaging activity of C5a.

## 7. Toll like receptor and complement system crosstalk in sepsis pathogenesis

To respond efficiently against pathogens or some other danger signals host's complement system uses both pattern recognition receptors (PRRs) and missing self recognition strategies (Hajishengalis and Lambris, 2010). For example, complement system coordinates innate immune system with TLRs to curtail the infection and spread of infectious agent by augmenting coagulation (Markiewski et al., 2007). Both complement and TLRs get swiftly activated in response to pathogens or their pathogenic components (i.e. LPS, PGN or microbial CpG DNA) (Ricklin et al., 2010). Complement system synergizes the TLR-induced production of pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$  and IL-6) *in vitro* and *in vivo* through C3aR, and more profoundly through C5aR signaling, thus, leading to more pronounced inflammatory immune response observed during sepsis (Zhang et al., 2007). TLR-4, TLR-2 and TLR-9 all are involved in potential crosstalk with complement system and converge at the level of anaphylatoxin signaling through the signaling molecules called mitogen activated protein Kinases (MAPKs) more specifically Erk1/2 and Jnk (Zhang et al., 2007). That may explain, why inhibiting C5a signaling protects animals from sepsis, induced by high doses of LPS or CLP (Guo et al., 2004).

C5a and TLR crosstalk involves C5aR as well as G-protein-independent C5L2, which may have both regulatory and pro-inflammatory roles (Chen et al., 2007; Hajishengalis and Lambris, 2008; Rittrisch et al., 2008). C5L2 induces HMGB1 release and contributes synergistically with C5aR to exaggerated inflammatory damage in CLP induced sepsis (Rittrisch et al., 2008). According to an *in vitro* study, induction of HMGB1 by C5a or LPS (or with their combination) is diminished in C5L2-/- but not C5aR-/- macrophages (Rittrisch et al., 2008). These studies suggest that, cooperation of C5L2 and TLR-4 crosstalk involves MAPK and phosphatidylinositol-3 pathways. C5L2 and TLR4 might also cooperate in the

induction of HMGB1 (Rittrisch et al., 2008). Alternatively, C5L2 also act as a co-receptor for TLR4 activation (Ricklin et al., 2010). A previous study has also indicated that complement derived C5a anaphylatoxin negatively regulates LPS-induced production of IL-12 family cytokines by macrophages, along with decrease in Th1 mediated immune response (Hawlich et al., 2005). Mice deficient in C5a receptor showed resistance against *Leishmania major* infection (Hawlich et al., 2005). C5a augments the release IL-6 from LPS-stimulated neutrophils *in vitro* while, blockade of C5a reduced IL-6 levels in septic rats (Riedemann et al., 2004). In addition to LPS, lipoteichoic acid (LTA), a TLR-2 agonist also induced complement activation with C5a generation in the human lung (Hoogerwerf et al., 2008). While, zymosan, a fungal TLR-2 agonist activated the complement system *in vivo* in an experimental septic peritonitis rat model (Mizumo et al., 2009). Another study by Zhang et al (2007) has also showed that complement activation augmented the pro-inflammatory cytokine mediated immune response to various TLR agonists in mice. Recently, Kaczorowski et al., (2010) showed the LPS and Poly I:C both up regulated the expression of factor B, a component of the alternative complement pathway. Thus, microbial metabolites and their products have a capability to induce exaggerated inflammatory cascade via activating cross talk between complement system and pattern recognition receptors (PRRs).

## 8. Cytokines in sepsis pathogenesis

Pro-inflammatory cytokines released due to activation of PRRs during sepsis serve as molecular messengers and result in the development of a constellation of clinical signs and symptoms characterizing the onset of sepsis. For example, TNF- $\alpha$  is a prototype mediator of sepsis and septic shock as its increased concentration in the bloodstream results in cardiovascular collapse (Van der Poll and Lowry, 1995). TNF- $\alpha$  is an important mediator of sepsis and multiorgan dysfunction syndrome which develop during sepsis as administration of TNF- $\alpha$  causes shock, hypotension, intravascular coagulation, hemorrhagic necrosis and organ failure in sepsis (Tracey and Cerami., 1994; Hotchkiss and Karl., 2003) (Figures 1 and 2). IL-1 is another pro-inflammatory cytokine which binds to IL-1R and results in activation of NF- $\kappa$  B and thus causes further increased release of pro-inflammatory cytokines during sepsis (Matsuda et al., 2006)

**High-mobility group box 1 protein (HMGB1)** has recently been identified as a late mediator of sepsis (Wang et al., 1999; Yang et al., 2001). It is known as late mediator of sepsis as macrophages release HMGB1 ~20 hour after activation and serum HMGB1 levels can become detectable 20-72 hours after sepsis development (Ombrellino et al., 1999; Czura et al., 2003). HMGB1 reaches the extracellular environment by its passive release after necrotic cell death or by active secretion from activated innate immune cells (Gardella et al., 2002; Rendon Mitchell et al., 2003; Erlandsson et al., 2004). The active secretion of HMGB1 from monocytes and macrophages occurs in response to inflammatory stimuli like LPS and TNF- $\alpha$ , IL-1 $\beta$  and IFN- $\gamma$ . Membrane bound HMGB1 binds to receptor for advanced glycation end product (RAGE) with a very high affinity. RAGE promotes leukocyte migration to inflamed tissue and its deletion in murine model of sepsis prevented the septic animals from lethality (Chavakis et al., 2003; Liliensiek et al., 2004). Along with these cytokines macrophage migration inhibitory factor (MIF) also plays an important role in the pathogenesis of sepsis and studies have indicated that mice with disrupted MIF gene are resistant to sepsis induced by LPS (Bozza et al., 1999). It was the first cytokine to be discovered for having a potential role in the pathogenesis of systemic as well as local inflammatory immune response (Calandra and Rogers., 2003). MIF also plays an important role in the pathogenesis



of Gram positive bacteria mediated sepsis i.e. toxic shock syndrome associated with *Staphylococcus aureus* (Calandra et al., 1998). Along with bacterial endo- and exotoxins other pro-inflammatory molecules like TNF- $\alpha$ , IFN- $\gamma$  and C5a are potent stimulators for the release of MIF from leukocytes or Immune cells (Calandra and Rogers., 2003; Riedemann et al., 2004). However, the pro-inflammatory activity of MIF is mediated by its tautomerase activity (i.e. ability to induce tautomerization), which is encoded by a domain containing an evolutionarily conserved catalytic site (Lubetsky et al., 2002). In addition to this it also amplifies the inflammation by stimulating the secretion of other pro-inflammatory cytokines, up regulating the expression of TLR-4 on immunological cells playing active role in the pathogenesis of sepsis along with suppressing the p53-dependent apoptosis of activated macrophages leading to sustained systemic inflammation at its higher concentration (Calandra and Rogers., 2003). Thus, targeting MIF can prove as an effective immunomodulatory target for sepsis management.

### 8.1 Earlier approaches for sepsis management

Corticosteroids were one of the most earliest used drugs of choice among patients suffering from sepsis and acute respiratory distress syndrome. However, several follow up clinical trials did not show any significant benefit in patients suffering from sepsis (Lefering et al., 1995), although a decrease in serum TNF- $\alpha$  and IL-6 levels of patients was observed those taking methylprednisolone. Thus, both the timing and doses of corticosteroid treatment are important for successful treatment of sepsis. LPS also plays a major role in the pathogenesis of sepsis but the clinical trial with anti-LPS antibody failed (Cohen, 1999). TNF- $\alpha$  is major cytokine involved in sepsis development but clinical trials comprising molecules inhibiting TNF- $\alpha$  failed and were not beneficial in the treatment of human sepsis (Reinhart et al., 2001). IL-1R inactivation with a recombinant IL-1R antagonist reduced mortality in an animal model of sepsis (Ohlsson et al., 1990) but the first human clinical trial of this IL-1R antagonist failed and did not show beneficial effects in human cases of sepsis (Opal et al., 1997).

Besides these, other anti-inflammatory therapies used in sepsis comprise platelet-activated factor (PAF) inhibitors, inhibitors of arachidonic acid metabolism pathway, and bradykinin pathway etc. For example, clinical trial for synthetic antagonists of PAF receptors (i.e. BN52021 (Ginkgolide B), TCV-309 and BB-882 (Lexipafant) was performed among 1,279 patients and a non significant decrease in mortality among septic patients was recorded (Placebo 51.5% and PAF receptor antagonists, 48.4%) (Dhainaut et al., 1994; Froom et al., 1996; Dhainaut et al., 1998; Poeze et al., 2000; Supottamongkol et al., 2000; Vincent et al., 2000). Interferon- $\gamma$  (IFN- $\gamma$ ) and granulocyte colony stimulating factor (G-CSF) have also been given to septic patients with little success in terms of survival (Vincent et al., 2001). To date drotrecogin alpha (recombinant activated Protein C) is the only drug which has been approved by the US FDA for treatment of patients with sepsis and associated high risk of death (Reidman et al., 2003)

### 8.2 Targeting Innate Immune system as a future Immunomodulatory approach for sepsis management

The picture presented above shows that current approaches to the treatment of sepsis have not worked effectively. Also, the inexorable rise of antibiotic resistance among bacterial species causing sepsis accompanied by a decrease in new antibiotic discoveries have made our healthcare system very helpless in terms of sepsis treatment. Thus, there is no doubt that we need effective drug targets and treatment opportunities to overcome these limitations. The development of sepsis is a consequence of increased and deleterious response of the



innate immune system against invading pathogens, this indicates that the innate immune system has evolved to contend with pathogens and not to develop sepsis. As a clinical problem, sepsis reflects an unusual situation in which the intolerable burden of bacterial pathogens causes increased activation of the innate immune system and damages the host. Thus, modulation of the innate immune response during sepsis in the proper direction could be used as a novel target for sepsis treatment and represents an important approach to the development of future sepsis therapeutics.

Cationic antimicrobial peptides are a class of anti infective modulators, which function independently of TLRs. These peptides have potent antimicrobial activity against pathogens with a potent tendency to modulate the innate immune system (Hancock et al., 2006). For example, antimicrobial peptide LL-37 neutralizes LPS both *in vitro* as well as *in vivo* leading to protection in animals against development of endotoxemia (Gough et al., 1996; Marra et al., 1990). Earlier, it was assumed that LL-37 directly binds to LPS and neutralizes. However, it is not true as it binds to CD14 and prevents development of sepsis (Nagoka et al., 2001). Bactericidal/permeability increasing protein (BPI) is another antimicrobial peptide which is effective against Gram-negative bacteria as well and also inhibits LPS-induced pro-inflammatory cytokine release (Weiss et al., 1984; Marra et al., 1990). Recombinant BPI<sub>21</sub> or rBPI<sub>21</sub> is effective in the treatment of sepsis in various murine and rat models (Jiang et al., 1998; Jiang et al., 1999). The initial phase I and II clinical trials of rBPI<sub>21</sub> conducted in pediatric patients suffering from meningococcal sepsis indicate that it can be used in pediatric sepsis patients (Bowdish et al., 2005). In phase III clinical trial, it also proved beneficial and has moderately improved the mortality rate with patients showing moderate improvement and requiring fewer amputations (Levin et al., 2000). Thus, rBPI<sub>21</sub> can be used as an adjunct therapy with standard antibiotic therapies during sepsis treatment to prevent sepsis-induced organ damage and amputation. In collaboration with investigators at University of Texas Southwestern medical Center, XOMA Ltd. is currently conducting a clinical trial of rBPI<sub>21</sub> for the treatment of patients with severe burn injury and sepsis (Hirsch et al., 2008). However, data regarding the levels of host defense peptides in human sepsis is very scarce. For example, Book et al (2007) reported a threefold increase in systemic plasma levels of human  $\beta$  defensin 2 in septic patients as compared with healthy control subjects. So more study is required in this field of host defense peptides and sepsis pathogenesis.

TLR4 plays a major role in recognition of LPS and downstream signaling leading to development of sepsis. Thus, decreasing or antagonizing the activity of TLR4 may be helpful in decreasing mortality associated with sepsis (Figure 1). Eritoran (E 5564) is a TLR4 specific antagonist and has been shown to be effective in human volunteers with sepsis (Lynn et al., 2003; Savov et al., 2005). This antagonist is now under phase II clinical trial (115). Ethyl (6R)-6-[N-(2-chloro-4-fluorophenyl) sulfamoyl] cyclohex-1-ene-1-carboxylate (TAK-242) inhibited TLR4-mediated cytokine production through suppression of intracellular signaling and is under preclinical investigation (Li et al., 2006).

Statins are a class of drugs which, inhibit 3-hydroxy- 3-methylglutaryl coenzyme (HMG-CoA) and are used to treat hypercholesterolemia but they also show immunomodulatory properties. Methe et al (2005) have shown that these agents decrease the TLR4 receptor expression on CD14<sup>+</sup> monocytes and macrophages and thus, the downstream signaling involved in sepsis. Pahan et al (1997) have shown the inhibitory effect of statin therapy on the release of TNF- $\alpha$  and IL-1 from macrophages and microglial cells. In addition, statins modify leukocyte-endothelial cell interactions by down regulating the expression of leukocyte function-associated antigen (LFA-1), CD11a and CD18. They also alter the binding capacity of LFA1 to ICAM-1 (Lee et al., 1999; Greenwood et al., 2006). Recently, a

randomized controlled trial for statin therapy is also done in patients with presumed infections (Kruger et al., 2011). Thus, statin therapy is capable of preventing both cytokine and neutrophil-induced tissue damage observed in sepsis and can be used as an adjuvant in the treatment of sepsis.

NF- $\kappa$ B is a major nuclear transcription factor, which is associated with the synthesis and release of various pro-inflammatory cytokines along with the expression of various adhesion molecules. Therefore, pharmacological inhibitors of NF- $\kappa$ B have been evaluated in murine and rodent models of sepsis and endotoxemia. Matsuda et al (2006) tested decoy oligonucleotides (ODNs) directed against NF- $\kappa$ B on inflammatory gene over expression and pulmonary derangements in mice with sepsis and they found an improved outcome with significant reduction in sepsis mediated acute lung injury (ALI). Pretreatment of septic animals with Pyrrolidine dithiocarbamate also prevented LPS-induced increased TNF- $\alpha$ , COX-II and adhesion molecules involved in neutrophil sequestration to various organs and decreased the mortality among septic animals. Pharmacological inhibition or genetic deletion of glycogen synthase kinase-3 $\beta$  down regulated the NF- $\kappa$ B DNA binding and expression of NF- $\kappa$ B dependent genes (Demarchi et al., 2003; Takada et al., 2004). GSK-3 $\beta$  inhibitors (i.e. TDZD-8, SB216763 and SB415286) proved beneficial to experimental animal model of sepsis (Dugo et al., 2005).

Since HMGB1 is a late mediator of sepsis, targeting HMGB1 after the onset of sepsis can be a useful treatment option. This can be explained as experimental studies have shown that other anti-inflammatory strategies worked only when they were administered very early or at the initial stages of sepsis development. Thus, drugs inhibiting HMGB1 may be a better option for treating patients with advanced and later stages of sepsis. Ethyl pyruvate is an important inhibitor of HMGB1 and improved the survival of mice when administered 24 hours after the onset of sepsis (Ulloa et al., 2002). Nicotine, by acting as cholinergic receptor agonist, inhibited HMGB1 release in an experimental murine model of sepsis and hence increased their survival (wang et al., 2004). Steroyl lysophosphatidylcholine (LPC) also inhibits HMGB1 in endotoxemic and septic mice, even when administered 10 hours after sepsis development. Steroyl LPC conferred protection against animals suffering from experimental sepsis partly by facilitating the elimination of the causative organism and partly by inhibiting HMGB1 activity (Yan et al., 2004). HMGB1 antagonists (i.e. anti-HMGB1 antibodies, recombinant A box) also proved beneficial in experimental models of sepsis (Yang et al., 2001), thus, HMGB1 inhibition promises as a future immunomodulatory therapy in clinical cases of sepsis.

MIF levels increase significantly during sepsis and play an important role in its pathogenesis and severity. Blockade of MIF for as long as 8 hours after experimental sepsis improved the survival rate of septic mice and its administration increased mortality of mice treated with LPS (Calandra et al., 2000). MIF also regulates TLR4 expression in macrophages (Roger et al., 2001). Thus MIF may be a potential therapeutic target in human sepsis.

Pepducins are newly synthesized lipidated (i.e. palmitic acid) cell-penetrating peptides that act by targeting either individual or multiple chemokine receptors. The hydrophobic group of the lipid group helps the peptide to get inside the lipid bilayer and allows the peptide to interact with receptor at intracellular surface of the plasma membrane (Lomas-Neira et al., 2005). Neutrophils are major innate immune cells and their increased activity during sepsis plays a major role in multiorgan dysfunction (Brown et al., 2006). IL-8 levels during sepsis rise abnormally and activate neutrophils and other inflammatory cells via binding to CXCR2 and CXCR1, thus causing increased infiltration of vital organs neutrophils, which correlates

with shock, lung injury and high rate of mortality (Reutershan et al., 2006). Kanieder et al (2005) have shown that pepducins by blocking the CXCR2 and CXCR1 prevented neutrophil infiltration and related organ damage. Pepducins prevented the mortality among septic mice when given eight hours after cecal ligation and puncture (CLP). As pepducins treatment does not suppress leukocyte trafficking towards other cytokines, its effect can be considered immunomodulatory instead of immunosuppressive. Thus, in the future Pepducins can be used as innate immune system modulators for the treatment of sepsis.

### 8.3 Future perspective

Despite extensive developments in the understanding of the sepsis pathogenesis, it remains one of the leading causes of mortality and morbidity in intensive care units worldwide and presents a major challenge for biomedical scientists involved in sepsis research. The earlier immunosuppressive agents targeting specific pro-inflammatory cytokines have controversial effects as they showed good results in preclinical studies but failed during clinical trials (Fisher et al., 1994; Abraham et al., 2001). Thus it has become important to understand more precisely the basic immunopathogenesis behind sepsis development so as to design better immunomodulatory agents which can be used as future sepsis therapy. The US FDA approval of drotrecogin alpha (recombinant activated protein C) as an antisepsis molecule has boosted a great interest in pharmaceutical and biotechnology companies to investigate the major factors involved in sepsis immunopathogenesis. With great efforts in that short period of time various new targets (i.e. TLR-4, CD14, MyD88, IRAK-1, HMGB1, NF- $\kappa$ B, MIF and C5a) for sepsis management have been discovered. However, inhibitors of these targets worked well in preclinical studies as well as in different phases of clinical trials. Recently, Ramos et al (2010) have also shown that mast cell stabilization provides therapeutic benefits during sepsis by inhibiting the extracellular release of HMGB1 from apoptotic cells and increased the survival of septic animals. Also as at later phase of sepsis there is immune cell depletion due to extensive apoptosis so another potential strategy may involve use of anti-apoptotic cytokines (i.e. IL-7 and IL-15), which have immunostimulatory properties (Opal, 2010). IL-7 has the potential to restore lymphocyte effector function and improves lymphocyte trafficking through increased integrin expression. Thus, this innate immune system based immunomodulatory approach will prove great as innate immune system is the major culprit behind the immunopathogenesis of sepsis and sepsis associated mortality. Thus, a better understanding of innate immune system function in the pathogenesis of sepsis can lead us to identify some novel targets for treating sepsis. But one thing should be kept in mind that innate immune system is a very complex system so precaution (i.e. system biology and translational approach) is needed when modulating or targeting this complex system, to prevent deleterious side effects.

**Abbreviations:** IL-1 Interleukin 1; IL-10 Interleukin 10; TNF- $\alpha$  Tumor Necrosis Factor- $\alpha$ ; CCR2 Chemokine receptor 2; CCR4 chemokine receptor 4; CXCR4 chemokine receptor 4; MIF Macrophage migration inhibitory factor; TLR2 Toll like receptor 2; TLR4 Toll like receptor 4; TREM-1 Triggering receptor expressed on myeloid cells; SIRS Systemic Inflammatory Response Syndrome; PAMPs Pathogen Associated Molecular Patterns; PRRs Pattern Recognition Receptors; LPS Lipopolysaccharide; LTA Lipoteichoic acid; GSK-3 Glycogen Synthase Kinase-3; LFA-1 Leukocyte function associated antigen-1; LRRs Leucine rich repeats; IFN, Interferon; CLP Cecal ligation and puncture; LBP Lipopolysaccharide binding protein; GPL Glycosylphosphatidylinositol; IRAK IL-1 Receptor associated kinase;

TAK-1 Transforming growth factor- associated kinase-1; HMG-B1 High mobility group box-1; NF B Nuclear factor kappa B; RAGE Receptor for advanced glycation end products; BP1 Bacterial permeability/Inhibitory protein 1; CARD Caspase recruitment domain; CARDIAK CARD associated ice-activated Kinase; HMG-CoA 3-Hydroxy-3-Methylglutaryl Coenzyme A; ICAM-1 Intercellular Adhesion Molecule-1; NOD Nucleotide-binding oligomerization domain; TIR Toll-interleukin-1 receptor; PGN Peptidoglycan; MDL-1 Myeloid DAP12-associating lectin-1.

Alarmins are structurally distinct endogenously released mediators which have a great potential to recruit and activate inflammatory cell as well as antigen-presenting cells (particularly dendritic cells) at the site of inflammation, and consequently possess the capacity to enhance innate and adaptive immune responses. These molecules are usually constitutively present in cells, such as leukocytes and epithelial cells (including keratinocytes), as a part of the cell component that can be either in granules, cytoplasm or nucleus. Most alarmins like cytokines can also be induced in response to pro-inflammatory cytokines and pathogen-associated molecular patterns (PAMPs). Unlike cytokines, alarmins are rapidly released by degranulation and/or cell necrosis in response to infection or tissue injury. Alarmins are endogenous peptides that are released in host defense against danger signals. For example,  $\alpha$ -defensins, Lactoferrin, Cathelicidins (i.e. LL-37), High Mobility Group Box-1 (HMG-B1), Granulysin, eosinophil-associated ribonucleases (e.g. eosinophil-derived neurotoxin) are some the well known alarmins.

Box. 1.

## 9. References

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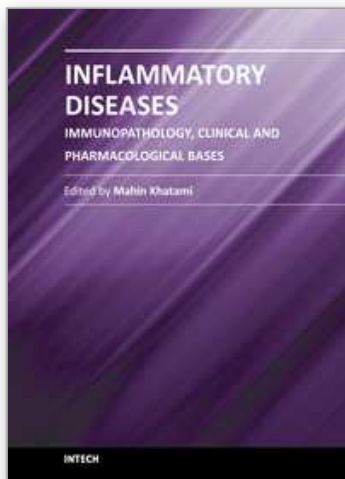
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