Discoveries

FORUM REVIEW ARTICLE

Targeting Toll-Like Receptors in Sepsis: From Bench to Clinical Trials

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Abstract

Significance: Sepsis is a critical clinical syndrome with life-threatening organ dysfunction induced by a dysregulated host response to infection. Despite decades of intensive research, sepsis remains a leading cause of in-hospital mortality with few specific treatments.

Recent Advances: Toll-like receptors (TLRs) are a part of the innate immune system and play an important role in host defense against invading pathogens such as bacteria, virus, and fungi. Using a combination of genetically modified animal models and pharmacological agents, numerous preclinical studies during the past two decades have demonstrated that dysregulated TLR signaling may contribute to sepsis pathogenesis. However, many clinical trials targeting inflammation and innate immunity such as TLR4 have yielded mixed results.

Critical Issues: Here we review various TLRs and the specific molecules these TLRs sense—both the pathogen-associated and host-derived stress molecules, and their converging signaling pathways. We critically analyze preclinical investigations into the role of TLRs in animal sepsis, the complexity of targeting TLRs for sepsis intervention, and the disappointing clinical trials of the TLR4 antagonist eritoran.

Future Directions: Future sepsis treatments will depend on better understanding the complex biological mechanisms of sepsis pathogenesis, the high heterogeneity of septic humans as defined by clinical presentations and unique immunological biomarkers, and improved stratifications for targeted interventions. *Antioxid. Redox Signal.* 35, 1324–1339.

Keywords: sepsis, toll-like receptors, danger-associated molecular patterns, pathogen-associated molecular patterns, innate immunity, inflammation, clinical trial

Introduction

TOLL-LIKE RECEPTORS (TLRs) represent an important component of the innate immune defense against microbial pathogens (2). They are among the first to sense pathogen invasion, activate innate immune response, control adaptive immune response (53), and maintain normal immune homeostasis. Depressed or overactivated immune responses *via* TLRs impair host defense against pathogens and play an important role in sepsis pathogenesis (21, 51).

Sepsis is a clinical syndrome with life-threatening organ dysfunction caused by a dysregulated host response to infection (115). In the United States, sepsis develops in more than 750,000 people annually and 210,000 of them die (9, 80). Despite the recent progress in sepsis man-

agement, such as early antibiotic coverage, aggressive fluid resuscitation, and vasopressors to maintain hemodynamics, sepsis remains the #1 cause of mortality in hospitals (32, 102, 110). There have been numerous phase 2 and 3 sepsis clinical trials of various therapies for sepsis, including anti-inflammation, anti-cytokine, and immune and coagulation modulations, most of which have failed (37, 77).

Among many possible causes, incomplete understanding of the biological mechanisms of sepsis pathogenesis may have contributed to the failed clinical trials (51, 77, 123). This article critically reviews the animal investigations on the role of TLR signaling in sepsis, the clinical trials testing the therapeutic efficacy of blocking TLR4, and the lessons we learn from these studies.

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TLRs: Pattern Recognition and Signaling Pathways

Following their invasion, microbes release various pathogen-associated molecular patterns (PAMPs), such as endotoxin, lipopeptide, and nucleic acid. PAMPs are sensed by the pattern recognition receptors such as TLRs in the host cells and activate the host proinflammatory responses such as leukocyte activation, complement activation, and coagulation activation (21, 51). These host responses are extremely important to contain and eliminate microbe dissemination. However, if the responses are dysregulated and excessive, it can cause collateral damage to the host. For example, too much cytokine production (cytokine storm) can cause septic shock, coagulation activation, and subsequent consumptive coagulopathy such as disseminated intravascular coagulation.

Dead cells release damage-associated molecular pattern (DAMP) that can act on TLRs and perpetuate proinflammatory responses, causing excessive inflammation and tissue damage. Almost at the same time, the anti-inflammatory responses are initiated. These include the neuroendocrine axis of parasympathetic outflow and adrenal glands, immune cell dysfunction or death, and anti-inflammatory cytokine production. The anti-inflammatory pathways are implicated in the enhanced susceptibility to secondary infections during the later stage of sepsis.

Discovery of toll and TLR

In 1985, Nusslein-Volhard identified the *Toll* gene critical for the embryonic development of the fruit flies *Drosophila* (7, 8). A decade later, Hoffmann and colleagues found that Toll was essential to innate immunity against pathogen (69). The following year, Medzhitov and Janeway at Yale discovered that a human Toll protein was a transmembrane protein with an extracellular domain consisting of a leucine-rich repeat and cytoplasmic domain, which they coined "Toll-like receptor" (79). Subsequently, several groups discovered that mice with naturally mutated *Tlr4* gene (97, 98), either a missense point mutation (Pro→His, C3H/HeJ strain) or null mutation (C57 BL/10 ScCr strain), or with specific *Tlr4* gene deletion (TLR4$^{-/-}$) (50), exhibited insensitivity to endotoxin, demonstrating TLR4 as the sensor for bacterial endotoxin.

TLR signaling pathways

At least 11 human and 13 mouse TLRs have been cloned and they are expressed on various types of immune and nonimmune cells, such as macrophages, monocytes, dendritic cells (DCs), lymphocytes, endothelial and epithelial cells, and cardiomyocytes. TLRs are single-spanning membrane glycoproteins with an intracellular Toll/interleukin-1 receptor (TIR) domain (58).

Based on their locations in the cell, TLRs are categorized into two groups: (i) those anchored on the plasma membranes including TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10, which mainly sense lipopeptides, peptidoglycan, lipopolysaccharide (LPS), or zymosan of bacterial and fungi origins, and (ii) those located inside the cell on the endosome membranes such as TLR3, TLR7, TLR8, TLR9, TLR11, TLR12, and TLR13, which are mainly associated with nucleic acid sensing (103), such as double-stranded (ds) RNA (TLR3) (4), single-stranded RNA (TLR7/8) (45, 73, 131), and DNA (TLR9) (49). Other known nucleic acid sensors are located in the cytoplasm and include the RNA sensors—retinoic-acid-inducible gene 1 (RIG-I) and melanoma-differentiation-associated gene 5 (MDA5), and the DNA sensors—cyclic GMP-AMP synthase and absent in melanoma 2 in the cytoplasm (103).

Following ligand binding, TLRs form dimers and the resulting TIR-TIR complexes trigger the downstream signaling (Fig. 1) through the specific adaptors (84), that is, MyD88 (myeloid differentiation factor 88), TIRAP (TIR domain-containing adaptor protein), Trif (TIR domain-containing adaptor inducing IFN-β–mediated transcription factor), SARM (sterile α- and heat-armadillo-motif-containing protein), and TRAM (Trif-related adaptor molecule).

TLR signaling can be further divided into two distinct but convergent pathways: MyD88-dependent and Trif-dependent pathways. MyD88-dependent pathway is activated by all TLRs with the exception of TLR3. MyD88 pathway leads to activation of the transcription factor nuclear factor kappa B (NF-κB) and mitogen-activated protein kinases. MyD88 recruits IL-1 receptor-associated kinase (IRAK). The IRAK1-TNF receptor-associated factor 6 (TRAF6) complex then activates transforming growth factor-α activated kinase 1 (TAK1). Activated TAK1 then phosphorylates I-κB kinase beta (IKKβ), leading to phosphorylation and degradation of I-κB, which releases the NF-κB p50/p65 subunits and results in the nuclear translocation and DNA binding of NF-κB. Trif-dependent pathway is utilized by TLR3 and TLR4. It induces type I interferon (IFN) and inflammatory cytokines through the transcription factor interferon regulatory factor 3 (IRF3).

PAMPs and DAMPs

TLRs specifically bind to a wide range of pathogens such as bacteria, fungi, and viruses through "PAMPs" recognition (Table 1) (2, 58). TLRs can also act as a stress sensor in response to noninfectious tissue injury and recognize a variety of endogenous danger molecules through "DAMPs" recognition (94).

LPS, one of the best-characterized bacterial ligand, is a wall component of gram-negative bacteria. The extracellular domain of TLR4 forms a complex to act as the LPS-binding site of TLR4 (88, 89). TLR2 senses a wide range of PAMPs—including lipopeptides, peptidoglycan, and lipoteichoic acid from gram-positive bacteria. TLR2 usually forms heterodimers with TLR1 or TLR6. TLR2/6 heterodimer senses diacylated lipopeptides (125), whereas TLR1/2 distinguishes triacylated lipopeptides (127). TLR5 responds to bacterial flagella through flagellin (43). TLR11 responds to the protozoan parasite and uropathogenic bacteria through a profilin-like molecule (137, 138). TLR3 recognizes double-strand RNA, which can activate immune responses to express IFN and cytokines to exhibit antiviral and antibacterial effects. TLR7 and TLR8 recognize single-strand RNAs, as well as imidazoquinoline compounds such as guanine analogues and imiquimod (142, 143). At last, TLR9 recognizes cytidine-phosphate-guanosine (CpG) DNA motifs with unmethylated dinucleotides from bacteria and viruses (11, 96) and mitochondrial DNA (36, 76, 141).

DAMPs are produced by injured cells under both infectious (*e.g.*, sepsis) and noninfectious (*e.g.*, trauma, ischemic injury, and autoimmune disease) conditions (18, 29, 39, 64, 65, 71, 118, 149, 151). Some of the reported examples include heat-shock proteins, hyaluronic acid, glycoprotein 96 (Gp96), heparan sulfate, fibrinogen, HMGB1, RNA, DNA,
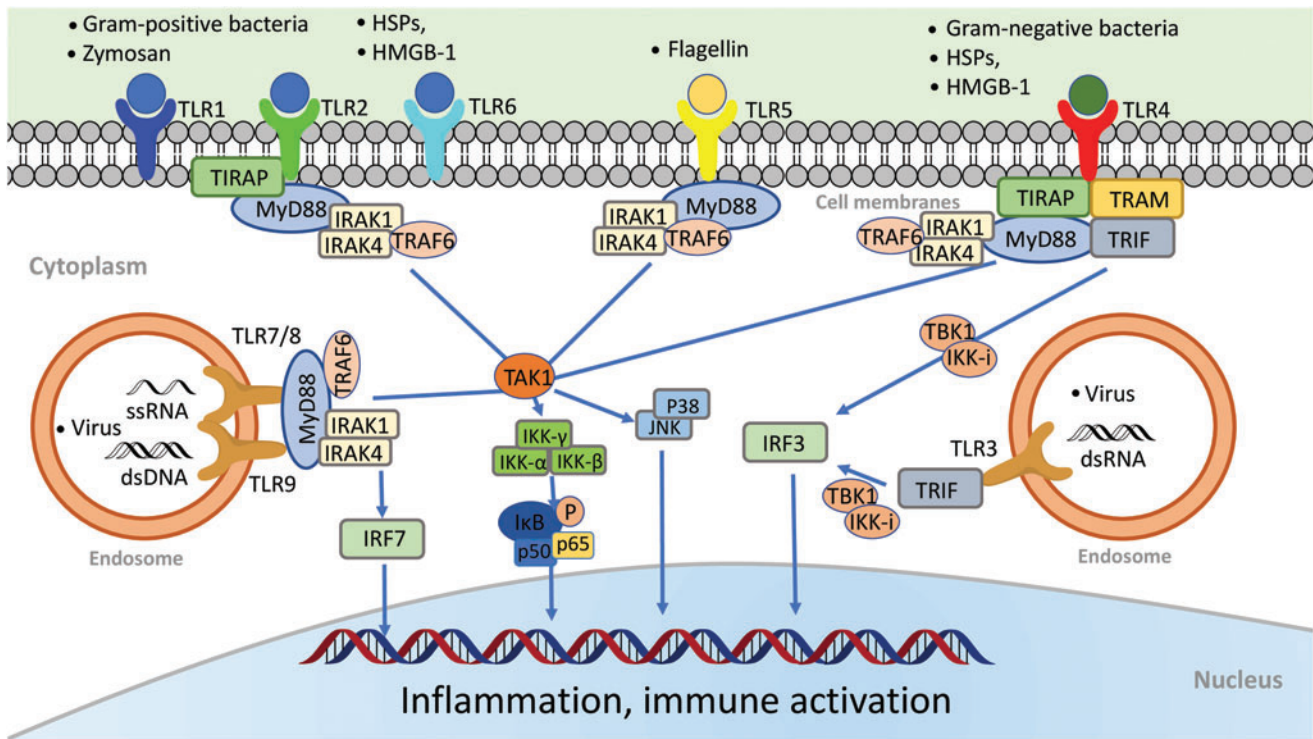
FIG. 1. TLRs: ligands and signaling pathways. All TLRs are transmembrane proteins. TLR1, TLR2, TLR4, TLR5, and TLR6 are expressed on the cell surface, whereas TLR3, TLR7/8, and TLR9 are located almost exclusively in endosomes. Different TLRs recognize different microbial components. For example, TLR2 recognizes lipopeptides or peptidoglycan, a wall component of gram-positive bacteria. It also recognizes DAMPs such as HSPs and HMGB1. TLR2 heterodimerizes either with TLR1 to recognize triacylated lipopeptide or with TLR6 to recognize diacylated lipopeptides. TLR4 senses endotoxin, a wall component of gram-negative bacteria. TLR5 senses bacterial flagellin, a protein component of flagella. TLR3 recognizes viral dsRNA, whereas TLR7 and TLR8 are the sensors for ssRNA. Finally, TLR9 senses bacterial CpG-rich hypomethylated DNA (CpG DNA) motifs. Upon ligand binding, TLRs form dimers and recruit one or more adaptor proteins, namely, MyD88, TIRAP, TRIF, or TRAM, to the cytoplasmic domains of the receptors through their TIR domain interactions. All TLRs with the exception of TLR3 signal *via* the MyD88-dependent pathway. TIRAP acts as a bridge to recruit MyD88 to TLR2 and TLR4 signaling, whereas TRIF is used in TLR3 signaling. In MyD88 signaling, MyD88 associates with IRAK4 and IRAK1. IRAK4 in turn phosphorylates IRAK1 and promotes their association with TRAF6, which serves as a platform to recruit and activate the kinase TAK1. Activated TAK1 activates the IKK complex, composed of IKKα, IKKβ, and IKKγ, which in turn catalyze phosphorylation and subsequent degradation of I-κB. I-κB degradation lets NF-κB (*i.e.*, p50/p65) free to translocate from the cytoplasm to the nucleus where it activates multiple inflammatory cytokine gene expressions. The transcription factor IRF7 is activated as the downstream signaling molecule of TLR7/8 and TLR9. It is directly phosphorylated by IRAK1 and then translocated into the nucleus to induce the expression of type I IFN-α and IFN-inducible genes. In the Trif-dependent pathway, Trif activates sTBK1 and IKK-i, resulting in the IRF3 activation and translocation into the nucleus to activate the transcription of IFN-β and IFN-inducible genes. CpG, cytidine-phosphate-guanosine; DAMP, damage-associated molecular pattern; dsRNA, double-stranded RNA; HMGB1, high-mobility group box 1; HSP, heat-shock protein; IFN, interferon; IKK, I-κB kinase; IL, interleukin; IRAK, IL-1 receptor-associated kinase; IRF, interferon regulatory factor; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor kappa B; ssRNA, single-stranded RNA; TAK1, transforming growth factor-α activated kinase 1; TIR, toll/interleukin-1 receptor; TIRAP, TIR domain-containing adaptor protein; TLR, toll-like receptor; TNF, tumor necrosis factor; TRAF6, TNF receptor-associated factor 6; TRAM, Trif-related adaptor molecule; TRIF, TIR domain-containing adaptor inducing IFN-β-mediated transcription factor. Color images are available online.

amyloid β, and oxidized low-density lipoprotein (Table 1). These endogenous pattern molecules, once released into the extracellular space, are sensed by various TLRs and elicit the host innate immune responses.

Severe acute respiratory syndrome coronavirus 2 and innate immune receptor recognition

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible virus and has caused the current global pandemic of acute respiratory disease, named ''coronavirus disease 2019'' (COVID-19), that threatens the global health and safety (52). SARS-CoV-2, like other coronaviruses, contains the dsRNA that is produced during viral genome replication and transcription (60, 146) and can be recognized by several nucleic acid sensors, including RIG-I and MDA5, in the cytoplasma (70, 109) and/or by TLR3 in the endosome (78, 124). Activation of these innate immune receptors leads to the antiviral type-I IFN signaling (68).

TABLE 1. TOLL-LIKE RECEPTORS AND LIGANDS

TLR	TLR localization	Adaptor molecule	PAMP	DAMP	References
TLR1	Plasma membrane	MyD88/TIRAP	Triacyl lipopeptide soluble factors	—	(117)
TLR2	Plasma membrane	MyD88/TIRAP	Lipoproteins, zymosan, atypical LPS, peptidoglycan, lipoarabinomannan	Hsp60, Hsp70, and glycoprotein 96, HMGB1, hyaluronic acid, matrix metalloproteinase 2, pancreatic adenocarcinoma upregulated factor	(5, 10, 19, 33, 38, 59, 91, 92, 112, 125, 127, 129)
TLR3	Endosome	TRIF	dsRNA	mRNA	(57)
TLR4	Plasma membrane and endosome	MyD88/TIRAP, TRIF/TRAM	LPS, taxol, fusion protein, envelope protein	Hsp60, Hsp70, Hsp72, Hsp22, hyaluronic acid, glycoprotein 96, heparan sulfate, fibrinogen, HMGB1	(3, 15, 25, 55, 56, 88, 89, 92, 100, 101, 106, 111, 121, 129, 130)
TLR5	Plasma membrane	MyD88	Flagellin	HMGB1	(43)
TLR6	Plasma membrane	MyD88/TIRAP	Di-acyl lipopeptides, lipoteichoic acid, β-glucan	Amyloid β and oxidized LDL	(23, 41, 63, 126)
TLR7	Endosome	MyD88	ssRNA, imidazoquinoline, loxoribine, bropirimine	ssRNA miRNA	(1, 44, 48, 73, 83, 131, 142, 143)
TLR8	Endosome	MyD88	ssRNA, midazoquinoline	ssRNA	(45, 83, 131)
TLR9	Endosome	MyD88	CpG-containing DNA, dsDNA, hemozoin	chromatin-IgG complex mtDNA	(11, 20, 36, 66, 67, 76, 81, 96, 141)
TLR10	Plasma membrane	MyD88	Lipopeptides		(42)
TLR11	Endosome	MyD88	Flagellin		(137, 138)
TLR12	Endosome	MyD88	Profilin-like protein		(99)
TLR13	Endosome	MyD88	23s ribosomal RNA		(85)

CpG, cytidine-phosphate-guanosine; DAMP, damage-associated molecular pattern; ds, double-stranded; HMGB1, high-mobility group box 1; Hsp, heat-shock protein; IFN, interferon; LDL, low-density lipoprotein; LPS, lipopolysaccharide; miRNA, microRNA; mtDNA, mitochondrial DNA; MyD88, myeloid differentiation factor 88; PAMP, pathogen-associated molecular pattern; TIRAP, TIR domain-containing adaptor protein; TLR, toll-like receptor; TRAM, Trif-related adaptor molecule; TRIF, TIR domain-containing adaptor inducing IFN-β-mediated transcription factor.

Role of TLRs in Sepsis

Extensive preclinical work has documented the possible role of TLRs in the sepsis pathogenesis. Many of these studies have taken *loss-of-function* approaches, either in genetically modified mouse models or pharmacological blocking, to manipulate TLR signaling. Others have used TLR ligands to activate TLR signaling. Table 2 summarizes some of these findings.

TLR2

Both animal and human studies support the role of TLR2 in sepsis-induced immune and multiple organ injuries, such as cardiac, endothelial, and neutrophil dysfunction, and coagulopathy (34, 47, 93, 103, 105, 114, 135, 136, 139, 145, 147, 150). For example, peptidoglycan-associated lipoprotein, a natural TLR2 ligand and a ubiquitous gram-negative bacterial membrane protein (72), inhibits cardiomyocyte function (145) and activates endothelial function and coagulation pathways (114). In mouse models of polymicrobial sepsis, TLR2$^{-/-}$ mice have better survival, improved cardiac function, attenuated blood and myocardial cytokine production

(147), improved clotting function (135), less immune cell depletion (82, 93), reduced mitochondrial reactive oxygen species production, and improved mitochondrial function (40) when compared with wild-type (WT) mice.

TLR3

TLR3 senses dsRNA and also endogenous RNA released from necrotic tissues and mediates acute inflammation (16). In a bacterial sepsis model, however, knockout of TLR3 confers a survival benefit and improved organ function (28, 35). Both bacterial and host RNAs are present in the circulation of septic mice and could potentially function as the agonists of TLR3. Interestingly, in a double-hit model, treatment with the TLR3 ligand poly(I:C) of animals before cecum ligation and puncture (CLP) enhances host immunity and improves the survival (24).

TLR4

Extensive reports have demonstrated the role of TLR4 in mediating cytokine storm, immune cell impairment, organ injury, and mortality in endotoxin shock or in polymicrobial

TABLE 2. ROLE OF TOLL-LIKE RECEPTORS IN ENDOTOXIN SHOCK AND BACTERIAL SEPSIS

TLRs	Sepsis
TLR2	TLR2$^{-/-}$ mice have better survival, markedly improved cardiac function, attenuated systemic and myocardial cytokine production (147), improved global clotting function, platelet counts, and near-normal plasma tissue factor levels compared with WT mice 24 h after CLP (135).
	Chimeric models demonstrate that it is nonhematopoietic TLR2 that contributes to neutrophil and cardiac function impairment in sepsis (150).
	Peptidoglycan-associated lipoprotein induces TLR2/MyD88 signaling cascade to activate cardiomyocyte dysfunction and inflammatory responses (145).
	TLR2 activation by bacterial lipoproteins stimulates endothelial function and coagulation pathways and contributes to endothelial activation, coagulopathy, and vascular leakage in sepsis (114).
	Complement factor B acts as a downstream effector of multiple TLRs and plays a critical role in bacterial sepsis (148).
	Circadian rhythms in immune cells mediate diurnal variations in murine sepsis severity *via* a TLR2-dependent mechanism (47).
	TLR2-mediated neutrophil depletion exacerbates bacterial sepsis (104).
	TLR2 and TLR4 contribute to sepsis-induced depletion of spleen dendritic cells (82, 93).
TLR3	Systemic deletion of TLR3 confers a survival benefit, improved cardiac function, and reduced inflammatory cell infiltration (28, 35).
TLR4	TLR4 mediates endotoxin-induced NF-κB activation, cytokine production, and cardiac dysfunction (61, 12).
	Chimeric studies indicate that TLR4 in bone marrow-derived hematopoietic cells is responsible for cardiac dysfunction during endotoxin shock (13, 119, 120).
	TLR4 signaling leads to neutrophil migration impairment and dendritic cell depletion in polymicrobial sepsis (6, 93).
	While TLR4 deletion clearly confers a survival benefit in endotoxin shock (97) or *lethal* gram-negative bacterial sepsis (105), it offers no survival benefit (26) or even deleterious effect in mild gram-negative or polymicrobial sepsis (105, 140).
TLR5	Bacterial flagellin activates innate immune response and induces marked myocardial inflammation and contractile dysfunction *via* TLR5 (107, 108).
	Flagellin-TLR5 signaling promotes endothelial repair and survival in sepsis (144).
TLR7	TLR7 mediates coagulation activation and coagulopathy in murine sepsis (135).
	Bone marrow-derived macrophages treated with TLR7 agonist (R837) exhibit marked increases in tissue factor expression (135).
	TLR7$^{-/-}$ mice have lower plasma cytokines, reduced circulatory shock, less organ injury, and significantly improved survival compared with WT mice after CLP (54).
TLR9	TLR9 contributes to cytokine production, splenic apoptosis, and kidney injury during polymicrobial sepsis (128).
	Compared with WT mice, TLR9$^{-/-}$ mice exhibited lower serum inflammatory cytokine levels, higher bacterial clearance, and greater survival after CLP (95).

CLP, cecum ligation and puncture; NF-κB, nuclear factor kappa B; WT, wild type.

sepsis (6). Animal endotoxemia induces NF-κB activation (61) that leads to robust myocardial cytokine response and myocardial dysfunction (12). This process involves signaling *via* TLR4, CD14, IRAK1, MyD88, and Trif. The endotoxin-mediated cardiac dysfunction may be an indirect effect secondary to immune cell activation rather than a direct effect on cardiomyocytes as *in vitro* treatment with LPS fails to inhibit cardiomyocyte function (120).

Chimeric models suggest that TLR4 in hematopoietic cells is responsible for cardiac dysfunction during endotoxic shock (13, 119, 120). Both MyD88 and Trif play an equally important role in endotoxin shock (31). Studies using tissue-specific MyD88 knockout models demonstrate that both cardiomyocyte- and myeloid-MyD88 play a role in mediating cardiac dysfunction and mortality during endotoxin shock (31). In animal models of bacterial sepsis, the role of TLR4 is complex and may depend on the type and severity of bacterial infection. For example, while TLR4 deletion clearly confers a survival benefit in endotoxin shock (97) or *lethal* gram-negative bacterial sepsis (105), it offers no survival benefit (26) or even deleterious effect in mild gram-negative or polymicrobial sepsis (105, 140).

These data seem to suggest that host may mobilize different innate immune mechanisms in endotoxemia and bacterial sepsis. Moreover, studies suggest that signaling *via* MyD88, but not Trif, plays a predominant role in mediating cardiac dysfunction, marked systemic inflammation, and mortality in a *lethal* model of bacterial sepsis, whereas MyD88 and Trif are equally important in systemic inflammation, organ dysfunction, and death during endotoxin shock (31).

Several possible mechanisms may explain the deleterious effect of TLR4 deficiency in low-grade bacterial sepsis. First, TLR4 is a part of the host immune defense against bacterial invasion. WT mice exhibit more robust neutrophil migratory and phagocytic functions compared with TLR4$^{-/-}$ mice. As a result of bacterial dissemination in the absence of TLR4, TLR4$^{-/-}$ mice have more cytokine production, bacterial load, and higher mortality. Second, TLR4 signaling may play a ''preconditioning-like'' role during low-grade bacterial infection. LPS pretreatment confers a cardioprotective effect against hypoxic injury (14, 17). Studies have shown that administration of low-dose endotoxin offers protection against both subsequent endotoxin challenges and poly-microbial infection (134). Finally, it should be pointed out that there is substantial difference in endotoxin sensitivity among different species. Controversies exist over whether or how well endotoxin-challenged mice mimic endotoxemia and acute inflammation in humans (22, 113, 116, 133).

TLR5

Bacterial flagellin, a TLR5 ligand, activates NF-κB-mediated inflammatory response and induces myocardial dysfunction (107, 108). *In vivo*, flagellin administration leads to cytokine storm, increased myocardial neutrophil infiltration, and reversible cardiac dysfunction.

TLR7/TLR8

In addition to sensing viral single-stranded RNA, TLR7 may also sense endogenous extracellular RNAs released from injured cells and plays a pivotal role in murine sepsis. In a CLP model of polymicrobial sepsis, studies have found an increased plasma RNA that is closely correlated with sepsis severity (149). Plasma microRNA (miRNA) array revealed upregulation of multiple miRNAs including miR-34, miR-122, miR-145, miR-146a, miR-210 (Fig. 2) (149). Tissue RNA extracts or RNA released from injured cells or miRNA mimics induces proinflammatory cytokine production and complement activation *via* a TLR7-dependent mechanism (29, 30, 149). Moreover, compared with WT mice, mice deficient of TLR7 had lower plasma cytokines, reduced circulatory shock (lower core temperature), and significantly improved survival (Fig. 3) (54). Finally, similar to humans, septic mice develop sepsis-induced coagulopathy characterized by global clotting dysfunction, severe thrombocytopenia, decreased fibrinogen, and increased plasma tissue factor (TF) and D-dimers (Fig. 4) (135). TLR7$^{-/-}$ septic mice exhibited preserved global clotting function, platelet counts, and near-normal plasma TF concentration (135).

TLR9

DNA and RNA isolated from *Staphylococcus aureus* and *Escherichia coli* induce rat cardiomyocyte dysfunction (87). Similarly, CpG-ODN, a TLR9 agonist, inhibits sarcomere shortening of isolated mouse cardiomyocytes. *In vivo*, CpG-ODN causes myocardial NF-κB activation and cytokine production. Both effects are abolished in TLR9-deficient mice (62). Compared with WT mice, TLR9$^{-/-}$ mice exhibited lower serum inflammatory cytokine levels, higher bacterial clearance, and greater survival after experimental peritonitis induced by CLP. Protection of TLR9$^{-/-}$ mice after CLP was associated with a greater number of peritoneal DCs and granulocytes than in WT controls. Adoptive transfer of TLR9$^{-/-}$ DCs was sufficient to protect WT mice from CLP and increased the influx of peritoneal granulocytes (95). Further studies indicate that host mitochondria-derived DNA may be responsible for TLR9 activation and contributes to sepsis-induced acute kidney injury (128).

Targeting TLRs in Sepsis: A Double-Edged Sword

It has long been recognized that systemic inflammatory response is a major contributor to sepsis pathogenesis leading to cardiovascular collapse, multiorgan injury, and mortality. Such systemic response is highly complex and caused by both infectious and noninfectious mediators. Manipulating this process as a therapeutic strategy, while seeming attractive and logical, has proven to be a double-edge sword. One such example is targeting TLR4. Blocking or genetically deleting TLR4 effectively protects animals from endotoxin-induced circulatory shock, cardiac depression, and high mortality (27, 31, 97, 98). Moreover, in a mouse model of *lethal* gram-negative bacterial sepsis with more than 80% mortality, TLR4 deletion or antibody blocking proves to be beneficial—damping systemic cytokine storm (TNFα and IL-6) and markedly improving the survival of septic animals (Fig. 5) (105). However, as an essential part of innate immunity, TLR4 also plays a critical role in host defense against bacterial invasion. In *nonlethal* gram-negative bacterial infection or mild-form of polymicrobial sepsis, TLR4 deletion or antibody blocking proves to be deleterious and leads to increased blood bacterial loading, worse cardiac dysfunction, and higher mortality (Fig. 5) (105, 140). Thus, it is evident that sepsis severity is an important factor in determining the outcome of TLR4-targeted sepsis intervention (Fig. 6).
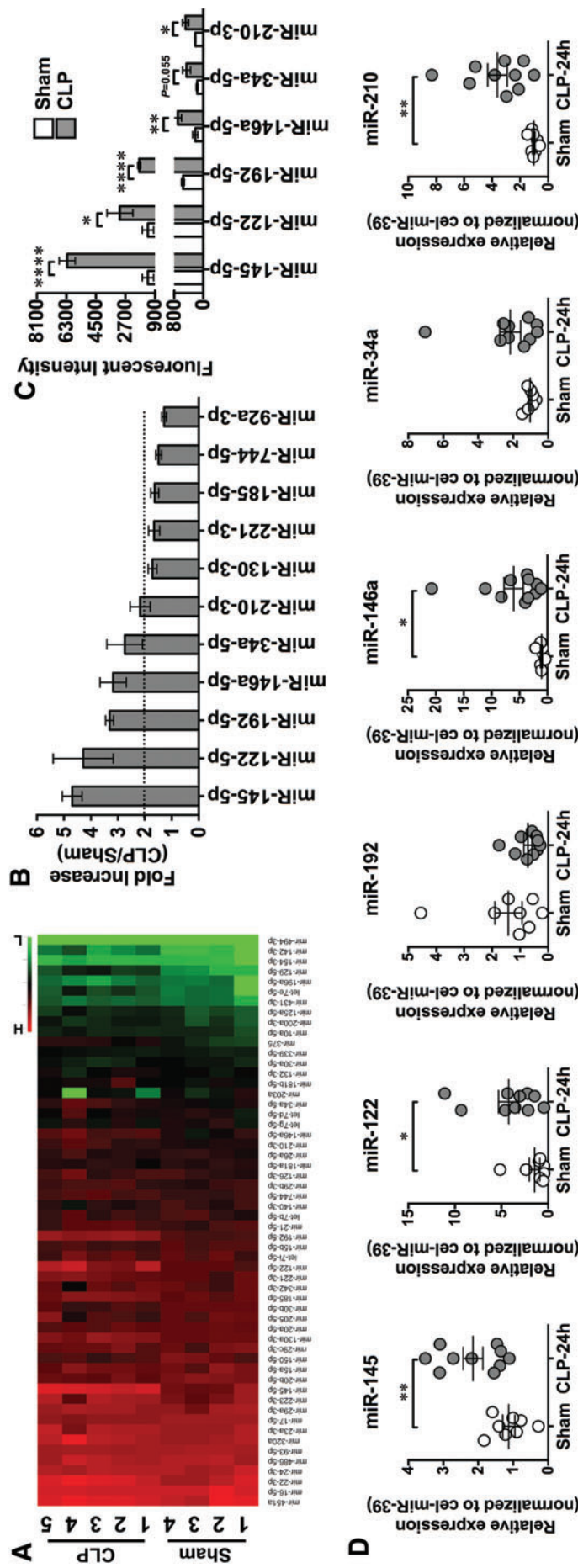
FIG. 2. Plasma miRNA profiling in septic mice. (A) Heat map of miRNA array. Mice were subjected to sham surgery ($n = 4$) or CLP ($n = 5$). At 24h, plasma was collected, and miRNAs were analyzed using a firefly miRNA array. The fluorescence intensity of 56 miRNAs was expressed from low (*green*) to high (*red*). (B) Fold change in the plasma miRNAs in CLP compared with sham mice (CLP/sham), as measured by miRNA array. (C) Mean fluorescent intensity of plasma miRNAs, as measured by miRNA array, in sham and CLP mice. Six miRNAs (miR-145, miR-122, miR-192, miR-146a, miR-34a, and miR-210) were significantly increased >2-fold in the septic mice compared with the sham control ($n = 4$ in sham group, $n = 5$ in CLP group). (D) qRT-PCR validation of miRNAs. The six target miRNAs were tested using qRT-PCR in a separate set of plasma ($n = 8$ in sham group, $n = 10$ in CLP group). $*p < 0.05$, $**p < 0.01$, $****p < 0.001$. [Zou *et al.* (149), used with permission]. CLP, cecum ligation and puncture; miRNA, microRNA; qRT-PCR, quantitative reverse-transcriptase–polymerase chain reaction. Color images are available online.
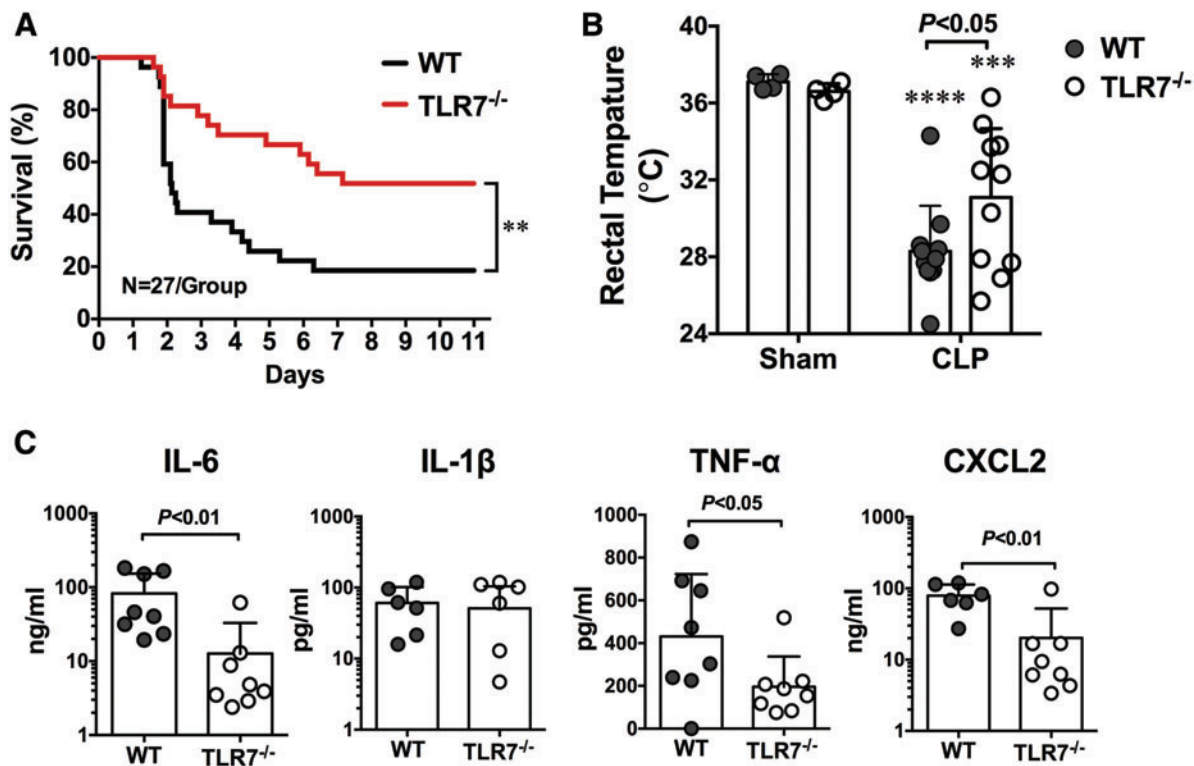
FIG. 3. TLR7-deficient mice have improved survival and attenuated plasma cytokine productions after polymicrobial sepsis. (A) Survival rate of WT and TLR7$^{-/-}$ mice during sepsis. Mice were subjected to CLP surgery and observed for survival for up to 11 days. **$p < 0.01$, $n = 27$ in WT and TLR7$^{-/-}$ group. **(B)** Rectal temperature at 24 h after CLP surgery. ***$p < 0.001$, ****$p < 0.0001$ *versus* sham group. Unequal variance *t*-test, $n = 11$ per group. **(C)** Plasma cytokines. IL-6 and TNFα are expressed as median with interquartile range and analyzed by Mann–Whitney *U* test. IL-1β and CXCL2 are expressed as mean ± SD and analyzed by unequal variance *t*-test [Jian *et al.* (54), used with permission]. WT, wild type. Color images are available online.

Targeting TLR4: The ACCESS Trials

There have been more than 100 phase 2 and phase 3 sepsis clinical trials (77). The strategies have been to manipulate the systemic inflammatory response by targeting the PAMP or endogenous inflammatory mediators such as TNFα, IL-1, eicosanoids, or platelet-activating factor, or by suppressing immune response or coagulating cascade. One of the most recent clinical trials for sepsis is eritoran. Eritoran is a synthetic lipodisaccharide, with a structure similar to LPS, that binds to MD2-TLR4 and competitively blocks LPS to TLR4 (89).

In a phase 1 trial (75), healthy volunteers were given eritoran before LPS. LPS, at a small dose of 3 ng/kg, induces very robust production of C-reactive protein, TNFα, and IL-6. Similar to animal studies, TLR4 blocking by eritoran in these healthy humans completely eliminated all clinical signs and cytokine production induced by LPS. In a subsequent phase 2 trial (122), a prospective, randomized, double-blinded, placebo-controlled multicenter study, two doses of eritoran were tested: 45 mg *versus* 105 mg over the course of 6 days, q12 hours, and given within 12 h of sepsis diagnosis.

The study patients had predicted risk of mortality between 20% and 80%. Among a total of 300 septic patients—100 in placebo and 200 in eritoran groups, 28-day all-cause mortality in the placebo group was 33.3%, low-dose group 32%, and high-dose group 26.6%, a substantial (20%) but not statistically significant reduction. Poststratification analysis of APACHE II

score on 28-day all-cause mortality revealed that patients with less severe sepsis (score 21) probably did worse with high-dose eritoran compared with placebo. In contrast, patients with high APACHE score 4—most severe sepsis—might have been benefited from eritoran, a finding very similar to what had been seen in animal studies noted above.

It was concluded that the trend toward a lower mortality rate in patients with severe sepsis and high predicted risk of mortality should be further investigated in the phase 3 trial (ACCESS trial, NCT00334828) that involved 1961 septic patients from 197 intensive care units (ICUs) worldwide (86). Like phase 2, it was a randomized, double-blinded, placebo-controlled multicenter study. Based on the phase 2 data, the study only tests high dose at 105 mg total and given every 12 h. The study patients were highly heterogenous in terms of (i) type of infection: G–, G+, mixed, fungi, and other unknown, and (ii) infection sites: mostly in the lungs, abdomen, and genitourinary track. At the end of the 5-year trial, there was no difference in the 28-day all-cause mortality, which was about 27%–28%. The 12-month mortality was also identical between the eritoran and control groups at 40%.

Sepsis Clinical Trials: Lessons Learned

The cause of the failed ACCESS trial might be multifactorial (86): (i) patient heterogeneity: the septic patients
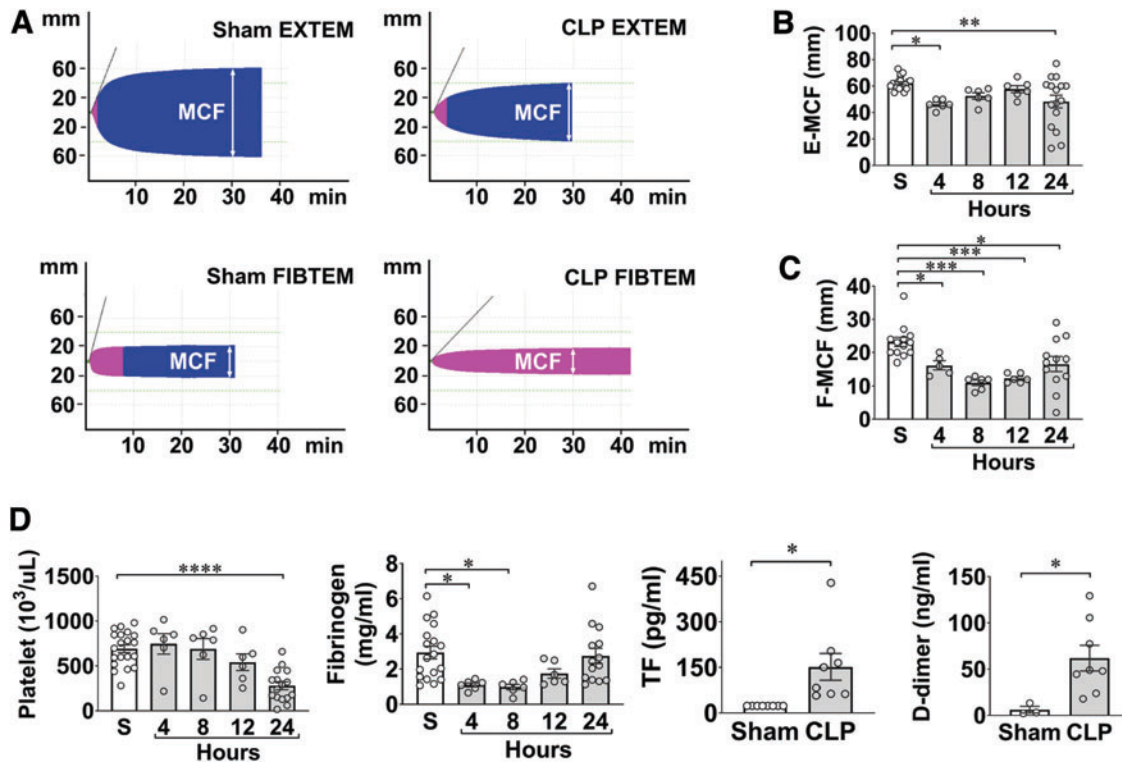
FIG. 4. **Septic mice develop global coagulopathy.** Wild-type C57BL/6 mice were subjected to CLP surgery, and killed at the indicated time points for blood collection. Sham mice were killed at 24 h. **(A)** Representative pictures of rotational thromboelastometry traces, a hemostatic viscoelastic test. Both representative EXTEM and FIBTEM traces from sham and CLP mice are shown. EXTEM to test tissue factor-initiated clot formation; FIBTEM to test EXTEM in the presence of a platelet inhibitor, cytochalasin. MCF is marked in each tracing at 30 min. **(B, C)** Time course of MCF values in EXTEM assays (E-MCF) and FIBTEM assays (F-MCF) following CLP. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. **(D)** Clotting factors in sham and septic mice. $*p < 0.05$, $**p < 0.01$, $****p < 0.0001$. [Williams *et al.* (135), used with permission]. EXTEM, extrinsic thromboelastometry; FIBTEM, fibrinogen thromboelastometry; MCF, maximum clot firmness. Color images are available online.

enrolled had various severity scores and comorbidities, which could have impacted how patients responded to the treatment; (ii) only a fraction (40.7%) of the septic patients had elevated plasma endotoxin levels. Considering TLR4 as the target, this might explain why some patients failed to respond to eritoran, although a *post hoc* analysis did not find survival benefit in endotoxin-positive patients either; (iii) lower than anticipated mortality rate in the placebo group. In the course of the trials, the sepsis mortality rate had been gradually decreased from 40% to ~27%, probably due to the
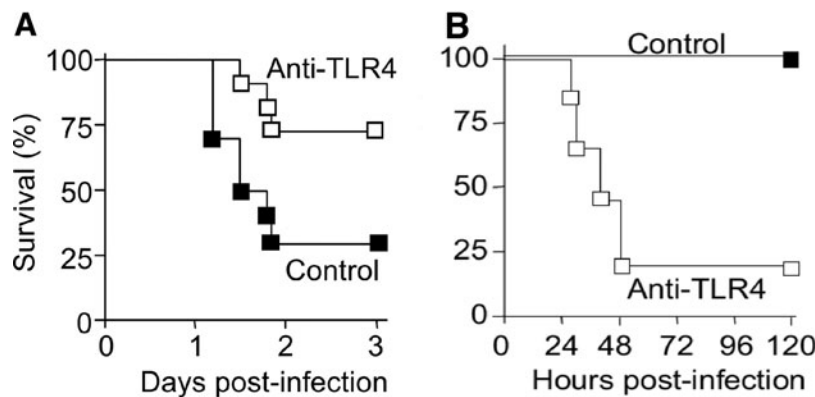

FIG. 5. **Effect of anti-TLR4 antibodies on the survival of mice with lethal or nonlethal gram-negative bacterial sepsis. (A)** Anti-TLR4 antibodies *decrease* the mortality of *lethal* gram-negative infection. BALB/c mice were injected i.p. with anti-TLR4 or control antibodies (200 mg/kg) given before an i.p. injection of 2×10^5 cfu inoculum of *Escherichia coli* O18. **(B)** Anti-TLR4 antibodies increase the mortality of nonsevere gram-negative infections. Survival of C57BL/6 mice ($n = 7$ or 8) injected intranasally with 5.6×10^2 cfu of *K. pneumoniae* and i.p. with 40 mg/kg of anti-TLR4 or control antibodies at 24 h postinfection. $p < 0.002$. [Roger *et al.* (105), modified and used with permission].
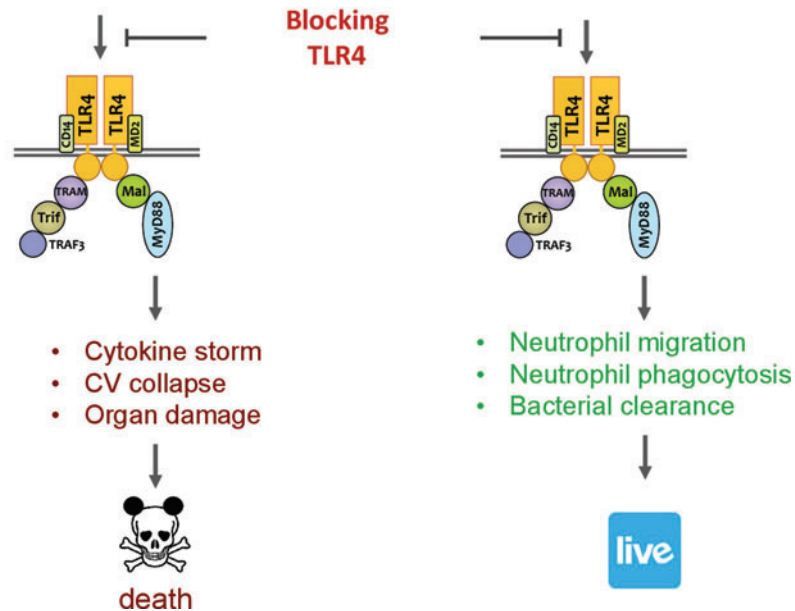
FIG. 6. Double-edged sword of targeting TLR4 for sepsis intervention. In lethal endotoxin shock or gram-negative bacterial sepsis, TLR4 plays a deleterious role in mediating cytokine storm, CV collapse, organ damage, and death. In contrast, in nonlethal or low-grade bacterial sepsis, TLR4 plays a beneficial defense role in neutrophil migration, neutrophil phagocytosis, and bacterial clearance. However, targeting TLR4 by genetic deletion or pharmacological inhibition would thus generate opposite outcomes. CV, cardiovascular. Color images are available online.

Surviving Sepsis Campaign, which, along with other interventions, might alter the responsiveness to eritoran; (iv) too late for intervention. The time for intervention was within 12 h after initial sepsis diagnosis, which might be too late for intervention as "genie was out of bottle"; and (v) different pathogens. The study patients were infected with gram-positive, gram-negative, fungi, and mixed ones. Each of these pathogens is sensed *via* different TLRs and thus understandably, blocking TLR4 alone might be less efficacious.

Moving forward, the main challenges facing sepsis research are multiple. The rodent models commonly used in sepsis research are quite different from the septic patients we see in hospital. Unlike young and healthy rodents used in most laboratory research, septic patients are often old with multiple comorbidities, such as diabetes, hypercholesterolemia, hypertension, or other systemic and metabolic diseases, and are often aggressively treated with multiple medications in ICU. These underlying conditions could profoundly impact how the body responds to infection as well as to treatment. Therefore, establishing animal models that closely simulate septic patients is of paramount importance. Septic humans are highly heterogeneous in their clinical presentations of infection (sites, pathogens, severity) and responses to treatments, the underlying comorbidities, the demographics, their genetic makeup and risk factor, and their immune response to pathogen infection. Delineating these complex biological, genetic, immunological, and clinical factors in human sepsis is essential for future sepsis intervention and trial design.

Summary

Sepsis is a deadly clinical syndrome induced by a host's dysregulated immune responses to infection. Acting *via* pattern-recognition and converging signaling pathways with a set of adaptor molecules, kinases, and transcriptional factors, TLRs play a pivotal role in host defense against microbe pathogens by launching a proinflammatory immune response. Preclinical rodent studies have established the mechanistic role of TLR signaling in sepsis pathogenesis. Targeting these innate immune receptors and manipulating host inflammatory responses in sepsis, while attractive and logical, may yield opposite results depending on the severity of sepsis at the time of intervention. Numerous clinical trials targeting innate immunity, inflammation, and coagulation, including the eritoran ACCESS trial, have failed to demonstrate therapeutic efficacy.

Future work will be needed to better understand the complex biological mechanisms of sepsis pathogenesis, establish animal sepsis models more closely related to human conditions, identify molecular basis—biochemical and immunological risk factors and biomarkers—for clinical heterogeneity of septic patients, and carefully design human trials with clear clinical and immune stratifications and various levels of clinical outcomes.

Authors' Contributions

F.C. drafted the article, L.Z. and B.W. critically edited the article, and W.C. instructed the review contents and finalized the article.

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References

1. Akira S and Hemmi H. Recognition of pathogen-
associated molecular patterns by TLR family. *Immunol
Lett* 85: 85–95, 2003.
2. Akira S, Uematsu S, and Takeuchi O. Pathogen recogni-
tion and innate immunity. *Cell* 124: 783–801, 2006.
3. Al-Ofi E, Coffelt SB, and Anumba DO. Fibrinogen, an
endogenous ligand of Toll-like receptor 4, activates
monocytes in pre-eclamptic patients. *J Reprod Immunol*
103: 23–28, 2014.
4. Alexopoulou L, Holt AC, Medzhitov R, and Flavell RA.
Recognition of double-stranded RNA and activation of
NF-kappaB by Toll-like receptor 3. *Nature* 413: 732–738,
2001.
5. Alonso H, Parra J, Malaga W, Payros D, Liu C-F, Berrone
C, Robert C, Meunier E, Burlet-Schiltz O, and Rivière M.
Protein O-mannosylation deficiency increases LprG-
associated lipoarabinomannan release by *Mycobacterium
tuberculosis* and enhances the TLR2-associated inflam-
matory response. *Sci Rep* 7: 1–14, 2017.
6. Alves-Filho JC, de Freitas A, Russo M, and Cunha FQ.
Toll-like receptor 4 signaling leads to neutrophil migra-
tion impairment in polymicrobial sepsis. *Crit Care Med*
34: 461–470, 2006.
7. Anderson KV, Bokla L, and Nusslein-Volhard C. Estab-
lishment of dorsal-ventral polarity in the *Drosophila* em-
bryo: the induction of polarity by the Toll gene product.
Cell 42: 791–798, 1985.
8. Anderson KV, Jurgens G, and Nusslein-Volhard C. Es-
tablishment of dorsal-ventral polarity in the *Drosophila*
embryo: genetic studies on the role of the Toll gene
product. *Cell* 42: 779–789, 1985.
9. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G,
Carcillo J, and Pinsky MR. Epidemiology of severe sepsis
in the United States: analysis of incidence, outcome, and
associated costs of care. *Crit Care Med* 29: 1303–1310,
2001.
10. Asea A, Rehli M, Kabingu E, Boch JA, Baré O, Auron PE,
Stevenson MA, and Calderwood SK. Novel signal trans-
duction pathway utilized by extracellular HSP70 role of
Toll-like receptor (TLR) 2 and TLR4. *J Biol Chem* 277:
15028–15034, 2002.
11. Bauer S, Kirschning CJ, Häcker H, Redecke V, Hausmann
S, Akira S, Wagner H, and Lipford GB. Human TLR9
confers responsiveness to bacterial DNA via species-
specific CpG motif recognition. *Proc Natl Acad Sci U S A*
98: 9237–9242, 2001.
12. Baumgarten G, Knuefermann P, Nozaki N, Sivasu-
bramanian N, Mann DL, and Vallejo JG. In vivo expres-
sion of proinflammatory mediators in the adult heart after
endotoxin administration: the role of toll-like receptor-4.
J Infect Dis 183: 1617–1624, 2001.
13. Binck BW, Tsen MF, Islas M, White DJ, Schultz RA,
Willis MS, Garcia JV, Horton JW, and Thomas JA. Bone

marrow-derived cells contribute to contractile dysfunction
in endotoxic shock. *Am J Physiol Heart Circ Physiol* 288:
H577–H583, 2005.
14. Brown JM, Grosso MA, Terada LS, Whitman GJ, Bane-
rjee A, White CW, Harken AH, and Repine JE. Endotoxin
pretreatment increases endogenous myocardial catalase
activity and decreases ischemia-reperfusion injury of
isolated rat hearts. *Proc Natl Acad Sci U S A* 86: 2516–
2520, 1989.
15. Byrd-Leifer CA, Block EF, Takeda K, Akira S, and Ding
A. The role of MyD88 and TLR4 in the LPS-mimetic
activity of Taxol. *Eur J Immunol* 31: 2448–2457, 2001.
16. Cavassani KA, Ishii M, Wen H, Schaller MA, Lincoln
PM, Lukacs NW, Hogaboam CM, and Kunkel SL. TLR3
is an endogenous sensor of tissue necrosis during acute
inflammatory events. *J Exp Med* 205: 2609–2621, 2008.
17. Chao W. Toll-like receptor signaling: a critical modulator
of cell survival and ischemic injury in the heart. *Am J
Physiol Heart Circ Physiol* 296: H1–H12, 2009.
18. Chen C, Feng Y, Zou L, Wang L, Chen HH, Cai JY, Xu
JM, Sosnovik DE, and Chao W. Role of extracellular
RNA and TLR3-Trif signaling in myocardial ischemia-
reperfusion injury. *J Am Heart Assoc* 3: e000683, 2014.
19. Chiu Y-C, Lin C-Y, Chen C-P, Huang K-C, Tong K-M,
Tzeng C-Y, Lee T-S, Hsu H-C, and Tang C-H. Pepti-
doglycan enhances IL-6 production in human synovial
fibroblasts via TLR2 receptor, focal adhesion kinase, Akt,
and AP-1-dependent pathway. *J Immunol* 183: 2785–
2792, 2009.
20. Coban C, Igari Y, Yagi M, Reimer T, Koyama S, Aoshi T,
Ohata K, Tsukui T, Takeshita F, and Sakurai K. Im-
munogenicity of whole-parasite vaccines against Plas-
modium falciparum involves malarial hemozoin and host
TLR9. *Cell Host Microbe* 7: 50–61, 2010.
21. Cohen J. The immunopathogenesis of sepsis. *Nature* 420:
885–891, 2002.
22. Copeland S, Warren HS, Lowry SF, Calvano SE, and Re-
mick D. Acute inflammatory response to endotoxin in mice
and humans. *Clin Diagn Lab Immunol* 12: 60–67, 2005.
23. Cot M, Ray A, Gilleron M, Vercellone A, Larrouy-
Maumus G, Armau E, Gauthier S, Tiraby G, Puzo G, and
Nigou J. Lipoteichoic acid in *Streptomyces hygro-
scopicus*: structural model and immunomodulatory activi-
ties. *PLoS One* 6: e26316, 2011.
24. Davis CG, Chang K, Osborne D, Walton AH, Ghosh S,
Dunne WM, Hotchkiss RS, and Muenzer JT. TLR3 ago-
nist improves survival to secondary pneumonia in a dou-
ble injury model. *J Surg Res* 182: 270–276, 2013.
25. Dybdahl B, Wahba A, Lien E, Flo TH, Waage A, Qureshi
N, Sellevold OF, Espevik T, and Sundan A. Inflammatory
response after open heart surgery: release of heat-shock
protein 70 and signaling through toll-like receptor-4.
Circulation 105: 685–690, 2002.
26. Echtenacher B, Freudenberg MA, Jack RS, and Mannel
DN. Differences in innate defense mechanisms in en-
dotoxemia and polymicrobial septic peritonitis. *Infect
Immun* 69: 7271–7276, 2001.
27. Ehrentraut S, Lohner R, Schwederski M, Ehrentraut H,
Boehm O, Noga S, Langhoff P, Baumgarten G, Meyer R,
and Knuefermann P. In vivo Toll-like receptor 4 antago-
nism restores cardiac function during endotoxemia. *Shock*
36: 613–620, 2011.
28. Fattahi F, Russell MW, Malan EA, Parlett M, Abe E,
Zetoune FS, and Ward PA. Harmful roles of TLR3 and

TLR9 in cardiac dysfunction developing during poly-microbial sepsis. *Biomed Res Int* 2018: 4302726, 2018.

29. Feng Y, Chen H, Cai J, Zou L, Yan D, Xu G, Li D, and Chao W. Cardiac RNA induces inflammatory responses in cardiomyocytes and immune cells via Toll-like receptor 7 signaling. *J Biol Chem* 290: 26688–26698, 2015.

30. Feng Y, Zou L, Yan D, Chen H, Xu G, Jian W, Cui P, and Chao W. Extracellular microRNAs induce potent innate immune responses via TLR7/MyD88-dependent mechanisms. *J Immunol* 199: 2106–2117, 2017.

31. Feng Y, Zou L, Zhang M, Li Y, Chen C, and Chao W. MyD88 and Trif signaling play distinct roles in cardiac dysfunction and mortality during endotoxin shock and polymicrobial sepsis. *Anesthesiology* 115: 555–567, 2011.

32. Fleischmann C, Scherag A, Adhikari NK, Hartog CS, Tsaganos T, Schlattmann P, Angus DC, and Reinhart K. Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. *Am J Respir Crit Care Med* 193: 259–272, 2016.

33. Frasnelli ME, Tarussio D, Chobaz-Péclat V, Busso N, and So A. TLR2 modulates inflammation in zymosan-induced arthritis in mice. *Arthritis Res Ther* 7: 1–10, 2005.

34. Gao JW, Zhang AQ, Wang X, Li ZY, Yang JH, Zeng L, Gu W, and Jiang JX. Association between the TLR2 Arg753Gln polymorphism and the risk of sepsis: a meta-analysis. *Crit Care* 19: 416, 2015.

35. Gao M, Ha T, Zhang X, Liu L, Wang X, Kelley J, Singh K, Kao R, Gao X, Williams D, and Li C. Toll-like receptor 3 plays a central role in cardiac dysfunction during polymicrobial sepsis. *Crit Care Med* 40: 2390–2399, 2012.

36. Garcia-Martinez I, Santoro N, Chen Y, Hoque R, Ouyang X, Caprio S, Shlomchik MJ, Coffman RL, Candia A, and Mehal WZ. Hepatocyte mitochondrial DNA drives non-alcoholic steatohepatitis by activation of TLR9. *J Clin Invest* 126: 859–864, 2016.

37. Gingo MR and Morris A. HIV infection and severe sepsis: a bitter pill to swallow. *Crit Care Med* 43: 1779–1781, 2015.

38. Godefroy E, Gallois A, Idoyaga J, Merad M, Tung N, Monu N, Saenger Y, Fu Y, Ravindran R, and Pulendran B. Activation of toll-like receptor-2 by endogenous matrix metalloproteinase-2 modulates dendritic-cell-mediated inflammatory responses. *Cell Rep* 9: 1856–1870, 2014.

39. Gong T, Liu L, Jiang W, and Zhou R. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nat Rev Immunol* 20: 95–112, 2020.

40. Gong Y, Zou L, Feng Y, Li D, Cai J, Chen D, and Chao W. Importance of Toll-like receptor 2 in mitochondrial dysfunction during polymicrobial sepsis. *Anesthesiology* 121: 1236–1247, 2014.

41. Goodridge HS, Wolf AJ, and Underhill DM. β-Glucan recognition by the innate immune system. *Immunol Rev* 230: 38–50, 2009.

42. Guan Y, Ranoa DRE, Jiang S, Mutha SK, Li X, Baudry J, and Tapping RI. Human TLRs 10 and 1 share common mechanisms of innate immune sensing but not signaling. *J Immunol* 184: 5094, 2010.

43. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, and Aderem A. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 410: 1099–1103, 2001.

44. Heil F, Ahmad-Nejad P, Hemmi H, Hochrein H, Ampenberger F, Gellert T, Dietrich H, Lipford G, Takeda K, and Akira S. The Toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily. *Eur J Immunol* 33: 2987–2997, 2003.

45. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, Lipford G, Wagner H, and Bauer S. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science* 303: 1526–1529, 2004.

46. This reference has been deleted.

47. Heipertz EL, Harper J, Lopez CA, Fikrig E, Hughes ME, and Walker WE. Circadian rhythms influence the severity of sepsis in mice via a TLR2-dependent, leukocyte-intrinsic mechanism. *J Immunol* 201: 193–201, 2018.

48. Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K, Horiuchi T, Tomizawa H, Takeda K, and Akira S. Small anti-viral compounds activate immune cells via the TLR7 MyD88–dependent signaling pathway. *Nat Immunol* 3: 196–200, 2002.

49. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, and Akira S. A Toll-like receptor recognizes bacterial DNA. *Nature* 408: 740–745, 2000.

50. Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, Takeda K, and Akira S. Cutting edge: toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J Immunol* 162: 3749–3752, 1999.

51. Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, and Vincent JL. Sepsis and septic shock. *Nat Rev Dis Primers* 2: 16045, 2016.

52. Hu B, Guo H, Zhou P, and Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol* 19: 141–154, 2021.

53. Iwasaki A and Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat Immunol* 5: 987–995, 2004.

54. Jian W, Gu L, Williams B, Feng Y, Chao W, and Zou L. Toll-like receptor 7 contributes to inflammation, organ injury, and mortality in murine sepsis. *Anesthesiology* 131: 105–118, 2019.

55. Johnson GB, Brunn GJ, Kodaira Y, and Platt JL. Receptor-mediated monitoring of tissue well-being via detection of soluble heparan sulfate by Toll-like receptor 4. *J Immunol* 168: 5233–5239, 2002.

56. Jung ID, Jeong SK, Lee C-M, Noh KT, Heo DR, Shin YK, Yun C-H, Koh W-J, Akira S, and Whang J. Enhanced efficacy of therapeutic cancer vaccines produced by co-treatment with *Mycobacterium tuberculosis* heparin-binding hemagglutinin, a novel TLR4 agonist. *Cancer Res* 71: 2858–2870, 2011.

57. Karikó K, Ni H, Capodici J, Lamphier M, and Weissman D. mRNA is an endogenous ligand for Toll-like receptor 3. *J Biol Chem* 279: 12542–12550, 2004.

58. Kawai T and Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol* 11: 373–384, 2010.

59. Kim S, Takahashi H, Lin W-W, Descargues P, Grivennikov S, Kim Y, Luo J-L, and Karin M. Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. *Nature* 457: 102–106, 2009.

60. Kindler E, Thiel V, and Weber F. Interaction of SARS and MERS coronaviruses with the antiviral interferon response. *Adv Virus Res* 96: 219–243, 2016.

61. Knuefermann P, Nemoto S, Baumgarten G, Misra A, Sivasubramanian N, Carabello BA, and Vallejo JG. Cardiac inflammation and innate immunity in septic shock: is there a role for toll-like receptors? *Chest* 121: 1329–1336, 2002.

62. Knuefermann P, Schwederski M, Velten M, Krings P, Ehrentraut H, Rudiger M, Boehm O, Fink K, Dreiner U, Grohe C, Hoeft A, Baumgarten G, Koch A, Zacharowski K, and Meyer R. Bacterial DNA induces myocardial inflammation and reduces cardiomyocyte contractility: role of toll-like receptor 9. *Cardiovasc Res* 78: 26–35, 2008.

63. Kruithof EKO, Satta N, Liu JW, Dunoyer-Geindre S, and Fish RJ. Gene conversion limits divergence of mammalian TLR1 and TLR6. *BMC Evol Biol* 7: 148, 2007.

64. Land W, Agostinis P, Gasser S, Garg A, and Linkermann A. Transplantation and damage-associated molecular patterns (DAMPs). *Am J Transplant* 16: 3338–3361, 2016.

65. Land WG, Agostinis P, Gasser S, Garg A, and Linkermann A. DAMP—induced allograft and tumor rejection: the circle is closing. *Am J Transplant* 16: 3322–3337, 2016.

66. Latz E, Schoenemeyer A, Visintin A, Fitzgerald KA, Monks BG, Knetter CF, Lien E, Nilsen NJ, Espevik T, and Golenbock DT. TLR9 signals after translocating from the ER to CpG DNA in the lysosome. *Nat Immunol* 5: 190–198, 2004.

67. Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ, Marshak-and Rothstein A. Chromatin–IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* 416: 603–607, 2002.

68. Lei X, Dong X, Ma R, Wang W, Xiao X, Tian Z, Wang C, Wang Y, Li L, Ren L, Guo F, Zhao Z, Zhou Z, Xiang Z, and Wang J. Activation and evasion of type I interferon responses by SARS-CoV-2. *Nat Commun* 11: 3810, 2020.

69. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, and Hoffmann JA. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. *Cell* 86: 973–983, 1996.

70. Li J, Liu Y, and Zhang X. Murine coronavirus induces type I interferon in oligodendrocytes through recognition by RIG-I and MDA5. *J Virol* 84: 6472–6482, 2010.

71. Li Y, Si R, Feng Y, Chen HH, Zou L, Wang E, Zhang M, Warren HS, Sosnovik DE, and Chao W. Myocardial ischemia activates an injurious innate immune signaling via cardiac heat shock protein 60 and Toll-like receptor 4. *J Biol Chem* 286: 31308–31319, 2011.

72. Liang MD, Bagchi A, Warren HS, Tehan MM, Trigilio JA, Beasley-Topliffe LK, Tesini BL, Lazzaroni JC, Fenton MJ, and Hellman J. Bacterial peptidoglycan-associated lipoprotein: a naturally occurring toll-like receptor 2 agonist that is shed into serum and has synergy with lipopolysaccharide. *J Infect Dis* 191: 939–948, 2005.

73. Lund JM, Alexopoulou L, Sato A, Karow M, Adams NC, Gale NW, Iwasaki A, and Flavell RA. Recognition of single-stranded RNA viruses by Toll-like receptor 7. *Proc Natl Acad Sci U S A* 101: 5598–5603, 2004.

74. This reference has been deleted.

75. Lynn M, Rossignol DP, Wheeler JL, Kao RJ, Perdomo CA, Noveck R, Vargas R, D'Angelo T, Gotzkowsky S, and McMahon FG. Blocking of responses to endotoxin by E5564 in healthy volunteers with experimental endotoxemia. *J Infect Dis* 187: 631–639, 2003.

76. Mallavia B, Liu F, Lefrancais E, Cleary SJ, Kwaan N, Tian JJ, Magnen M, Sayah DM, Soong A, Chen J, Saggar R, Shino MY, Ross DJ, Derhovanessian A, Lynch JP, 3rd, Ardehali A, Weigt SS, Belperio JA, Hays SR, Golden JA, Leard LE, Shah RJ, Kleinhenz ME, Venado A, Kukreja J, Singer JP, and Looney MR. Mitochondrial DNA stimulates TLR9-dependent neutrophil extracellular trap formation in primary graft dysfunction. *Am J Respir Cell Mol Biol* 62: 364–372, 2020.

77. Marshall JC. Why have clinical trials in sepsis failed? *Trends Mol Med* 20: 195–203, 2014.

78. Mazaleuskaya L, Veltrop R, Ikpeze N, Martin-Garcia J, and Navas-Martin S. Protective role of Toll-like receptor 3-induced type I interferon in murine coronavirus infection of macrophages. *Viruses* 4: 901–923, 2012.

79. Medzhitov R, Preston-Hurlburt P, and Janeway CA, Jr. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* 388: 394–397, 1997.

80. Minino AM, Heron MP, Murphy SL, and Kochanek KD. Deaths: final data for 2004. *Natl Vital Stat Rep* 55: 1–119, 2007.

81. Nasu K and Narahara H. Pattern recognition via the Toll-like receptor system in the human female genital tract. *Mediators Inflamm* 2010: 976024, 2010.

82. Navarini AA, Lang KS, Verschoor A, Recher M, Zinkernagel AS, Nizet V, Odermatt B, Hengartner H, and Zinkernagel RM. Innate immune-induced depletion of bone marrow neutrophils aggravates systemic bacterial infections. *Proc Natl Acad Sci U S A* 106: 7107–7112, 2009.

83. Nuhn L, Van Hoecke L, Deswarte K, Schepens B, Li Y, Lambrecht BN, De Koker S, David SA, Saelens X, and De Geest BG. Potent anti-viral vaccine adjuvant based on pH-degradable nanogels with covalently linked small molecule imidazoquinoline TLR7/8 agonist. *Biomaterials* 178: 643–651, 2018.

84. O'Neill LA and Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat Rev Immunol* 7: 353–364, 2007.

85. Oldenburg M, Krüger A, Ferstl R, Kaufmann A, Nees G, Sigmund A, Bathke B, Lauterbach H, Suter M, Dreher S, Koedel U, Akira S, Kawai T, Buer J, Wagner H, Bauer S, Hochrein H, and Kirschning CJ. TLR13 recognizes bacterial 23S rRNA devoid of erythromycin resistance–forming modification. *Science* 337: 1111, 2012.

86. Opal SM, Laterre PF, Francois B, LaRosa SP, Angus DC, Mira JP, Wittebole X, Dugernier T, Perrotin D, Tidswell M, Jauregui L, Krell K, Pachl J, Takahashi T, Peckelsen C, Cordasco E, Chang CS, Oeyen S, Aikawa N, Maruyama T, Schein R, Kalil AC, Van Nuffelen M, Lynn M, Rossignol DP, Gogate J, Roberts MB, Wheeler JL, and Vincent JL. Effect of eritoran, an antagonist of MD2-TLR4, on mortality in patients with severe sepsis: the ACCESS randomized trial. *JAMA* 309: 1154–1162, 2013.

87. Paladugu B, Kumar A, Parrillo JE, Der S, Osman J, Mensing J, Falvo L, Xu X, and Kumar A. Bacterial DNA and RNA induce rat cardiac myocyte contraction depression in vitro. *Shock* 21: 364–369, 2004.

88. Park BS and Lee J-O. Recognition of lipopolysaccharide pattern by TLR4 complexes. *Exp Mol Med* 45: e66-e66, 2013.

89. Park BS, Song DH, Kim HM, Choi B-S, Lee H, and Lee J-O. The structural basis of lipopolysaccharide recognition by the TLR4–MD-2 complex. *Nature* 458: 1191–1195, 2009.

90. This reference has been deleted.

91. Park H, Lee Y, Oh Y, Jung J, Park YW, Myung K, Kim K, Koh SS, and Lim D-S. Pancreatic adenocarcinoma upregulated factor promotes metastasis by regulating TLR/CXCR4 activation. *Oncogene* 30: 201–211, 2011.

92. Park JS, Svetkauskaite D, He Q, Kim J-Y, Strassheim D, Ishizaka A, and Abraham E. Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. *J Biol Chem* 279: 7370–7377, 2004.

93. Pene F, Courtine E, Ouaaz F, Zuber B, Sauneuf B, Sirgo G, Rousseau C, Toubiana J, Balloy V, Chignard M, Mira JP, and Chiche JD. Toll-like receptors 2 and 4 contribute to sepsis-induced depletion of spleen dendritic cells. *Infect Immun* 77: 5651–5658, 2009.

94. Piccinini AM and Midwood KS. DAMPening inflammation by modulating TLR signalling. *Mediators Inflamm* 2010: 672395, 2010.

95. Plitas G, Burt BM, Nguyen HM, Bamboat ZM, and DeMatteo RP. Toll-like receptor 9 inhibition reduces mortality in polymicrobial sepsis. *J Exp Med* 205: 1277–1283, 2008.

96. Pohar J, Yamamoto C, Fukui R, Cajnko M-M, Miyake K, Jerala R, and Benčina M. Selectivity of human TLR9 for double CpG motifs and implications for the recognition of genomic DNA. *J Immunol* 198: 2093–2104, 2017.

97. Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, and Beutler B. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 282: 2085–2088, 1998.

98. Qureshi ST, Lariviere L, Leveque G, Clermont S, Moore KJ, Gros P, and Malo D. Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). *J Exp Med* 189: 615–625, 1999.

99. Raetz M, Kibardin A, Sturge CR, Pifer R, Li H, Burstein E, Ozato K, Larin S, and Yarovinsky F. Cooperation of TLR12 and TLR11 in the IRF8-dependent IL-12 response to *Toxoplasma gondii* profilin. *J Immunol* 191: 4818, 2013.

100. Rallabhandi P, Phillips RL, Boukhvalova MS, Pletneva LM, Shirey KA, Gioannini TL, Weiss JP, Chow JC, Hawkins LD, and Vogel SN. Respiratory syncytial virus fusion protein-induced toll-like receptor 4 (TLR4) signaling is inhibited by the TLR4 antagonists *Rhodobacter sphaeroides* lipopolysaccharide and eritoran (E5564) and requires direct interaction with MD-2. *mBio* 3: e00218–e00212, 2012.

101. Ramirez SR, Singh-Jasuja H, Warger T, Braedel-Ruoff S, Hilf N, Wiemann K, Rammensee H-G, and Schild H. Glycoprotein 96–activated dendritic cells induce a CD8-biased T cell response. *Cell Stress Chaperones* 10: 221, 2005.

102. Rhee C, Dantes R, Epstein L, Murphy DJ, Seymour CW, Iwashyna TJ, Kadri SS, Angus DC, Danner RL, and Fiore AE. Incidence and trends of sepsis in US hospitals using clinical vs claims data, 2009-2014. *JAMA* 318: 1241–1249, 2017.

103. Roers A, Hiller B, and Hornung V. Recognition of endogenous nucleic acids by the innate immune system. *Immunity* 44: 739–754, 2016.

104. Roger T and Calandra T. TLR2-mediated neutrophil depletion exacerbates bacterial sepsis. *Proc Natl Acad Sci U S A* 106: 6889–6890, 2009.

105. Roger T, Froidevaux C, Le Roy D, Reymond MK, Chanson AL, Mauri D, Burns K, Riederer BM, Akira S, and Calandra T. Protection from lethal gram-negative bacterial sepsis by targeting Toll-like receptor 4. *Proc Natl Acad Sci U S A* 106: 2348–2352, 2009.

106. Rolland A, Jouvin-Marche E, Viret C, Faure M, Perron H, and Marche PN. The envelope protein of a human endogenous retrovirus-W family activates innate immunity through CD14/TLR4 and promotes Th1-like responses. *J Immunol* 176: 7636–7644, 2006.

107. Rolli J, Loukili N, Levrand S, Rosenblatt-Velin N, Rignault-Clerc S, Waeber B, Feihl F, Pacher P, and Liaudet L. Bacterial flagellin elicits widespread innate immune defense mechanisms, apoptotic signaling, and a sepsis-like systemic inflammatory response in mice. *Crit Care* 14: R160, 2010.

108. Rolli J, Rosenblatt-Velin N, Li J, Loukili N, Levrand S, Pacher P, Waeber B, Feihl F, Ruchat P, and Liaudet L. Bacterial flagellin triggers cardiac innate immune responses and acute contractile dysfunction. *PLoS One* 5: e12687, 2010.

109. Roth-Cross JK, Bender SJ, and Weiss SR. Murine coronavirus mouse hepatitis virus is recognized by MDA5 and induces type I interferon in brain macrophages/microglia. *J Virol* 82: 9829–9838, 2008.

110. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, Colombara DV, Ikuta KS, Kissoon N, and Finfer S. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. *Lancet* 395: 200–211, 2020.

111. Schaefer L, Babelova A, Kiss E, Hausser H-J, Baliova M, Krzyzankova M, Marsche G, Young MF, Mihalik D, and Götte M. The matrix component biglycan is proinflammatory and signals through Toll-like receptors 4 and 2 in macrophages. *J Clin Invest* 115: 2223–2233, 2005.

112. Scheibner KA, Lutz MA, Boodoo S, Fenton MJ, Powell JD, and Horton MR. Hyaluronan fragments act as an endogenous danger signal by engaging TLR2. *J Immunol* 177: 1272–1281, 2006.

113. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L, Finnerty CC, López CM, Honari S, Moore EE, Minei JP, Cuschieri J, Bankey PE, Johnson JL, Sperry J, Nathens AB, Billiar TR, West MA, Jeschke MG, Klein MB, Gamelli RL, Gibran NS, Brownstein BH, Miller-Graziano C, Calvano SE, Mason PH, Cobb JP, Rahme LG, Lowry SF, Maier RV, Moldawer LL, Herndon DN, Davis RW, Xiao W, and Tompkins RG. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci U S A* 110: 3507–3512, 2013.

114. Shin HS, Xu F, Bagchi A, Herrup E, Prakash A, Valentine C, Kulkarni H, Wilhelmsen K, Warren S, and Hellman J. Bacterial lipoprotein TLR2 agonists broadly modulate endothelial function and coagulation pathways in vitro and in vivo. *J Immunol* 186: 1119–1130, 2011.

115. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche J-D, and Coopersmith CM. The Third International Consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 315: 801–810, 2016.

116. Takao K and Miyakawa T. Genomic responses in mouse models greatly mimic human inflammatory diseases. *Proc Natl Acad Sci U S A* 112: 1167–1172, 2015.

117. Takeuchi O, Sato S, Horiuchi T, Hoshino K, Takeda K, Dong Z, Modlin RL, and Akira S. Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. *J Immunol* 169: 10–14, 2002.

118. Tang D, Kang R, Coyne CB, Zeh HJ, and Lotze MT. PAMP s and DAMPs: signal 0s that spur autophagy and immunity. *Immunol Rev* 249: 158–175, 2012.

119. Tavener SA and Kubes P. Cellular and molecular mechanisms underlying LPS-associated myocyte impairment. *Am J Physiol Heart Circ Physiol* 290: H800–H806, 2006.

120. Tavener SA, Long EM, Robbins SM, McRae KM, Van Remmen H, and Kubes P. Immune cell Toll-like receptor 4 is required for cardiac myocyte impairment during endotoxemia. *Circ Res* 95: 700–707, 2004.

121. Termeer C, Benedix F, Sleeman J, Fieber C, Voith U, Ahrens T, Miyake K, Freudenberg M, Galanos C, and Simon JC. Oligosaccharides of hyaluronan activate dendritic cells via toll-like receptor 4. *J Exp Med* 195: 99–111, 2002.

122. Tidswell M, Tillis W, Larosa SP, Lynn M, Wittek AE, Kao R, Wheeler J, Gogate J, and Opal SM. Phase 2 trial of eritoran tetrasodium (E5564), a toll-like receptor 4 antagonist, in patients with severe sepsis. *Crit Care Med* 38: 72–83, 2010.

123. Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, and Katze MG. Into the eye of the cytokine storm. *Microbiol Mol Biol Rev* 76: 16–32, 2012.

124. Totura AL, Whitmore A, Agnihothram S, Schafer A, Katze MG, Heise MT, and Baric RS. Toll-Like receptor 3 signaling via TRIF contributes to a protective innate immune response to severe acute respiratory syndrome coronavirus infection. *mBio* 6: e00638-15, 2015.

125. Triantafilou M, Gamper FG, Haston RM, Mouratis MA, Morath S, Hartung T, and Triantafilou K. Membrane sorting of toll-like receptor (TLR)-2/6 and TLR2/1 heterodimers at the cell surface determines heterotypic associations with CD36 and intracellular targeting. *J Biol Chem* 281: 31002–31011, 2006.

126. Tsan M-F. Toll-like receptors, inflammation and cancer. *Semin Cancer Biol* 16: 32–37, 2006.

127. Tsan MF and Gao B. Endogenous ligands of Toll-like receptors. *J Leukoc Biol* 76: 514–519, 2004.

128. Tsuji N, Tsuji T, Ohashi N, Kato A, Fujigaki Y, and Yasuda H. Role of mitochondrial DNA in septic AKI via Toll-like receptor 9. *J Am Soc Nephrol* 27: 2009–2020, 2016.

129. Vabulas RM, Ahmad-Nejad P, da Costa C, Miethke T, Kirschning CJ, Häcker H, and Wagner H. Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. *J Biol Chem* 276: 31332–31339, 2001.

130. Vabulas RM, Braedel S, Hilf N, Singh-Jasuja H, Herter S, Ahmad-Nejad P, Kirschning CJ, Da Costa C, Rammensee H-G, and Wagner H. The endoplasmic reticulum-resident heat shock protein Gp96 activates dendritic cells via the Toll-like receptor 2/4 pathway. *J Biol Chem* 277: 20847–20853, 2002.

131. Vollmer J, Tluk S, Schmitz C, Hamm S, Jurk M, Forsbach A, Akira S, Kelly KM, Reeves WH, Bauer S, and Krieg AM. Immune stimulation mediated by autoantigen binding sites within small nuclear RNAs involves Toll-like receptors 7 and 8. *J Exp Med* 202: 1575–1585, 2005.

132. This reference has been deleted.

133. Warren HS, Fitting C, Hoff E, Adib-Conquy M, Beasley-Topliffe L, Tesini B, Liang X, Valentine C, Hellman J, Hayden D, and Cavaillon JM. Resilience to bacterial infection: difference between species could be due to proteins in serum. *J Infect Dis* 201: 223–232, 2010.

134. Wheeler DS, Lahni PM, Denenberg AG, Poynter SE, Wong HR, Cook JA, and Zingarelli B. Induction of endotoxin tolerance enhances bacterial clearance and survival in murine polymicrobial sepsis. *Shock* 30: 267–273, 2008.

135. Williams B, Neder J, Cui P, Suen A, Tanaka K, Zou L, and Chao W. Toll-like receptors 2 and 7 mediate coagulation activation and coagulopathy in murine sepsis. *J Thromb Haemost* 17: 1683–1693, 2019.

136. Williams DL, Ha T, Li C, Kalbfleisch JH, Schweitzer J, Vogt W, and Browder IW. Modulation of tissue Toll-like receptor 2 and 4 during the early phases of polymicrobial sepsis correlates with mortality. *Crit Care Med* 31: 1808–1818, 2003.

137. Yarovinsky F, Zhang D, Andersen JF, Bannenberg GL, Serhan CN, Hayden MS, Hieny S, Sutterwala FS, Flavell RA, Ghosh S, and Sher A. TLR11 Activation of dendritic cells by a protozoan profilin-like protein. *Science* 308: 1626, 2005.

138. Zhang D, Zhang G, Hayden MS, Greenblatt MB, Bussey C, Flavell RA, and Ghosh S. A Toll-like receptor that prevents infection by uropathogenic bacteria. *Science* 303: 1522, 2004.

139. Zhang J, He Y, Song W, Lu Y, Li P, Zou L, and Zhong W. Lack of association between factor v leiden and sepsis: a meta-analysis. *Clin Appl Thromb Hemost* 21: 204–210, 2015.

140. Zhang M, Zou L, Feng Y, Chen YJ, Zhou Q, Ichinose F, and Chao W. Toll-like receptor 4 is essential to preserving cardiac function and survival in low-grade polymicrobial sepsis. *Anesthesiology* 121: 1270–1280, 2014.

141. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, and Hauser CJ. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* 464: 104–107, 2010.

142. Zhang Z, Ohto U, Shibata T, Krayukhina E, Taoka M, Yamauchi Y, Tanji H, Isobe T, Uchiyama S, Miyake K, and Shimizu T. Structural analysis reveals that Toll-like receptor 7 is a dual receptor for guanosine and single-stranded RNA. *Immunity* 45: 737–748, 2016.

143. Zhang Z, Ohto U, Shibata T, Taoka M, Yamauchi Y, Sato R, Shukla NM, David SA, Isobe T, Miyake K, and Shimizu T. Structural analyses of Toll-like receptor 7 reveal detailed RNA sequence specificity and recognition mechanism of agonistic ligands. *Cell Rep* 25: 3371–3381.e5, 2018.

144. Zhu J, Duan G, Lang L, Liu Y, Zhu J, Wang H, and Liu Y. The bacterial component flagellin induces anti-sepsis protection through TLR-5, IL-1RN and VCAN during polymicrobial sepsis in mice. *Cell Physiol Biochem* 36: 446–456, 2015.

145. Zhu X, Bagchi A, Zhao H, Kirschning CJ, Hajjar RJ, Chao W, Hellman J, and Schmidt U. Toll-like receptor 2 activation by bacterial peptidoglycan-associated lipoprotein activates cardiomyocyte inflammation and contractile dysfunction. *Crit Care Med* 35: 886–892, 2007.

146. Zielecki F, Weber M, Eickmann M, Spiegelberg L, Zaki AM, Matrosovich M, Becker S, and Weber F. Human cell tropism and innate immune system interactions of human

respiratory coronavirus EMC compared to those of severe acute respiratory syndrome coronavirus. *J Virol* 87: 5300–5304, 2013.

147. Zou L, Feng Y, Chen YJ, Si R, Shen S, Zhou Q, Ichinose F, Scherrer-Crosbie M, and Chao W. Toll-like receptor 2 plays a critical role in cardiac dysfunction during polymicrobial sepsis. *Crit Care Med* 38: 1335–1342, 2010.

148. Zou L, Feng Y, Li Y, Zhang M, Chen C, Cai J, Gong Y, Wang L, Thurman JM, Wu X, Atkinson JP, and Chao W. Complement factor B is the downstream effector of TLRs and plays an important role in a mouse model of severe sepsis. *J Immunol* 191: 5625–5635, 2013.

149. Zou L, Feng Y, Xu G, Jian W, and Chao W. Splenic RNA and MicroRNA mimics promote complement factor B production and alternative pathway activation via innate immune signaling. *J Immunol* 196: 2788–2798, 2016.

150. Zou L, Feng Y, Zhang M, Li Y, and Chao W. Non-hematopoietic toll-like receptor 2 contributes to neutrophil and cardiac function impairment during polymicrobial sepsis. *Shock* 36: 370–380, 2011.

151. Zou N, Ao L, Cleveland JC, Jr., Yang X, Su X, Cai GY, Banerjee A, Fullerton DA, and Meng X. Critical role of extracellular heat shock cognate protein 70 in the myocardial inflammatory response and cardiac dysfunction after global ischemia-reperfusion. *Am J Physiol Heart Circ Physiol* 294: H2805–H2813, 2008.

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Abbreviations Used

APACHE II = Acute Physiology And Chronic Health Evaluation II
CLP = cecum ligation and puncture
COVID-19 = coronavirus disease 2019
CpG = cytidine-phosphate-guanosine
CV = cardiovascular
DAMPs = damage-associated molecular patterns
DCs = dendritic cells
ds = double-stranded
EXTEM = extrinsic thromboelastometry
FIBTEM = fibrinogen thromboelastometry
Gp96 = glycoprotein 96
HMGB1 = high-mobility group box 1
HSP = heat-shock protein
ICU = intensive care unit
IFN = interferon
IKK = I-κB kinase
IL = interleukin
IRAK = IL-1 receptor-associated kinase
IRF = interferon regulatory factor
LDL = low-density lipoprotein
LPS = lipopolysaccharide
MCF = maximum clot firmness
MD2 = myeloid differentiation factor 2
MDA5 = melanoma-differentiation-associated gene 5
miRNA = microRNA
mtDNA = mitochondrial DNA
MyD88 = myeloid differentiation factor 88
NF-κB = nuclear factor kappa B
ODN = oligodeoxynucleotides
PAMPs = pathogen-associated molecular patterns
poly(I:C) = polyinosinic-polycytidylic acid
qRT-PCR = quantitative reverse-transcriptase–polymerase chain reaction
RIG-I = retinoic-acid-inducible gene 1
SARM = sterile α- and heat-armadillo-motif-containing protein
SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2
ss = single-stranded
TAK1 = transforming growth factor-α-activated kinase 1
TBK1 = TRAF family member-associated NF-κB activator (TANK) binding kinase-1
TF = tissue factor
TIR = toll/interleukin-1 receptor
TIRAP = TIR domain-containing adaptor protein
TLRs = toll-like receptors
TNFα = tumor necrosis factor α
TRAF6 = TNF receptor-associated factor 6
TRAM = Trif-related adaptor molecule
Trif = TIR domain-containing adaptor inducing IFN-β–mediated transcription factor
WT = wild type