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REVIEW

Severe sepsis and Toll-like receptors

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Abstract Severe sepsis dominates the mortality of non-cardiac intensive care units. The ingenious Toll-like receptor (TLR) system can recognise many infectious organisms through relatively few receptors to trigger pro-inflammatory and anti-inflammatory cytokine release. Further complexity arises from positive and negative signalling feedback loops. Severe sepsis may be a consequence of an inappropriately excessive response or inadequate endogenous negative feedback. Therapies targeting these pathways are currently being evaluated. Alternatively, in clinical scenarios such as compensatory anti-inflammatory response syndrome, chronic viral sepsis or inadequate vaccine function, TLR signalling may be inadequate. TLR agonists may augment the innate response and are being investigated.

Keywords Sepsis · Toll-like receptors · Gram-positive bacterial infections · Gram-negative bacterial infections · Viral infections · Fungal infections

Introduction

The host response to infection or sepsis represents a continuum from a localised tissue response to widespread systemic inflammation. In its most extreme form, when infection is overwhelming or the host response inappropriately excessive, then there is septic shock, hypotension, vascular dysfunction and multi-organ failure. Sepsis is a common reason for patients to present to the emergency department [1], and patients with severe sepsis, septic shock and multi-organ failure dominate the case load and mortality of non-coronary intensive care units [2]. It has been estimated that the annual incidence of severe sepsis in the USA is around 715,000, with a cost of around $16.7 billion and a crude mortality of 17–28% [3–5]. This equates to around 215,000 deaths per annum. These epidemiological data also revealed a higher incidence in older age groups, suggesting that sepsis will be an increasing healthcare problem in an ever aging population. Although sepsis is a potentially modifiable disease, specific therapies for severe sepsis are limited. Indeed, only one anti-inflammatory mediator [drotrecogin alfa (activated)] has been demonstrated to reduce mortality in clinical studies [6]. Patients who survive severe sepsis have considerable morbidity and a reduced health-related quality of life [7, 8].

Louis Pasteur was the first to link microorganisms and human disease when he elucidated the streptococcal aetiology of puerperal sepsis [9]. Subsequently, much research in sepsis has focused on the bacterial aetiology of infection. Thus, when Richard Pfeiffer described heat-stable fragments (that he called endotoxins) of *Vibrio cholerae* that caused shock in guinea pigs [10], there was a considerable effort to identify the active component of endotoxin which later transpired to be lipopolysaccharide (LPS). Tiny doses of LPS induce a sepsis-like syndrome in normal human subjects [11]. In the late 1990s, the laboratory of Bruce Beutler identified Toll-like receptor-4 (TLR4) as the receptor specific for LPS [12, 13]. This was proclaimed as a key milestone in our understanding of bacterial sepsis. Modern descriptions of sepsis also encom-

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pass viruses and fungi as agents of infectious disease. The identification of other TLR has revealed that TLR activate the innate immune response to these microorganisms also.

The TLR system represents an ingenious method of activating the immune system in response to an enormous range of infectious agents through a relatively few number of receptors. This obviates the need for previous exposure to an organism or many receptors encoded on the genome such as occurs with VDJ recombination of T cell receptors. Indeed, only ten TLR have been identified in humans. These ten TLR recognise molecular structures common to infectious organisms (pathogen-associated molecular patterns, PAMPs) that are very infrequently found in the host [14, 15]. For example, double-stranded ribonucleic acid (RNA) is rarely found in humans, but is common in many viruses and is recognised by TLR3. Similarly, LPS, lipoteichoic acid (LTA) and flagellin are exclusive to Gram-negative, Gram-positive and motile organisms, respectively. As different TLR recognise different PAMPs (Table 1), collectively, they detect most infectious organisms including protozoa, bacteria, fungi and viruses [16]. Studies regarding the specificities of ligands for various receptors have been complicated by inadvertent contamination of experimental agents. For example, it was long considered that LTA triggered TLR4 and peptidoglycan triggered TLR2 observations that are no longer considered to be accurate [17, 18]. Some ligands recognised by TLR are structurally within microorganisms. However, they are often released during organism replication or antibiotic-induced bacterial lysis [19], and thus, accessible to surface TLR receptors. Some TLR are intracellular. Intense and ongoing investigations have mapped the signalling cascades activated by the various TLR (Fig. 1). The end result is the activation of pro-inflammatory transcription factors such as nuclear factor kappa B (NF-kB), activator protein-1 (AP-1) and interferon response factor-3 (IRF-3). After gene transcription, cytokines are released, which results in the clinical manifestations of sepsis. There are other families of receptors that recognise PAMPs, which include RIG-like helicases (RLHs) and nucleotide-oligomerisation domain (NOD) receptors. These recognise pathogens that have invaded intracellularly.

Table 1 TLRs and pathogen-associated molecular patterns

Toll receptor	Micro-organism	Structural component
TLR1	Neisseria meningitides Mycobacteria	Triacyllipopeptides
TLR 2	Gram-positive bacteria	Lipoteichoic acid, lipopetpides
	Cytomegalovirus	Glycoprotein gB and gH
	Leptospirosis interrogans	Atypical lipopolysaccharide
	Porphyromonas gingivalis	Atypical lipopolysaccharide
	Yeast	Zymosan
	Measles	HA
	Herpes simplex virus	Unknown
	Varicella zoster virus	Unknown
TLR 3	Various viruses	ds RNA
TLR4	Gram negative bacteria	Lipopolysaccharide
	Respiratory Syncytial virus	Fusion protein
TLR5	Motile bacteria	Flagellin
TLR6	Mycoplasma	Diacyl lipopeptides
	Gram-positive bacteria	Lipoteichoic acid
	Yeast	Zymosan
TLR 7	Influenza A	Single-stranded RNA containing uridines and ribose
	HIV	Single-stranded RNA containing uridines and ribose
	Varicella Stomatitis virus	Single-stranded RNA containing uridines and ribose
TLR 8	HIV	Single-stranded RNA
TLR9	Viruses and bacteria	CpG-containing DNA
TLR10	Unknown	Unknown

TLR signalling is more than a linear cascade, as different receptors share adaptor proteins (Fig. 1) that link them to intracellular kinases and ultimately transcription factors. Sharing adaptor proteins creates potential for cross-talk as organisms ligate multiple receptors. At present, this is poorly defined, with only few studies examining the effects of simultaneously activating more than one receptor [20, 21]. Moreover, positive and negative feedback loops exist on the various cascades.

This review discusses the implications of TLRs for our understanding and management of sepsis. In particular, it focuses on sepsis in its most severe form, namely septic shock and multi-organ failure. It explores the therapeutic opportunities that the discovery of TLRs have heralded.

Sepsis: one disease or several?

The current consensus definition of sepsis makes no reference to the infecting organism [22]. However, Fig. 1 illustrates that different TLRs are activated by different infectious organisms and trigger different transcription factors, and hence, different genes. This implies that the host response may not only vary in its manifestations but also probably in its response to any therapeutic intervention. There are data that corroborate this concept for different bacteria [23]. Observational studies show that cytokine profiles differ according to the infecting organism. Thus, Gram-negative disease is associated with greater plasma levels of tumour necrosis factor alpha (TNF-α) and

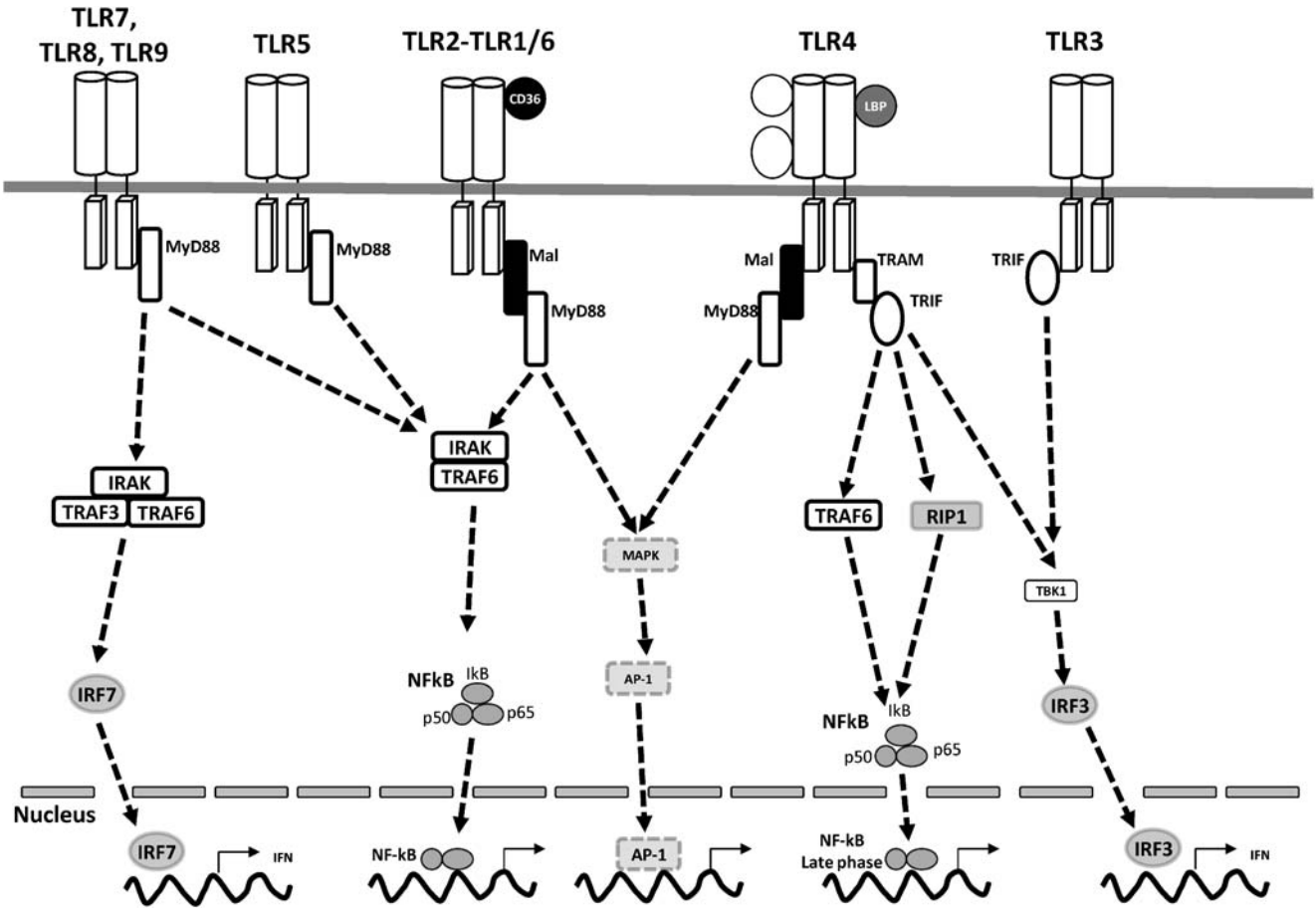

Fig. 1 Simplified intracellular TLR cascades. *AP-1* Activator protein 1, *CD* cluster differentiation molecule, *IkB* I kappa B, *IRAK* interleukin 1 receptor associated kinase, *LBP* lipopolysaccharide binding protein, *Mal* MyD88 adaptor like, *MyD88* myeloid differentiation factor 88, *NF-kB* nuclear factor kappa B, *RIP* receptor interacting protein, *TBK* TANK binding kinase, *TLR* Toll-like receptors, *TRAF* tumour necrosis factor receptor associated factor, *TRAM* translocating associating membrane protein, *TRIF* TIR domain-containing adaptor inducing interferon beta

interleukin 6 (IL-6) when compared to Gram-positive disease [24, 25]. In another study, meningococcal sepsis was associated with greater plasma IL-10 and lower interferon gamma (IFN-γ) than Gram-positive sepsis [26]. Different therapeutic responses have been detected in studies of a TNF-α receptor fusion protein, the platelet-activating factor receptor antagonist BN52021 and the bradykinin antagonist CP-0127 [27–29]. None of these therapies were demonstrated to be advantageous in sepsis. By contrast, drotrecogin alfa (activated) is equally effective in both Gram-positive and negative sepsis [6, 30].

It is likely that severe sepsis due to fungal, protozoan or viral infection differ also. However, clinical differentiation is presently difficult in early sepsis. Newer DNA-based assays may allow more expedient identification of the causative microbe [31] but are not validated presently for clinical use. Thus, the Surviving Sepsis Campaign which endeavours to harmonise the quality of care internationally by summarising existing evidence does not suggest any different therapeutic strategy according to infecting organism [32].

Bacterial sepsis

Bacteria are the most frequently isolated organisms responsible for severe sepsis [33]. Historically, Gram-negative organisms dominated the clinical picture due to many cases of intra-abdominal sepsis. However, recent data from 49 US hospitals demonstrate that Gram-positive organisms now account for 65% of nosocomial bacteraemias, with only 25% due to Gram-negative organisms alone [33]. The increased use of invasive vascular lines and implanted devices, the altered case mix of intensive care units and the emergence of Gram-positive organisms that are resistant to many antibiotics [33] may account for this change.

The search for an "LPS-receptor" leads to the discovery of TLR4. TLR1, TLR2 and TLR5 are also central to bacterial recognition. Although methodological difficulties in preparing pure ligands complicated the picture for some time, it is generally considered that pure LTA and pure LPS (with the possible exception of some cylindrical forms of LPS) ligate TLR2 and TLR4, respectively, with no cross-over [34, 35].

LTA and LPS are components of bacterial cell walls that are exclusive to Gram-positive and Gram-negative bacteria, respectively. By contrast, it is unlikely that the whole bacteria ligate the respective receptors in such an exclusive manner. The impurities in preparations of LTA and LPS alluded to above demonstrate that the cell walls can activate both types of TLR [17, 36, 37]. A lack of dependence on a single cell type has obvious evolutionary benefits. Nevertheless, the balance of the two types of signal may differ according to the Gram staining characteristics of the organism. Thus, Gram-positive infection may have a TLR2-dominant signal, whereas Gram-negative infections have a TLR4-dominant signal. This is reflected in murine data which demonstrate that TLR2-deficient mice have increased susceptibility to *Staphylococcus aureus* [38], whereas TLR4-deficient mice are particularly prone to Salmonella infection [39]. TLR1 recognises lipoproteins common to Mycobacteria. TLR5 is activated by flagellin, a component of motile bacteria [40].

In patients with sepsis, the mRNA levels and surface expression of TLR2 increase on neutrophils and monocytes with respect to healthy controls or non-septic critically ill patients [41, 42]. Whether TLR4 expression on monocytes is upregulated is debated [41, 42], although mRNA levels do increase [42]. These represent a possibly very significant positive feedback loop that may either prime the immune system for subsequent infection or amplify the response. These are scenarios often seen in clinical sepsis when the course of a second infection can be more fulminant or the response to a first infection apparently inappropriately excessive. In these scenarios, attenuating the TLR response may be therapeutically attractive.

Endogenous mechanisms exist that inhibit TLR signalling [43]. These include soluble TLR receptors that may compete for ligand. Soluble TLR4 has been detected in mice and treatment of cells with a recombinant form reduced LPS-induced IL8 release from a human monocyte cell line [44]. A human version has been implicated from RNA studies [45]. Soluble TLR2 has also been identified in humans [46]. It is able to inhibit TLR2-mediated IL-8 and TNF-α production in human cell lines. There are also many intracellular inhibitory mechanisms that include MyD88s, IRAKM, A20 and ST2L [47]. These target the adaptor proteins such as MyD88 and IRAK to attenuate TLR signalling. Many are induced over a period of hours after LPS stimulation of monocytes and/or macrophages [47]; others are expressed constitutively. MyD88s is interesting, as despite acting proximally, it appears to have an effect primarily on NF-kB but little effect on AP-1 [48]. Undoubtedly, these negative regulatory mechanisms add a layer of complexity to TLR signalling that allows greater diversity in the host response. They also provide a brake to excessive inflammation. Whether they are impaired in some patients with severe sepsis is not clear. To date, none has been exploited therapeutically.

Polymorphisms within TLR genes may influence a patient's susceptibility to bacterial infection. Two common TLR4 polymorphisms are D299G and T399I. Both are found more commonly in patients who are hyporesponsive to LPS [61]. However, the implications for susceptibility for Gram-negative disease are conflicting [49]. By contrast, there is an excess of (each individually rare) missense mutations of TLR4 in patients with systemic meningococcal disease in comparison to ethnically matched controls [50]. Relatively common polymorphisms of TLR2 include R753Q, R677W and P631H. Whilst R753Q impairs TLR-2-induced NF-kB induction in vitro [51], it is not associated with increased risk of severe *Staphylococcus aureus* infection [52]. By contrast, it may be associated with an increased susceptibility to *Mycobacterium tuberculosis* infection [53]. R677W may be associated with an increased incidence of lepromatous leprosy [54] and tuberculosis [55]. P631H may be more common in meningococcal disease [50]. A polymorphism that encodes a premature stop codon in the ligand binding domain of TLR5 may increase susceptibility to pneumonia caused by *Legionella pneumophilia* [56]; this study is rather small and warrants confirmation in a larger population. The I602S polymorphism of TLR1 reduces cellular responses in vitro but protects against leprosy, a disease due to *Mycobacteria leprae* infection [57].

Fungal sepsis

Fungi are increasingly common causes of nosocomial infection accounting for at least 11.7% of blood stream infections on the ICUs of 49 US hospitals [33]. The associated mortality was around 45%. Candida and Aspergillus are ubiquitous in humans and the environment and can cause invasive infections in the setting of breached mucosal barriers and immunological dysfunction. The increased incidence is likely related to the increased use of intravascular catheters, corticosteroids and broad-spectrum antibiotics along with a greater severity of illness, higher incidence of diabetes mellitus and the undertaking of complex surgery.

Many fungal structures are recognised by TLR4 and TLR2 (and its heterodimeric partners TLR1 and TLR6). Several of these PAMPs are based on the polysaccharides chitin (a polysaccharide synthesised from *N*-acetyl-D-glucosamine) and mannan (an α-linked polysaccharide of mannose). For example, phospholipomannan is recognised by TLR2 and TLR4, mannan by TLR4 [58] and glucuronoxylomannan by TLR4 [59]. Yeast zymosan is recognised by TLR2/TLR6 heterodimers [60].

The importance of TLR4 is illustrated by the increased dissemination and replication of *Candida albicans* in TLR4

knockout mice after intravenous infection [61]. By contrast, TLR2 knockout mice appear less susceptible to death and exhibit lower fungal loads than their wild-type counterparts [61]. In the latter study, the authors propose that this is due to increased fungicidal effects seen with reduced IL-10 production. Considering another common clinical isolate, mice with genetic deletions of TLR2, TLR4 or MyD88 are not more susceptible to die from invasive aspergillosis after intranasal inoculation. Indeed, MAP kinase signalling appeared more critical for aspergillus killing in vitro. This implies that a non-TLR2 non-TLR4 pathway is also important in invasive aspergillosis [62].

Protozoan sepsis

Malaria, a protozoan disease, represents a major health risk to 40% of the world's population, particularly in Africa, South America and South Asia [63]. There are 300–500 million cases per year and more than one million deaths per year [64]. Individuals present with anaemia and 'flu-like' symptoms, which can progress to shock, multi-organ failure and death. More than 95% of fatalities follow infection with *Plasmodium falciparum*, which is recognised by three TLRs. TLR2 and TLR4 sense glycosylphophatidylinositol, the component of the parasite which is thought to be responsible for the pathogenesis of malaria [65]. It was initially thought that TLR9 was activated by hemozoin. However, recent evidence suggests that hemozin is not a ligand for TLR9 but acts as a carrier and plays a role in presenting malarial DNA to intracellular TLR9 [66]. TLR9 activation is inhibited by chloroquine, a commonly prescribed anti-malaria drug [67]. Whether this is an advantageous or disadvantageous effect of chloroquine is unknown.

It is possible that overactivity of some or all of these TLR pathways account for the clinically more severe course. Indeed, TLR2 and TLR9 knockout mice are protected against the development of cerebral malaria and death in a mouse malarial model [68]. Mice deficient in MyD88 (which is required for TLR2, TLR4 and TLR9) did not develop cerebral malaria, but died of parasitaemia later on. In humans, heterozygosity for the S180L polymorphism in the TLR4 adaptor protein Mal is protective against severe malaria [69]. S180L heterozygotes are also afforded a degree of protection against invasive pneumococcal disease, bacteraemia and tuberculosis. By contrast, S180L homozygotes appear to have a greater incidence of invasive pneumococcal disease. S180L homozygotes have not been reported in Africa where infectious disease is prevalent. The S180L polymorphism reduced murine TLR2 signalling in vitro. This suggests that attenuated Mal signalling may be advantageous, whilst either normal or greatly reduced signalling is detrimental. Whether pharmacological inhibition of this pathway is possible and advantageous is not known. Moreover, the interactions are likely more complex, as in a study of Ghanaian children, TLR4 variants were associated with increased severity of malaria [70].

Viral sepsis

Viruses are least often identified as causing severe sepsis in critically ill patients. It is unclear whether this is due to their different pathogenicity, the different spectra of diseases seen in developed countries, complicating intercurrent bacterial infection obscuring the clinical picture or difficulties in identifying viruses in clinical samples. Certainly, bacteriological samples are frequently negative in patients with severe sepsis, and it is likely that a subset of these represent viral illnesses. Nevertheless, systemic inflammation does occur in otherwise healthy patients with Yellow fever, viral haemorrhagic fevers [71], adenoviral infections [72] and varicella. The range of pathogenic viruses is much greater in those patients with deficiencies in their immune system.

Viruses enter host cells either directly by fusion of viral membrane and the host's cell membrane or via receptor-mediated endocytosis and endosomal fusion [73]. Subsequent viral uncoating usually takes place within endosomal compartments, thus, exposing the viral genome to the intracellular environment where viral replication takes place. Recognition by either TLR, NOD or RLH proteins can occur anywhere along these steps and in several cellular compartments. TLR2 and TLR4 are expressed on cell surfaces where they recognise viral surface proteins on entry into the cell. By contrast, TLR7, TLR8 and TLR9 are expressed on endosomes where they encounter viral nucleic acid after viral uncoating. Thus, viral PAMPS activate a number of TLR [73] which depend on which ligands they express and their intracellular location [74]. Responses also vary according to host cell type, with RIG-1 being important after infection of conventional dendritic cells and fibroblasts by RNA viruses, whereas TLRs are more critical in plasmacytoid dendritic cells in blood [74]. The various TLR, RIG-1 and NOD cascades converge on the induction of type I interferons and other cytokines that mediate antiviral defences.

The first TLR found to be activated by a virus was TLR4. A fusion protein of respiratory syncytial virus, which is a common cause of lower respiratory tract infections in children, stimulated the secretion of interleukin-6 from wild-type murine cells but not from those isolated from C57BL10/ScCr or C3H/HeJ mice which are both deficient in TLR4 [75, 76]. Viruses that bind TLR2 include cytomegalovirus [77], measles [78], varicella stomatitis virus [79] and herpes simplex virus [80]. TLR7 and TLR8 recognise viral single-stranded RNA in the endosome [81] to induce an anti-viral response. TLR7 detects uridine and ribose-rich

RNA common to influenza A, human immunodeficiency virus, vesicular stomatitis virus. TLR8 is more specific to HIV and human parechovirus [82]. TLR9 is an endosomal receptor for DNA sequences that contain unmethylated CpG motifs found commonly in both viral and bacterial DNA. Finally, TLR3 is localised predominantly on cytoplasmic vesicles such as endosomes and the endoplasmic reticulum. It is responsible for recognising viruses expressing double-stranded RNA or Poly I:C, a synthetic double-stranded RNA [83]. Double-stranded RNA is released by most viruses during replication, including vesicular stomatitis virus and West Nile virus. TLR3 is unique in functioning independently from the adaptor protein MyD88 and using only TIR-domain-containing adapter-inducing interferon-β (TRIF) instead. TRIF is also used by TLR4, and this may account for some of the similarities observed between the pathophysiology of severe systemic viral infection and that of the inflammatory response to LPS [81]. As TLR3 plays a major role in anti-viral defence stimulating, it may enhance anti-viral effects [34]. However, TLR3 is implicated in the neurological complications of West Nile virus [84]. Thus, TLR3-deficient mice have a lower viral load in their brain, although a greater load in peripheral tissues. This is associated with reduced cytokine production in the brain and reduced neuropathology. It is possible that West Nile virus entry into the brain is mediated through TLR3-mediated cytokines [81, 85]. Thus, TLR3 antagonists could be beneficial in the prevention or treatment of this condition.

Viral proteins may exploit or disable TLR for their own benefit to gain cell entry or replicate [71]. For example, hepatitis C secretes a serine protease NS3/4a which cleaves two adaptor proteins: TRIF essential for TLR3 signalling and IPS-1 which is essential for RIG-1 signalling [86]. Both TLR3 and RIG-1 pathways are essential for an effective anti-viral host response against hepatitis C. Another example is provided by Vaccinia, which produces two proteins A52R and A46R that disrupt TLR signalling, and thus, minimises the host anti-viral response. A52R inhibits TRAF6 and IRAK2, thereby inhibiting TLR3, whereas A46R competitively binds MyD88, Mal, TRIF and TRIF related adaptor molecule (TRAM) reducing activation of both NF-kB and IRF pathways. Vaccinia with mutations within either protein have reduced virulence[87, 88].

Therapeutic opportunities

Inhibition of TLR signalling

Severe sepsis is characterised by a seemingly excessive pro-inflammatory response resulting in widespread vascular dysfunction and organ failure. Many investigations have aimed to damp this response either through corticosteroids or targeting pro-inflammatory cytokines. Targeting single cytokines such as TNF-α or IL-1 has not been effective [89, 90]. By contrast, supra-physiological doses of recombinant activated protein C (drotecogin alfa, Eli Lilly) have been shown to reduce mortality, possibly because its effect is directed proximally in the inflammatory process and at a point on a positive feedback loop. Could targeting TLR be equally attractive? Other scenarios where TLR inhibition may be beneficial include minimising the cerebral effects of malaria and West Nile virus. Possible approaches include the advent of small molecules that can block protein–protein interactions [91], the use of monoclonal antibodies against TLR or augmenting endogenous inhibitory mechanisms such as decoy receptors. To date, attention has centred around three TLR4 antagonists: TAK-242, E-5564 and CRX-526.

TAK242 is a novel small molecule antagonist that suppresses production of nitric oxide, IL-1β, IL-6 and TNF-α by human blood mononuclear cells in response to LPS [92, 93]. As it does not influence binding of LPS to cells and does not influence IL-1, TLR2, TLR3 or TLR9-mediated signalling, it is likely that TAK242 acts very proximally and selectively on the TLR4 pathway. Indeed, it protects mice from LPS induced mortality, reduces the rise in nitric oxide, IL-6, IL-1β, IL-10 and the chemokine MIP-2, as well as attenuating increases in markers of liver and renal dysfunction. This effect occurs even when TAK242 is administered 4 h after the LPS challenge and at a time when increased cytokine levels and clinical signs of sepsis are already evident [94]. A phase III multicentre randomised placebo-controlled study of TAK242 is currently recruiting patients within 36 h of the onset of severe sepsis (Clinicaltrials.gov reference: NCT00143611). The primary endpoint of the study will be 28-day all cause mortality. Directed at TLR4, it is likely to be primarily efficacious in bacterial and possibly only Gram-negative sepsis.

E5564, or Eritotran, is a synthetic lipodisaccharide that antagonises the effects of endotoxin [95]. Specifically, it reduces the release of TNF-α, IL-1β, IL-6, IL-8 and IL-10 from human primary cultures and human whole blood activated with LPS. It has a relatively long half-life of 40 h [96]. In vitro, E5564 dose-dependently inhibited LPS-mediated activation of primary cultures of human myeloid cells and mouse tissue culture macrophage cell lines as well as human or animal whole blood as measured by production of cytokines including tumour necrosis factor-α or interleukins-1β, IL-6, IL-8 and IL-10. E5564 also blocked the ability of Gram-negative bacteria to stimulate human cytokine production in whole blood. In vivo, E5564 blocked induction of LPS-induced cytokines and LPS or bacteria-induced lethality in primed mice. [95]. Moreover, in a double blind placebo-controlled study, a single dose of

E5564 caused a dose-dependent reduction in temperature, heart rate, clinical symptoms, C-reactive protein, white cell counts, TNF-α and IL-6 after a bolus infusion of LPS [96]. These effects do not appear to be influenced by other serum lipids such as cholesterol [97]. Currently, a phase III double blind placebo-controlled study is recruiting patients with severe sepsis within 12 h of onset (Clinicaltrials.gov reference: NCT00334828). The primary outcome measure is 28-day survival.

CRX-526 is a synthetic lipid A mimetic molecule which antagonises TLR4. It blocks the ability of LPS to trigger TNF-α release from human monocytes both in vitro and systemically in mice in vivo. [98]. It has been investigated in a murine colitis model, but remains unexamined in models of sepsis.

There are fewer data regarding TLR2 antagonists, despite the greater preponderance of Gram-positive infections in the clinical arena. An IgG_1 anti-TLR2 monoclonal antibody (T2.5) inhibits the TLR2-mediated release of TNF-α from primary murine macrophages and a human cell line. It also reduces mortality in mice stimulated with lethal doses of lipopeptide or intraperitoneal heat-killed *Bacillus subtilis* [99]. This effect still occurred even when the monoclonal antibody was administered up to 4 h after the infectious stimulus. The agent has not been studied in humans at present.

Augmentation of TLR signalling

It has been proposed that activating TLR signalling may help in situations where microbial infection appears to circumvent the immune system and enter a phase of chronic sepsis. It is possible in this setting that the TLR activation and the subsequent immune response was inadequate on initial infection. Examples of this include some viral infections and chronic or latent infections such as Lyme disease, mycobacterial infections, ricketssial disease and spirochetal disease. Moreover, some patients, often those who have been admitted for a prolonged time, appear to develop an anergy to new infectious stimuli. This has been termed the compensatory anti-inflammatory response syndrome. Indeed, it can be shown that a subset of patients develop impaired neutrophil or monocyte/macrophage function [100, 101]. Whether TLR agonists have any role in this group of patients is not known. Finally, considerable interest has focused on the use of TLR agonists as adjuvants to increase the efficacy of vaccines.

TLR3 agonists such as the synthetic compound Poly I:C [83] may have a role in the treatment of genital herpes infections caused by herpes simplex virus 2 (HSV-2). Indeed, local application of PolyI:C provided protection against genital Herpes simplex virus 2 in mice [102]. Unfortunately, toxicity limits its clinical use [103]. Thiolation appears to reduce the toxicity of PolyI:C whilst not impairing its ability to induce the release of interferons [103]. These modified compounds have not been evaluated clinically.

The imidazoquinoline drugs, imiquimod and resiquimod (R848), appear to mimic viral nucleic acid and are recognised by TLR7 and TLR8 [104, 105], resulting in the activation of type I interferons, thereby exerting an anti-viral effect [104, 105]. Indeed, these are the first TLR agonists used clinically. Imiquimod enhances the cutaneous immune response to genital warts induced by human papillomavirus, resulting in a 50–60% clearance rate [106]. Imiquimod is formulated as a cream and may also be beneficial in the treatment of cutaneous Herpes simplex infections [107], molluscum contagiosum [108] and cutaneous leishmaniasis [109]. Intravenous agents such 852A and S28690 are being evaluated as potential chemotherapy [110, 111] but have not been investigated as anti-viral agents. Finally, another TLR7/TLR8 agonist 3M-011 significantly inhibited influenza serotype H3N2 viral replication in the nasal passages even when administered after inoculation in rats; this may be a potential therapeutic agent in influenza [112].

IMO-2125 (IMO-2125, Idera Pharmaceuticals, Cambridge, MA, USA) is an oligonucleotide that is agonist at TLR9. It is currently undergoing phase I evaluation as a potential stimulus for IFN-α production and a therapy for hepatitis C infection.

CpG-containing DNA fragments are being investigated as vaccine adjuvants [113], although none are presently licensed for human use. Phase I clinical trials are being undertaken for a malaria vaccine (NCT00427167, NCT00320658) and phase II studies for a vaccine containing recombinant hepatitis B surface antigen; the latter also contains the TLR7/TLR8-activating imidazoquinoline resiquimod. Finally, a two-dose hepatitis C vaccine is currently being investigated [114, 115]. TLR4 agonists such as monophosphoryl lipid A are also being investigated as adjuvants for respiratory synctial virus, hepatitis B, and leishmania vaccines [116].

Finally, therapies directed at specific virulence factors that allow viruses to avoid the innate immune response may be valuable. Thus, NS3/4a inhibitors, such as BILN 2061 and telapravir, may be effective in chronic hepatitis C infection [117]. These have entered preclinical trials in which they seem to be well tolerated and reduce plasma HCV RNA levels [118–120]. Pharmacological inhibitors of A52R and A46R produced by vaccinia are also attractive therapeutic targets.

Implications for patients possessing polymorphisms of TLR genes

In the above discussion, several polymorphisms have been highlighted that increase or decrease a patient's susceptibility to infection or modulate the clinical course. It is possible that

with prior knowledge of a specific patient's genotype, it may be able to tailor perioperative antibiotic prophylaxis, anti-malarial agents or post-exposure anti-tuberculous drugs. However, such interventions are far from being realized, as it remains unclear as to the relevance of certain polymorphisms and some, such as those of TLR, are individually rare although collectively common. By contrast, genetic variations are likely to have greater impact on vaccine efficacy. Thus, it has been demonstrated in some countries that there is evolutionary pressure for S180L heterozygosity of the Mal adapter protein, as it is protective against severe malaria. Predominance of this polymorphism may impair the clinical efficacy of any vaccine that requires signalling via this pathway. These include those based on glycosylosphatidylinositol.

Summary

The TLR are fascinating molecules that recognise an enormous range of infectious agents and initiate the innate immune response. It is increasingly evident that multiple levels of control enrich and diversify this response. Some infectious organisms produce virulence factors that specifically circumvent this system. There is great interest in inhibitory agents that may prevent the excessive inflammatory response seen in severe sepsis, septic shock and multi-organ failure, and some have now entered clinical trials. To date, more clinical success has been achieved in settings where enhancing the TLR response is appropriate, such as with vaccine adjuvants and chronic viral infections. The corollary of the value of both approaches is that therapies must achieve appropriate activation that is neither too great nor inadequate. Nevertheless, the TLR have opened up an enormous range of therapeutic opportunities in severe sepsis, a condition that still results in the death of many thousands of patients each year.

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