

## Review Article

# The role of immunostimulatory nucleic acids in septic shock

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**Abstract:** Sepsis and its associated syndromes represent the systemic host response to severe infection and is manifested by varying degrees of hypotension, coagulopathy, and multiorgan dysfunction. Despite great efforts being made to understand this condition and designing therapies to treat sepsis, mortality rates are still high in septic patients. Characterization of the complex molecular signaling networks between the various components of host-pathogen interactions, highlights the difficulty in identifying a single driving force responsible for sepsis. Although triggering the inflammatory response is generally considered as protective against pathogenic threats, the interplay between the signaling pathways that are induced or suppressed during sepsis may harm the host. Numerous surveillance mechanisms have evolved to discriminate self from foreign agents and accordingly provoke an effective cellular response to target the pathogens. Nucleic acids are not only an essential genetic component, but sensing their molecular signature is also an important quality control mechanism which has evolved to maintain the integrity of the human genome. Evidence that has accumulated recently indicated that distinct pattern recognition receptors sense nucleic acids released from infectious organisms or from damaged host cells, resulting in the modulation of intracellular signalling cascades. Immunoreceptor-mediated detection of these nucleic acids induces antigen-specific immunity, secretion of proinflammatory cytokines and reactive oxygen/nitrogen species and thus are implicated in a range of diseases including septic shock.

**Keywords:** Sepsis, septic shock, immunostimulatory nucleic acid, signaling networks, therapy

## Introduction

Sepsis is a life-threatening disease which develops through the systemic inflammatory response to severe infection. In addition to being triggered by an overwhelming initial response, sepsis is also characterized by hyperactivation of cellular immunity. Neutrophils, macrophages, lymphocytes, and non-professional immune cells produce and respond to a storm of proinflammatory cytokines which include TNF- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-6 and secondary mediators (oxygen and nitrogen species) that further enhance the dysregulated inflammatory network [1-3]. During the early stages of sepsis, the

complement system is a defense mechanism involved in clearing pathogenic organisms and cellular debris. However, the complement activation enhances cytokine and chemokine secretion, and promotes reactive oxygen species (ROS) that ultimately lead to injury at the late stage of sepsis [4]. Although this dynamic response is a key component of this complex condition, recent studies have proposed other molecular mechanisms to explain the substantial heterogeneity which exists in sepsis patients. For example, sepsis-induced apoptosis does not only impair the cellular function of immune and non-immune cells, but may also contribute to both immunosuppression and multiple organ

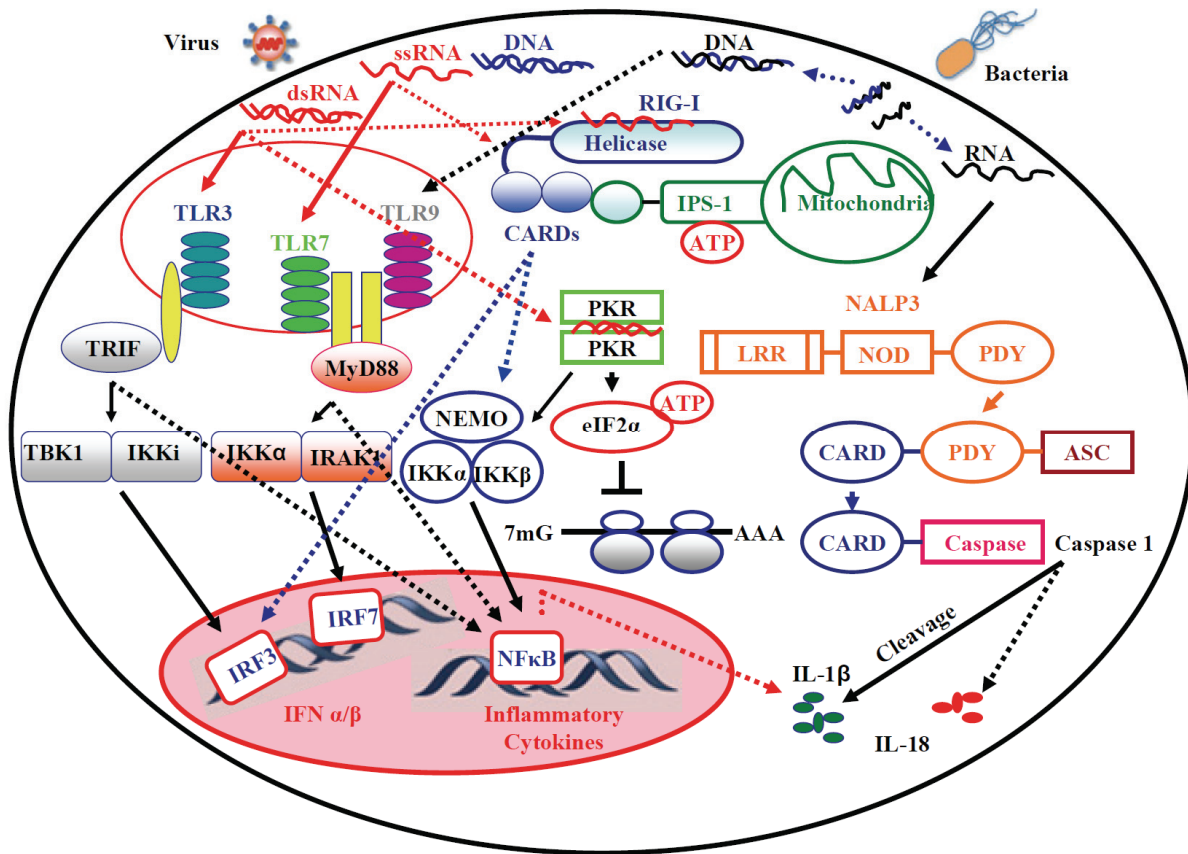
failure that characterizes severe septic patients [5,6]. We previously reported that sera derived from septic shock patients induces apoptosis, activates transcriptional factors Stat1, NF- $\kappa$ B, IRF1, and differentially regulates apoptosis related genes [7-9]. The fine-tuned coordination between the release of proinflammatory mediators and the regulatory anti-inflammatory molecules which is believed to mediate this immunosuppression is a crucial factor in determining the magnitude of the early injury phase and the subsequent risk of complications. While some septic patients die during the early hyperinflammatory stage, high death rates have been also reported in patients displaying prolonged immunosuppression [10-12]. Another level of the complexity correlated with sepsis is the dysregulation of the homeostatic systems including fibrinolysis and coagulation pathways. Hyperactivation of these cascades results in disseminated intravascular coagulation (DIC), depletion of coagulation factors and platelets, which leads to decreased flow rate and hydrostatic pressure of the blood. These conditions will progressively develop to hypoperfusion, hypoxia, ischemia and ultimately multiple organ failure and death [13,14].

Sepsis initiates when the pathogenic insults or their molecular signatures gain systemic access to sterilized human tissues and is detected by pattern recognition receptors (PRRs). Understanding the molecular interactions between the host and pathogens is not only important for exploring the molecular mechanisms underlying septic events, but also in the identification of potential therapeutic targets. Several classes of PRRs are employed by host cells to specifically recognize pathogen associated molecular patterns (PAMPs) and provoke appropriate cellular responses to eliminate pathogenic invaders [15-20]. Sensing the molecular signatures of nucleic acids is also an important quality control mechanism evolved to discriminate self from nonself and in maintaining the integrity of the human genome. In addition to microbial cell wall components and toxins which are considered as principal triggers of early sepsis phases, nucleic acids are now implicated in a range of diseases and syndromes including septic shock. Evidence that has accumulated recently indicated that distinct PRRs sense nucleic acids released from infectious organisms and leads to the activation of intracellular signalling cascades [21-25].

The Toll like receptor (TLR) 3, TLR7, TLR8, and TLR9 detect natural nucleic acids derived from various pathogenic origins and synthetic oligonucleotides. While TLR9 senses unmethylated CpG motifs, which is characteristic of bacterial and viral DNA, viral double-stranded RNA (dsRNA) activates TLR3 and single-stranded RNA (ssRNA) activates TLR7 and TLR 8. TLR-mediated recognition of these immunostimulatory molecules induces antigen-specific immunity, massive secretion of proinflammatory cytokines and interferon, activation of coagulation and the complement cascades, and release of reactive oxygen and nitrogen intermediates [22-24,26-34]. In addition to TLRs, recent reports have identified other cellular receptors such as the nucleotide binding and NOD-like receptors (NLRs) and the RIG-I-like receptors (RLRs) as additional non-redundant surveillance mechanisms which are involved in the detection of various ligands including nucleic acids. For example, while NOD1 and NOD2 sense the cytosolic presence of bacterial peptidoglycan, NALP3 is activated in response to several structurally non-related molecules such as bacterial and viral RNAs, LPS, bacterial toxins, and very recently by microbial and mammalian DNA [35-40]. In addition to the RNA-activated protein kinase (PKR) which has been known as a specific cytosolic sensor for viral dsRNA, the RLRs such as retinoic acid-inducible gene RIG-I and melanoma differentiation-associated gene (MDA) are also key cytosolic receptors involved in the recognition of molecular patterns of pathogenic dsRNA and ssRNA to induce antiviral defence mechanisms [41-46]. Recent studies have also demonstrated that antigens of abundant self nucleic acids released from injured or apoptotic host tissues (named damage associated molecular patterns, DAMPs), activate innate signaling pathways including the TLRs and the complement system to promote the development of autoimmune diseases [47-49]. Here, we review the current knowledge in nucleic acid research in relation to their discrimination, immune activation, and signaling pathways. We further describe the potential of nucleic acids to cause septic shock. See **Figure 1** for a diagram of the cellular immunostimulatory nucleic acid signal transduction.

### The immunostimulatory potential of DNA

The potential of microbial DNA as an immunostimulatory molecule was first identified by



**Figure 1.** Recognition of Pathogenic Nucleic Acids by Pattern Recognition Receptors and Signaling. Upon stimulation, the endosomal TLR3 recognizes viral dsRNA while TLR7 detects ssRNA derived from virus-infected cells. TLR9 recognizes DNA derived from both viruses and bacteria. TLR7 and TLR9 activate the MyD88-dependent pathway to regulate inflammatory responses by activating NF-κB. IRF7 forms a signaling complex with MyD88, IRAK1 and IRAK4 and translocates to the nucleus. TRAF6-dependent ubiquitination is also essential for IRF7 activation that regulates expression of type I IFN together with NF-κB. TLR3 utilizes TRIF but not MyD88, TIRAP/Mal and TRAM for its signaling and interacts with TBK1 to induce IRF3. Activation of IRF3 and NF-κB is required for the induction of type I IFN. In addition to TLRs, the cytosolic RIG1 detects the dsRNA or the 5'-triphosphate ssRNA that have accumulated in the cytoplasm after viral infection as a part of a RNA-quality control mechanism. RIG-I is essentially expressed in the cytoplasm as an inactivated molecule, in which CARD and helicase domains are masked. Upon infection, viral RNA is detected by the helicase domain of RIG-I which results in conformational changes to allow interaction with the downstream adaptor, IPS-1, on the mitochondria and signal transduction, leading to the activation of both NF-κB and IRF3. Binding of viral dsRNA to the kinase PKR, causes the protein to undergo activation and subsequent phosphorylation of the eIF2α which leads to the inhibition of cellular protein synthesis. PKR has also been implicated in NF-κB signaling induced by viral dsRNA. TLR-mediated recognition of nucleic acids can induce synthesis of IL-1β through the activation of NF-κB. Bacterial RNA that is presented to the cytosol through the release from intracellular bacteria or active transport stimulates the NALP3 inflammasome through the adaptor ASC. Stimulation of cryopyrin/Nalp3 mediates caspase-1 activation that process IL-1β and IL-18 into the mature cytokine.

Tokunaga et al. [50] when they reported that bacterial DNA itself was a component of Bacillus Calmette-Guerin (BCG) vaccine. While mammalian DNA is not stimulatory, the ability of bacterial DNA to provoke the immune system is partially dependent on unmethylated CpG dinu-

cleotides in a base context named CpG motif [51,52]. The immunomodulatory effects of bacterial DNA have been mimicked by synthetic oligodeoxynucleotides containing a CpG-motif (CpG-ODN). Unmethylated CpG DNA activates monocytes and macrophages to induce cytokine

production and modulates cellular responses. Treatment with CpG DNA upregulates the expression of CD40 and CD69 and induces the secretion of cytokines which include IFN- $\alpha/\beta$ , IL-1 $\beta$ , IL-6, TNF- $\alpha$  and IL-12 [53-55]. However, human monocytes deficient in the TLR9 failed to be activated by CpG DNA. In addition to production of cytokines, macrophage and monocytes challenged with CpG DNA displayed enhanced expression of the inducible nitric oxide synthase (iNOS) and production of nitric oxide. Cell line models such as RAW 264 exposed to CpG DNA and LPS produce IL-12 which was enhanced by IFN- $\gamma$  treatment [56,57].

CpG DNA also stimulates the B cells to enter the proliferative cell cycle and induce polyclonal immunoglobulin production and thus they are potent mitogenic molecules. It has been also shown that CpG-mediated induction of IL-6 is essential for the subsequent secretion of IgM by B cells. Furthermore, CpG DNA also induces B cells to express Fc $\gamma$  receptor and costimulatory molecules such as CD80, and CD86 [51,58,59]. It has been also shown that treating WEHI-231 or BKS-2 B cell lines with CpG DNA protects against apoptosis by upregulating NF- $\kappa$ B and c-Myc expression levels and protects B cells against Fas-mediated cytotoxicity [54,59]. There have been no reports indicating that resting T cells can directly respond to CpG DNA. However, the activity of resting T cells can be affected by type I IFNs and cytokines produced by other cell types in response to CpG DNA. In highly purified murine T cells, CpG DNA synergistically enhances the proliferative response of T cells to T cell receptor (TCR) ligation and can also induce IL-2 production, IL-2 receptor expression and proliferation of purified T cells [60,61]. CpG DNA can directly stimulate both immature and mature dendritic cell (DCs) populations derived from bone marrow. However, human DCs and their precursors are expressing different subsets of TLRs that enable them to sense different PAMPs derived from various pathogens [62,63]. CpG DNA can directly activate human plasmacytoid DC (pDC) to induce major histocompatibility complex (MHC) class II, ICAM-1, CD40, CD54, CD80, production of cytokines, resistance to apoptosis, and growth factor-independent survival in cultured cells [62-65]. These observations indicated that CpG-ODN alone could replace the cytokine requirement for survival of these primary DCs. CpG DNA promotes immune response patterns similar to the T helper cells type I (Th1) by direct effects on B cells or through

indirect effects on other cytokines which are considered as critical elements to the use of CpG DNA either alone or as part of a DNA vaccine [64,66]. Initially, it has been observed that mycobacterial DNA stimulated natural killer (NK) cells to produce IFN- $\gamma$  which was shown to be dependent on palindromic sequences in mycobacterial DNA [67,68]. Palindromes are not essential for NK stimulation but their presence may result in the formation of a double-stranded duplex region that protects the structure against nuclease degradation. Purified NK cells do not respond directly to CpG DNA, but their stimulation seems to be mediated by cytokines such as IL-12, TNF- $\alpha$ , and type I IFNs which are induced by this stimulant [68,69].

### The immunostimulatory potential of RNA

Several species of RNA are considered as potent stimulants for the immune system and represent danger signals for PRRs such as TLRs. DsRNA can serve as a viral associated signature recognized by TLR3 on effector cells as nonself. Stimulation of TLR3 by this ligand activates NF- $\kappa$ B and induces the production of type I IFNs and other proinflammatory cytokines [70,71]. Poly I:C (a synthetic analogue for viral dsRNA) which was identified as a TLR3 agonist can efficiently induce the maturation of DCs and enhances the antigen specific immune responses. It has been also reported that polyI:C was an effective stimulus for the *in vitro* generation of mature DCs capable of secreting IL-12 [72-74]. Knockout mice studies have shown that TLR3 is required for the NK cell response to viral infection. The potential of polyI:C to direct the adaptive immune response and enhance antigen-specific CD8 T-cell responses has been established in investigations. Recently, it has been shown that polyI:C stimulates post-vaccination CD8 T-cell responses and attenuates the contraction of antigen-specific T cells which was associated with a decrease in apoptosis. Administration of polyI:C was also associated with antitumor immunity in tumor challenge models [75-77].

Recent reports have also reported that naturally occurring bacterial RNA and modified single-stranded RNA (ssRNA) detected by TLRs can induce antigen-specific immunity, secretion of proinflammatory cytokines and type I IFNs [30-32]. The chemical modifications and structural elements that are distributed on either bacterial or mammalian RNA, offers a molecular recogni-

tion patterns for immune cells to discriminate differences between the immunomodulatory and inert RNA [17,27,29].

In contrast to DNA and dsRNA, the use of naturally occurring RNA and single-stranded synthetic oligoribonucleotides as ligands for TLRs has been restricted by its susceptibility to nuclease degradation. Most of the investigations reported with these species of RNA as agonists of TLR7/8 used cationic liposomes, polymers, or chemical modifications to enhance nuclease stability [78-80]. It has been observed that human monocytes-derived DC precursors transfected with bacterial RNA, but not with mammalian RNA, induces high-levels of IL-12 secretion in conjunction with dendritic cell maturation [28]. Through the extensive examination of the effects of RNA modifications on TLR-mediated innate immune activation, it has been observed that among natural RNAs, bacterial but not mammalian RNA, activated human DCs to secrete cytokines [29]. It has been shown that CpG oligoribonucleotides (ORN) stimulate monocytes to produce large amounts of IL-12 [81]. CpG ORN also activates NF- $\kappa$ B and p38 MAPK and it has been suggested that activation of these cells was not mediated through either TLR3 or TLR7/8. However, it has been recently reported that synthetic single-stranded ORNs are potent inducers of interferon- $\gamma$  via the TLR7. The immunostimulatory ORNs activates dendritic cells and induces production of cytokines that results in bystander activation of T and B cells. Furthermore, it has been observed that RNA induced antibodies of the IgG isotype indicating a Th2 type of immune response which is characterized by a potent cytotoxic T cell response to infection [32].

### Toll like receptors (TLRs)

TLRs are a key component of the immune sensors involved in the recognition of PAMPs and triggering the host responses. Toll receptor was originally identified as a molecule essential for the embryogenic patterning of *Drosophila* and subsequently as a key receptor for the antifungal immune response [17,82,83]. The mammalian homology of Toll receptor, designated TLR4, was subsequently shown to induce the expression of genes involved in the inflammatory response. Since then, several structural and functional proteins related to TLR4 were identified and named as TLRs [84-86]. So far, ten distinct TLRs family members have been identified in

humans and the corresponding ligands derived from different microbial origins have also been reported [87,88]. TLRs are conserved transmembrane proteins characterized by an ectodomain, a transmembrane region, and a cytoplasmic signaling domain homologous to that of the interleukin 1 receptor (IL-1R) which is known as Toll/IL-1R homology (TIR) domain. TLRs contain leucine-rich repeats (LRRs) in their extracellular domain. Recognition of molecular patterns from pathogens and TLR binding to their corresponding ligand occurs through LRR motifs, which allows for the subsequent activation of signal transduction pathways that regulate the host's immune response.

Based on the type of ligands, TLRs can be further categorized into several subclasses, each of which recognizes related pathogenic signatures. Microbial proteins such as flagellin and respiratory syncytial virus (RSV) fusion protein can activate TLR5 and TLR4, respectively [89,90]. The lipids and lipopeptides responsive TLRs are comprised of TLR1, TLR2, TLR4, and TLR6. Although lipopolysaccharide (LPS) from the outer membrane of gram-negative bacteria is the major activator for TLR4, TLR2 recognizes a variety of lipids and lipoproteins derived from gram-positive bacteria and parasites as well as responding to atypical LPS from certain gram-negative bacteria [91]. Recognition of diacylated or triacylated lipoproteins which are contained within lipoteichoic acid (LTA) is generally mediated by various heterodimeric combinations of TLR2 with either TLR1 or TLR6 [92-94]. Despite this specificity, some TLRs can sense several structurally unrelated ligands. For example, ligands such as LPS, fibronectin, and heat-shock proteins can be recognized by TLR4 [17,95]. Although the majority of TLRs sense pathogenic ligands on the cell surface, TLR3, TLR7, TLR8 and TLR9 recognize nucleic acids and their mimics and thus form a distinct class of TLRs based on functional and structural homology. Within the endosomal compartments, TLR3 senses dsRNA and both TLR7 and TLR8 recognize ssRNA, while DNA is a ligand for TLR9. In addition to their expression on professional immune cells; epithelial cells and fibroblasts also express functional TLRs and can directly sense PAMPs [17,18,20,87,96].

### TLR signaling pathways

Upon stimulation with PAMPs, TLRs undergo conformational changes and subsequent

dimerization to initiate signaling pathways that result in the activation of proinflammatory cytokines through either MyD88-dependent or MyD88-independent pathways [87,97]. Activation of these two distinct pathways initially depends on the recruitment of selective adaptor molecules to the TIR domain. Four adaptor molecules have been identified which includes MyD88, TIR-associated protein (TIRAP), TIR domain containing adaptor protein-inducing IFN- $\beta$  (TRIF) and TRIF-related adaptor molecules (TRAM). The adaptors MyD88 and TIRAP contribute to the induction of proinflammatory genes whereas TRIF and TRAM are responsible for the induction of the IFNs [98-100].

Activation of TLR3 and TLR4 recruits TIR to initiate the antimicrobial response. Although TRIF recruitment mediates TLR3 and TLR4 signaling, TLR4 activation uses another adaptor protein, TRAM. For NF- $\kappa$ B induction, TRIF recruits both receptor-interacting protein 1 (RIP1) and TRAF6 and with the cooperation of these two proteins; they activate NF- $\kappa$ B to induce the expression of proinflammatory cytokines. Furthermore, TRIF-mediated signaling also activates IRF3 and IRF7 to induce type-I IFN gene expression. TRIF interacts with both TBK1 and IKK $\epsilon$ , which phosphorylates IRF3 and IRF7. Phosphorylated IRF3 then dimerize and translocates into the nucleus to induce expression of type I IFN and IFN-inducible genes.

All TLRs except for TLR3, recruit the general TLR adapter protein MyD88, which possesses a death domain in the N-terminus and a TIR domain in the C-terminus. MyD88 recruitment is followed by the activation of IL-1 receptor-associated kinases (IRAKs) family members including IRAK1, IRAK2, IRAK4 and IRAK6. When phosphorylated, IRAKs are activated and subsequently associate with TRAF6 to activate the downstream pathways involving the IKK complex and the MAPK family. Phosphorylation of I $\kappa$ Bs by the IKK complex results in its degradation and the subsequent nuclear translocation of the transcription factor NF- $\kappa$ B, which controls the expression of various inflammatory cytokine genes. In addition, the MAPK family of kinases phosphorylates and activates the transcription factor AP-1 which is involved in the inflammatory responses to TLR signaling. MyD88 is required for the signaling pathways of all TLR family members that lead to the production of inflammatory cytokines since MyD88-

deficient mice showed no production of inflammatory cytokines in response to all TLR ligands.

### Discrimination of self and nonself DNA by TLRs

Discriminating self from foreign is an essential feature of innate immunity that employs PRRs to sense molecular signatures associated with pathogens [21-24,87,101]. Detection of pathogen-derived nucleic acids is important for maintaining the integrity of the genome and for survival. The immunomodulatory effects of bacterial DNA have been mimicked by synthetic oligodeoxynucleotides containing a CpG-motif (CpG-ODN). Subsequently, it has been reported that CpG-DNA signaling is abolished in knockout mice deficient in MyD88 which suggested that TLR 9 is a cognate receptor for CpG-DNA [102]. In parallel experiments, Hemmi et al. [26] have also shown that macrophages from TLR9 knockout mice did not respond to CpG-DNA. These observations demonstrated that TLR9 is the direct immune sensor for CpG-DNA. In addition to bacterial DNA, the genomes of DNA viruses such as herpes simplex virus 1 (HSV-1) and murine cytomegalovirus (MCMV) are rich in CpG-DNA motifs, and therefore activates inflammatory cytokines and type I IFN secretion through TLR9 [72,103-105]. In contrast to macrophages that produce less IFN- $\alpha$  in response to viral infections, HSV-2-induced expression of TNF- $\alpha$  and RANTES is mainly dependent on TLR9, whereas type I IFN is induced in a TLR9-independent manner [103]. In addition to DNA modifications, the innate surveillance mechanisms of TLRs are also utilized by the endosomal compartments to tolerate self nucleic acids [17,106]. Since TLR9 is selectively expressed in the endosome, pathogenic DNA must be delivered intracellularly where the acidic environment within the endosome results in the degradation of dsDNA into multiple fragments containing ss-CpG-motifs that subsequently triggers TLR9 [107,108]. In contrast to foreign DNA, the self abundant DNA is sequestered within the nucleus and therefore can not access the endosomal compartments. The sequestration of self DNA and the selective intracellular localization of TLR9 are proposed to have an important role in self tolerance and autoimmunity.

The structural requirements and species specificity for the activation of TLR9 by DNA were initially explored by synthetic ODNs that have a

modified phosphorothioate (PS) backbone [106,109,110]. Because the synthetic ODNs contain natural phosphodiester linkages (PD-ODN) they are rapidly digested by DNase resulting in poor uptake by cells. It was proposed that stabilized PS-ODNs that are flanked by two 5'-purines and two 3'-pyrimidines mimic natural ssDNA fragments for activating TLR9. Therefore, the discrimination of endogenous DNA from nonself pathogenic DNA was explained by the fact that unmethylated CpG motif serves as a molecular signature which is sensed by TLR9. However, recent studies have reported contradictory results to the sequence-specific manner in which DNA is recognized by TLR9. For example, not only microbial DNA, but also mammalian DNA that contains less or no unmethylated CpG motifs can also stimulate pDCs and provoke an antiviral-like state in a TLR9-independent fashion [111]. Several further investigations have supported the idea that self DNA is an autoimmune molecule which activates autoreactive B cells through TLR9. For example, the CpG-mediated proliferation of B cells was abrogated by TLR inhibitory oligonucleotides in transgenic 3H9 B cells that have DNA-specific B cell receptors (BCR) [47,112-114].

These contradictory observations may imply that host DNA contains DNA sequences that serve as ligands for TLR9. Contrary to stabilized PS-ODN, it has been demonstrated that natural PD-ODN lacking CpG motifs stimulate DCs to secrete IFN- $\alpha$  and proinflammatory cytokines via TLR9 which was confirmed by ligand-binding studies [111]. These observations raise the question of what governs the recognition of natural PD-DNA by TLR9. Recently, it has been shown that the sugar backbone is a major determinant of recognition of natural DNA by TLR9. PD 2-deoxyribose polymers lacking DNA bases (abasic) are shown to act as a TLR9 agonist while abasic PS 2-deoxyribose functions as TLR9 antagonist. The authors also show that natural PD DNA drives TLR9 activation in a CpG-independent manner but DNA sequence can modulate the strength of the response. This response is similar to that for dsRNA recognized by TLR3 and ssRNA recognized by TLR7 and TLR8 [115].

### **Discrimination of self and nonself RNA by TLRs**

Naturally occurring viral and bacterial RNAs and synthetic oligoribonucleotides recognized by

TLRs can induce antigen-specific immunity, secretion of proinflammatory cytokines and type I IFNs [27-32]. For example, RNA derived from bacteria, but not from mammalian sources, prime for high-levels of IL-12 secretion and activation of human myeloid dendritic cells (DCs) [28]. Bacterial RNA also activates human DCs to secrete TNF- $\alpha$  and other cytokines [29]. These observations raise the question of how abundant self-RNA species are discriminated from non-self RNA. Sequestration of self nucleic acids and the selective expression of TLRs within intracellular compartments is one of the explanations that is proposed to answer this question [116-121]. Under normal circumstances, the abundant self-RNA is contained within the nucleus or in the cytoplasm and therefore can not access the endosomal TLR3, TLR7, or TLR8 that sense various species of RNA. However, RNA released from viral infection or damaged host cells under pathological conditions could be delivered to and activate endosomal TLRs and thus circumvent this safety mechanism [30,122].

In addition to this hypothesis, recent reports have indicated that specific nucleoside modifications are differentially represented in either microbial or mammalian RNA and therefore provide a molecular mechanism for the immune sensors to discriminate differences between the pathogenic and abundant self RNA [20,27,29,123]. In contrast to most cellular species of RNA that are heavily modified, unmodified motifs or low degree of nucleoside modifications are abundant in bacterial and viral RNAs [124]. For example, cellular primary transcripts undergo various modifications to acquire a 7-methylguanosine (7mG) cap structure at its 5'-end while bacterial and viral mRNAs possess a triphosphate at their terminal end [125-127]. In addition to capping, up to 25% of nucleosides may be modified in some eukaryotic tRNA [128]. Other posttranscriptional modifications such as pseudouridine and methylated nucleosides are significantly higher in eukaryotic rRNA than that of microbial origins [128,129]. Recently, Kariko et al have examined the effects of RNA modifications on TLR-mediated innate immune activation and established that among natural RNAs, bacterial RNA activated human DCs to secrete cytokines [29]. The TLR-mediated recognition of natural and synthetic RNAs is primarily governed by certain nucleoside modifications such as pseudouridine, 5-methyluridine, 2-thiouridine, or 2-O-

methylation.

### The nucleotide binding and oligomerization domain (NOD)-like receptors (NLRs)

Recognition of multiple pathogenic patterns by PRRs expressed in and on host tissues triggers several signaling pathways to target the infecting pathogens [130]. Recent studies have identified and characterized the NLRs as an additional mechanism which contributes to intracellular recognition of PAMPs and regulation of the immune response [35-37]. NLRs are a family of structurally related receptors that are characterized by conserved presence of Nod. The general domain structure of this family includes carboxyl-terminal LRRs that recognize specific PAMPs and modulates NLR activity, an intermediate Nod that is responsible for oligomerization of these proteins, and finally an N-terminal effector binding region consisting of protein-protein interaction domains which includes the caspase recruitment domain (CARD), pyrin (PYD) domain, or the baculovirus inhibitor repeat (BIR) domain [131-133]. The expression of these receptors in cytosolic compartments enables them to recognize invaders that have evaded detection through invasiveness or escape

The NLRs family is divided into three categories based on their N-terminal domains and are referred to as CARD-containing NODs, PYD-containing NALPs, or BIR-containing NAIPs. Although recent studies have identified specific ligands for several NLRs, it is still unclear whether NLRs directly bind to their ligand that they sense. Nod1 (Card4) and Nod2 (Card15) are involved in the recognition of bacterial components produced during the synthesis or lyses of peptidoglycan [135-137]. NOD1 detects pathogenic bacteria through sensing peptidoglycan containing meso-diaminopimelic acid (meso-DAP), unique to most Gram-negative and only specific Gram-positive bacteria [135-137]. Despite this activation by meso-DAP, recent studies have revealed that the optimal peptidoglycan motifs detected by human Nod1 is a structure containing L-Ala- $\gamma$ -D-Glu-meso-DAP and the minimal activating structure is the dipeptide  $\gamma$ -D-Glu-meso-DAP [132,134]. In contrast, Nod2 is activated by muramyl dipeptide (MDP) conserved in all bacteria [136,138]. In addition, NOD2 is considered as a general sensor involved in recognition of intracellular pathogenic bacteria such as *Listeria monocytogenes* and *Streptococcus pneumoniae* [139,140]. These

ligands must be delivered intracellularly through either the cytosolic bacterial invasion or through some cellular uptake mechanisms to activate NOD1 or NOD2. In addition to being detectors for microbial structures, NALP-containing proteins can also mediate activation of the immune system in response to endogenous danger signals. The most studied members of this subfamily are NALP1 and NALP3 which are implicated in the formation of the inflammasome [141]. The inflammasome is a multiprotein complex involved in processing and activation of caspase-1. Caspase-1 which is present in cells in an inactive form undergoes proteolytic cleavage upon activation. Activated caspase-1 is essential for maturation of proinflammatory cytokines such as IL-1 $\beta$  and IL-18 for converting them to the active forms in response to infection or injury. IL-1 $\beta$  is also implicated in inflammation, fever, tumor progression and sleep [133,141]. NALP3 is activated in response to several diverse molecules such as bacterial RNA, LPS, peptidoglycan, bacterial toxins (Nigericin, aerolysin) and whole bacteria [131-133]. In addition to classical microbial moieties, NALP3 is also capable of sensing endogenous danger and stress situations such as damage associated molecular patterns (DAMPs). Exposure of mammalian cells to ATP but not to a PAMP, stimulates caspase-1 activation and subsequent IL-1 $\beta$  secretion. Furthermore, the inflammatory disorders induced in response to monosodium urate (MSU) and calcium pyrophosphate dehydrate (CPPD) crystals can also be mediated by NALP3 and caspase-1 release [142,143]. In addition to NOD and NALP proteins, the third subclass of NLRs family is Ipaf/CARD12. Ipaf/CARD12 has been shown to be implicated in sensing intracellular pathogens such as *Salmonella typhimurium* or *Legionella pneumophila* which then rapidly activates caspase-1. The molecular patterns recognized by Ipaf have been shown to be flagellin. Recent studies have demonstrated that upon infection, *S. typhimurium* or *L. pneumophila* deliver flagellin and other virulence factors to the host cytosol by the type III or type IV secretion systems. *Salmonella* and *Legionella* mutants that do not express flagellin failed to activate caspase-1 [131,144-146].

### NLR signaling pathway

Activation of NLRs by microbial PAMPs activates two distinct signaling pathways and proinflammatory intracellular responses. The first path-



way involves stimulation of Nod1 or Nod2 by specific bacterial cell wall components which results in their oligomerization and recruitment of RICK/RIP2/CARD/IAK cascade through CARD-CARD interactions. The binding of RICK kinase to Nod1 or Nod2 leads to activation of NF- $\kappa$ B through phosphorylation and ubiquitin-proteasome-dependent degradation of the I $\kappa$ B $\alpha$ . The signaling proteins TRIP6 and CARD6 positively modulate Nod1- and Nod2-dependent signaling to NF- $\kappa$ B. The activated NF- $\kappa$ B translocates to the nucleus and induces transcription from targeted genes. In addition to the NF- $\kappa$ B pathway, MAP family kinases are also activated in response to stimulation of Nod1 or Nod2 through RIP2 that results in the activation of kinases including p38, Erk, and JNK [147-151]. This pathway modulates the expression of pro-inflammatory cytokines to regulate both innate and adaptive immune responses. The second pathway involves activation of the inflammasome in response to either classical PAMPs or endogenous danger stress signals. It has been shown that Ipaf, Naip and NALP proteins all can participate in the formation of distinct inflammasomes including the NALP1 inflammasome, the NALP3 inflammasome and the Ipaf inflammasome. In response to microbial or danger signals, the autorepressed NALP3 is activated, oligomerizes in an ATP-dependent manner and this results in caspase-1 processing and activation. This complex is composed of a Pyrin domain, and the adaptor-proteins apoptosis-associated speck-like protein (ASC) and Cardin. Activated caspase-1 then cleaves and activates the precursor forms of IL-1 $\beta$  and IL-18. Recently, it has been demonstrated that Cryopyrin, Ipaf, as well as the adaptor ASC, are required for the activation of caspase-1 in response to pathogenic components and intracellular bacteria which is TLR-independent [36,38,39,130,131,145,152].

### **Sensing of self and nonself nucleic acids by NLRs**

Recent investigations have indicated that NALP3 can also detect RNA derived from bacterial and viral origins in TLR-independent manner. Bacterial RNA derived from *E. coli*, *Listeria* and *Legionella* induced IL-1 $\beta$  and IL-18 secretion from macrophages in a Nalp3-dependent fashion [38]. It has been also shown that natural RNA of viral origin and polyI:C are also able to activate the Nalp3 inflammasome [38,39].

Viral dsRNA also stimulates the release of type-I IFNs, TNF- $\alpha$  and IL-6 through other RNA sensors (TLR3 and RIG-I). However, activation of the NALP3 inflammasome by RNA species only occurs in the presence of ATP which itself is an activator of the inflammasome. There has also been recent reports showing that Nalp3 can sense DNA from bacteria, virus and even from mammalian sources. Internalized adenoviral DNA induces maturation of pro-IL-1 $\beta$  in macrophages in a NALP3 and ASC-dependent mechanism and this inflammatory response is depressed in NALP3- and ASC-deficient mice [40]. Activation of the inflammasome also occurs in response to transfected cytosolic bacterial, viral and mammalian DNA. However, the cytosolic recognition of DNA in this case is dependent on ASC but not on NALP3. This surveillance mechanism for detecting DNA is independent of TLRs and interferon regulatory factors (IRFs). The authors suggested that the IL-1 production most likely reflects a general inflammatory state triggered by adenoviruses, rather than a direct antiviral activity.

### **RIG-I-like receptors (RLRs)**

In addition to the protein kinase R (PKR) which was proposed as the first cytoplasmic sensor for triggering IFN-induced signaling in response to viral dsRNA, screening of an expression cDNA library identified the RIG-I-like receptors (RLRs) as a novel innate receptor family that recognizes viral RNA in intracellular compartments [36,37]. The discovery of these cytosolic sensors suggests that microbes evading extracellular surveillance encounter another line of detection in the host cytosol. RLRs are RNA helicases expressed ubiquitously in the cytoplasm and are strongly induced by IFNs. RLRs family consists of three members designated retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated antigen 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2). These members have a high degree of similarities among their helicase domains [153]. RIG-I contains two repeats of a caspase activation and recruitment domain (CARD)-like motif at its N terminus that functions as a signaling domain. The CARD domain is followed by helicase motifs (I to VI) including Walker's ATP-binding motif (motif I) and TAS (motif III) which are essential for RIG-I to function [154-157]. The C-terminal domain of RIG-I, termed the repressor domain (RD), is responsible for inhibiting RIG-I signaling

and is responsible for autorepression through interaction with both CARD and helicase domains [158,159]. Although RIG-I and MDA5 share some common structural and signalling features, recent evidence indicated that the two helicases may discriminate among different ligands to trigger the innate immune response to RNA viruses [43,160]. CARD-like repeats are shared by RIG-I and MDA5 but are lacking in LGP2. The C-terminal region have partial conservation among all 3 helicases, but the repression function was only observed in RIG-I and LGP2 [156,161].

### RLRs signaling

The CARD motifs of RIG-I and MDA5 are the structures responsible for initiating the signaling events. When the cells are not infected, the CARD domain is masked by the RD of RIG-I but MDA-5 is silenced by unknown mechanism [162-164]. Overexpression of the N-terminal CARD of either RIG-I or MDA5 constitutively activates the transcription factors NF- $\kappa$ B and IRF-3 and subsequently induces IFN- $\beta$  expression which suggests the direct contribution of CARD in signaling [157]. Recent investigations identified IFN promoter stimulator-1 (IPS-1) as CARD-containing adaptor required for RIG-I/MDA5 signaling [162,165,166]. IPS-1 is a protein expressed in the outer mitochondrial membrane which suggests that mitochondria not only contribute to apoptosis and metabolism but also regulate the IFN responses [167-169]. When cells are infected with RNA viruses, RIG-I and MDA5 sense 5'-triphosphate ssRNA and dsRNA through their RNA-binding domains. This recognition causes RLRs to undergo conformational changes in an ATP-dependent manner to free the CARD domain from autoinhibition and allows downstream signaling to IPS-1. In turn, IPS-1 interacts with downstream adaptors which include TRAF3 to activate two IKK-related kinases, designated TANK-binding kinase 1 (TBK1) and inducible I $\kappa$ B kinase (IKK-i) which then phosphorylate IRF-3 and IRF-7 [170-172]. However, IFN- $\beta$  could also activate IKKi to recruit and phosphorylate STAT1, and thus it regulates the expression of a number of IFN-inducible genes [173]. IKK-I and TBK1 interact with the molecules TANK, and NAK-associated protein 1 (NAP1) which are essential components for RLH signaling [174-176]. Phosphorylation of IRF-3 and IRF-7 by these kinases induces their translocation into the nucleus and binding

to IFN-stimulated response elements (ISREs). This allows for the regulated expression of type I IFN genes and IFN-inducible genes. NF- $\kappa$ B is also activated by IPS-1 via a FADD (FADD/RIP1/IKK $\alpha$ /IKK $\beta$ ) and a caspase-8/caspase-10-dependent pathway [177,178].

### Discrimination of self and nonself nucleic acids by RLRs

RLRs play important roles in sensing viral RNA and elicit the antiviral defence mechanisms. It is initially proposed that upon accumulation of viral dsRNA in the infected cells, both RIG-I and MDA-5 become activated and trigger signaling pathways that result in an immune response [43,44]. However, subsequent analysis of RIG-I and MDA5 knockouts revealed that MDA5, but not RIG-I, was the sensor responsible for recognition of poly I:C. Interestingly, infection with influenza A results in IFN gene expression without detectable dsRNA accumulation [179]. Recent studies have reported that ssRNA with 5'-triphosphate terminus, rather than dsRNA is a substrate for RIG-I. These studies demonstrated that RIG-I is specifically sensed by and binds to ssRNA bearing a 5'-triphosphate end, but not ssRNA with 5'-monophosphate or 5'-diphosphate end [45]. These investigators hypothesized that RIG-I utilizes the 5'-triphosphate end, which is primarily present in foreign transcripts such as viral and bacterial mRNA and absent from host RNA, as a part of a RNA-quality control mechanism to distinguish endogenous from pathogenic RNA. Recent studies further indicated that RLRs are differentially detecting cytoplasmic viral RNAs and they differ in their substrate specificity [43]. For example, when RIG-I-deficient embryonic fibroblast cells (MEFs) were infected with viruses such as Newcastle disease virus (NDV), vesicular stomatitis virus (VSV) or influenza virus, the induction of IFN was significantly abrogated whereas MDA5-deficient cells were not defective. However, transfection with poly I:C or infection with encephalomyocarditis virus (EMCV) inhibited IFNs induction in MDA5-KO cells, but not in RIG-I-KO cells. West Nile virus and Dengue virus were shown to induce type I IFN even in the absence of either RIG-I or MDA5 [43,180,181].

### The contribution of nucleic acids to sepsis pathogenesis

The host surveillance mechanisms sense vari-

ous components derived from different pathogenic origins as foreign and hence target them. TLR3, TLR7, TLR8, and TLR9 comprise a distinct subclass of TLRs expressed within endosomal compartment where they can recognize bacterial or viral nucleic acid [24,101]. DNA containing unmethylated CpG motifs is detected by TLR9 and produce proinflammatory cytokines such as TNF- $\alpha$ , IL-1, IL-6, IL-12, and reactive nitrogen and oxygen intermediates. Furthermore, DNA can also trigger antiviral-like innate responses which include production of IFN- $\alpha/\beta$  and IFN- $\gamma$ . When detected in the cytoplasm, not only pathogenic, but also self-DNA can trigger an antiviral-like state. Therefore, the physical residence of DNA within the nucleus is used to avoid the immune response to self DNA [24,88,101]. In addition to DNA, several other PRRs have been implicated in the sensing of different RNA species including TLRs, dsRNA-activating protein kinase (PKR), RIG-I, MDA5, and the NALP3 inflammasome. These RNA sensors differ mainly in their ligand specificity, cellular expression and signaling pathways. The diversity in the receptors suggests that host cells employ multiple, nonredundant surveillance mechanisms to detect invading pathogens. While RIG-I, MDA-5, and NALP3 are cytosolic receptors, the TLR family members involved in RNA recognition are expressed in the endosomal compartments. Recognition of RNA patterns by these receptors activates immune and non-immune cells to induce the production of proinflammatory cytokines and IFNs [35,182,183].

Sepsis develops when the invading pathogens gain access to the circulation, are recognized by the host immune surveillance mechanisms and accordingly respond systemically by generating exaggerated amounts of inflammatory mediators and proinflammatory cytokines. In addition to bacterial cell wall components and exotoxins which are considered as the principal PAMPs responsible for sepsis, recent studies have indicated that both purified microbial nucleic acids and synthetic oligonucleotides can also induce the production of cytokines and reactive oxygen/nitrogen species and nitric oxide and therefore are also implicated in the development of sepsis [25,184]. *In vivo* and *in vitro* studies demonstrated that natural DNA and synthetic oligonucleotides can cause septic shock. DNA derived from both Gram-positive and Gram-negative bacteria, or synthetic oligonucleotides

trigger high and rapid TNF- $\alpha$  production in macrophages and thus cause TNF- $\alpha$ -mediated septic shock in D-galactosamine-sensitized mice [185]. At high concentration of DNA, the magnitude of TNF- $\alpha$  responses induced was similar to that of LPS. Mice sensitized with TNF- $\alpha$  and injected with these DNAs, developed lethal toxic shock which was due to acute liver failure mediated by TNF- $\alpha$  triggered apoptosis. Furthermore, it has been shown that macrophages challenged with plasmid DNA induced TNF- $\alpha$  mRNA. Mammalian DNA, DNase- treated plasmid DNA or methylated CpG dinucleotides did not induce TNF- $\alpha$  mRNA in the macrophages [53]. Interestingly, DNA is taken up by macrophages and characteristic bacterial DNA sequences activate a signaling cascade that leads to the activation of NF- $\kappa$ B and inflammatory gene induction. Although TNF- $\alpha$  is considered to be a central element in the pathogenesis of endotoxic shock, the involvement of IFNs in this process have been also supported by several line of evidence. Besides bacterial DNA, synthetic viral RNA analogues poly d(I:C) and poly I:C also induced TNF- $\alpha$  secretion from macrophages [53]. These data therefore support the concept that microbial nucleic acids alone can elicit a lethal inflammatory response.

The multi-hit synergistic threshold hypothesis of lethal septic shock provides an additional proposal to explain the complex inter-relationships that occurs in sepsis. For example, the host immune system which has been primed by the previous release of LPS, responds further with a fatal exaggerated systemic inflammatory response induced by other PAMPs such as DNA [184]. Alternatively, the release of multiple pathogenic components may synergistically reach a threshold that can provoke an undesirable inflammatory immune response in individuals with an existing genetic predisposition to an exaggerated immune response. It has been reported that viral infection with VSV or treatment with poly I:C sensitizes mice to LPS-induced shock [186]. This hypersensitivity to LPS correlated with hyperproduction of TNF- $\alpha$  which was completely abrogated in IFN- $\alpha/\beta$  receptor-deficient mice. This result indicated that the principal mechanism underlying rapid virus or poly I:C-induced sensitization to LPS is an IFN- $\alpha/\beta$ -mediated priming of mice for an augmented production of TNF- $\alpha$  in response to LPS. The impact of antiviral responses on the inflammatory responses to septic shock has been

studied by several independent investigators. For example, injection of mice with the TLR3 ligand (poly I:C) caused robust induction of IFN- $\alpha$  with IFN- $\alpha$ / $\beta$ -dependent major histocompatibility antigen class II up-regulation of peritoneal macrophages [187]. Poly I:C pretreatment before septic shock resulted in augmentation of TNF- $\alpha$ , IL-6, and IL-10 and a potentially heightened lethality as compared with septic shock alone. DNA from bacteria interacts synergistically with subthreshold concentrations of LPS to elicit TNF- $\alpha$  production and NO via enhanced inducible NO synthase gene expression in macrophages [188,189]. High-mobility group protein 1 (HMGB1) functions as a cytokine that induces proinflammatory responses which includes the expression of TNF- $\alpha$ , IL-1, NO, and maturation of DC, which mediates sepsis. It has been shown that LPS and poly I:C treatment resulted in HMGB1 secretion from macrophages whereas CpG ODN failed to induce this response [190]. The extent of HMGB1 release correlated with the occurrence of apoptosis with the treatment of both LPS and poly I:C. In human Jurkat T cells, poly I:C induced apoptosis and HMGB1 release and this suggested that apoptotic cells can contribute to HMGB1-mediated events in sepsis.

### Nucleic acids in cardiac myocyte dysfunction

Cardiac myocytes, whose primary function is contraction, proliferate rapidly during fetal life but exit the cell cycle to terminally differentiate. In response to pathophysiological insults, cardiac myocytes undergo hypertrophy and remodelling which is characterized by progressive changes in ventricular size, shape, function and further loss of cardiac myocytes by necrosis and apoptosis [191]. The inflammatory response is an important component of cardiac pathology and is associated with a number of heart diseases including microbial infections, sepsis, myocarditis, and myocardial infarction. Cardiac dysfunction is clearly identified as a serious and frequent complication of human sepsis and septic shock. Several mediators have been described as the initiators of cardiac function impairment during sepsis. These mediators include microbial components, cytokines, and reactive oxygen/nitrogen species [1-3,192]. These immunological mediators have been increasingly thought to influence not only the inflammatory molecules but also cardiac function [193-196]. In addition to the immune system,

there is accumulating evidence suggesting that the coagulation, endocrine, and nervous systems also contribute to depression of cardiac function under inflammatory conditions. Experimental and clinical reports demonstrated that TLRs expressed on various cell lines contributes to the development and progression of sepsis. For example, monocytes/macrophages expression of TLR2 and TLR4 in septic patients and in cecum ligation and puncture (CLP) sepsis models are significantly up-regulated compared with the expression in healthy individuals [197-199]. In addition, genes involved in TLR signaling pathway are differently regulated in peripheral mononuclear cells (PBMC) and neutrophils of septic patients, and that they are dynamically modulated in every cell population throughout the different stages of sepsis [200]. These mechanisms may contribute to the organ dysfunction and mortality that occurs in sepsis.

Although there is clear evidence of how immune cells sense danger signals of foreign nucleic acids and accordingly respond, only recently has it been reported that cardiac myocytes have other functions similar in some respects to innate immune cells with a complex inflammatory response. In addition to being expressed in immune cells, cardiovascular cells including cardiac myocytes, endothelial cells, and vascular smooth muscle also express TLRs and can directly sense the molecular signatures associated with pathogens. TLRs family members including TLR2, TLR3, TLR4, TLR5, TLR6, TLR7 and TLR9 are expressed in both whole heart tissue and cardiac myocytes cell lines [201-204]. Stimulation of these receptors with their cognate ligands results in cardiomyocyte expression of the proinflammatory cytokines, chemokines and cell surface adhesion molecules. Furthermore, activation of these TLRs is associated with induction of NF- $\kappa$ B and decreased cardiac contractility. In addition to cardiac myocytes, endothelial cells and the underlying vascular smooth muscle cells in vessels also express functional TLRs and sense pathogens. TLR-mediated specific recognition of PAMPs activates these cells to release cytokines, chemokines, vasoactive and dilatory hormones [205-207].

With regard to microbial nucleic acids, we demonstrated that bacterial DNA and RNA derived from clinical *S. aureus* and *E. coli* isolates significantly depress myocardial contraction of rat

neonatal myocytes in a concentration-dependent fashion [208]. Challenging of neonatal myocytes with polyI:C also results in highly significant depression of myocardial contractility. In contrast, bacterial nucleic acids isolated from non-pathogenic *E. coli* have also shown to induce significant cardiac depression. These data suggested that bacterial and viral DNA and RNA may contribute to myocardial depression during bacterial sepsis and septic shock. *In vivo* studies demonstrated that treatment of wild type mouse model (C57BL/6) with bacterial DNA caused a robust response in hearts as indicated by increased levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, iNOS, and activation of NF- $\kappa$ B [209]. This response was mediated through recognition of CpG-ODN by TLR9. However, such inflammatory responses were absent in TLR9 knockout mice. In addition, contractility measurements of isolated ventricular cardiac myocytes demonstrated a TLR9-dependent loss of sarcomeric shortening after CpG-ODN stimulation which was dependent on production of iNOS. These observations suggest that bacterial DNA contributes to myocardial cytokine production and loss of cardiac contractility via TLR9.

### Autoantigens of nucleic acids

Although self nucleic acids are supposed to be tolerant to receptor-mediated detection, recent studies have demonstrated that TLRs can also recognize self nucleic acids released from damaged host tissues and potentially promote the development of autoimmune diseases. These observations suggested that the host receptors might not be able to fully distinguish nonself from endogenous nucleic acids under certain circumstances [47,48,101]. Nucleic acid molecules are also considered exclusively as autoantigens and a unique target of immune reactivity in systemic lupus erythematosus (SLE). SLE is a prototypic autoimmune disease characterized by high serum levels of type I IFNs that correlate with disease severity. The immune complexes of this disease are comprised of host DNA and anti-DNA IgG or host RNA and anti-RNA or protein-nucleic acid complexes [210,211]. These autoantibodies (anti-DNA and anti-RNA) which can bind both single- and double-stranded nucleic acids resulted from the loss of tolerance to restricted set of nuclear antigens (antinuclear antibodies or ANA).

The ability of TLRs to sense nucleic acids and

the production of autoantibodies against these molecules was established in studies of transgenic mice where the B cells express antibodies specific for self-IgG which is also named as, rheumatoid factor (RF) [47]. Immune complexes (ICs) consisting of IgG bound to mammalian chromatin have been shown to effectively stimulate transgenic RF via involvement of B cell antigen receptor (BCR) recognition of the IC. Inhibition of TLR9 abrogates the response of the autoreactive B cells and hence suggested that this stimulation was TLR9-dependent. These observations established a synergistic cooperation between the BCR which binds and internalizes DNA and its recognition by TLR9. Several further investigations have supported the idea that nucleic acids are autoimmune activators which stimulate autoreactive B cells through TLRs. For example, the CpG-mediated proliferation was abrogated by TLR inhibitory oligonucleotides in transgenic 3H9 B cells that have DNA-specific BCR. Anti-DNA chromatin ICs translocates to lysosomal compartments through Fc $\gamma$ R-mediated endocytosis and stimulated DCs to secrete cytokines and IFNs via a TLR-dependent manner [112-114]. Further evidence that TLR9 plays a critical role in autoantibody responses came from the demonstration that autoimmune lupus-prone mice lacking TLR9 failed to generate anti-DNA antibodies. The importance of DNA as an autoimmune molecule was also demonstrated in mice lacking DNase I, which developed a lupus-like syndrome [212,213].

Not only microbial DNA, but also mammalian DNA when formulated with cationic lipids or which exists in the cytoplasm can also stimulate pDCs and provoke antiviral-like responses in a TLR9-dependent or independent manner [111]. In addition to being activated by autoantigens associated with self DNA, recent studies extended the activation of autoreactive B cell by self RNA. RNA and RNA/protein complexes such as small nuclear ribonucleotide proteins (Sn/RNP) also constitute a major class of autoantigens implicated in autoimmune diseases. Activation of TLR7 by ICs containing uridine-rich U1 RNA can stimulate pDCs to produce IFNs which was potentially linked to SLE and other proinflammatory cytokines [30,214,215]. TLR7-deficient mice bearing anti-RNA BCR knock-in allele are protected from a lupus like syndrome. Further evidence that TLR7 plays a key role in autoantibody responses came from the findings

that autoimmune-prone mice, lacking the TLR adaptor protein MyD88, had markedly reduced chromatin, Sn/RNP, and RF autoantibody titers [30,216].

### Conclusion

Sepsis is a life-threatening condition which can develop through the misregulation of complex cellular signaling networks. Several surveillance mechanisms have evolved to discriminate self from foreign and accordingly trigger effective cellular responses to target the pathogenic threats. Since, the misregulation of complex immunopathological cellular signaling pathways contribute to the high mortality and morbidity rates of sepsis, several efforts have been made to understand the role of pattern recognition receptors (PRRs) and nucleic acids in this condition. Understanding these molecular interactions is not only important for exploring the molecular mechanisms underlying sepsis pathophysiology, but also in the development of novel therapeutics. Targeting a single inflammatory mediator has not showed efficacy in clinical trials and most of the single-hit therapeutic strategies have not been successful. The current sepsis treatments are mainly limited to measures directed at the infectious agents (e.g. antibiotics, surgical and supportive therapies) rather than altering the pathophysiologic processes responsible for its initiation and progression. The application of interdisciplinary approaches that integrate high-throughput genomics, proteomics, biostatistics, and computational biology will open avenues to novel multi-hit therapeutic approaches for sepsis.

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