Opinion TRENDS in Molecular Medicine Vol.12 No.1 January 2006

The etiology of sepsis: turned inside out

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The sepsis syndrome is thought to occur when microbial products activate Toll-like receptors stimulating widespread inflammation, in turn causing organ failure, shock and death. However, recent discoveries reveal that: (i) not only microbial substances but also endogenous molecules can trigger Toll-like receptors; (ii) Toll-like receptor-4, the endotoxin receptor, is constitutively suppressed; and (iii) the first step in sepsis could be the release of Toll-like receptor-4 from suppression. These discoveries suggest that endotoxin might not always initiate the sepsis syndrome and they explain why anti-endotoxin therapies fail. The discoveries also suggest new therapeutic targets – endogenous agonists and Toll-like receptor regulators – for treatment of sepsis.

Introduction

Sepsis, the sepsis syndrome and the systemic inflammatory response syndrome (SIRS), which are characterized by fever, tachycardia, tachypnea, shock and often death, are subjects of pre-eminent medical and scientific interest. Together, the three conditions account for greater use of critical health care and more deaths than any condition other than coronary artery disease [1]. The molecular pathways underlying sepsis also underlie ischemia [2], immune activation [3] and atherosclerosis [4]. Therefore, recent advances in basic and clinical science that apparently reveal a complete molecular picture for the etiology and pathogenesis of these conditions, and the promise of new highly specific and incisive therapies, generate acclaim in both clinical and scientific communities [5,6]. However, despite these advances, sepsis, the sepsis syndrome and SIRS continue to puzzle and challenge physicians [1,7]. Here, we consider how the canonical model of sepsis fails and how new insights could improve understanding and treatment of these disorders.

In this article, we use the term 'sepsis syndrome' to refer to a condition characterized by fever, tachycardia, tachypnea and shock, regardless of the cause. We use the term 'sepsis' to refer to the sepsis syndrome associated with bacteria in the blood or large focus of infection [8]. We use the term 'systemic inflammatory response syndrome' or SIRS to refer to the sepsis syndrome without demonstrable infection [9].

The canonical model of sepsis

What sepsis is and how to explain it have been subjects of interest for ~3000 years [10]. The ancients associated sepsis with putrescence and abnormality of the intestine that poisoned the affected individual [10,11]. Consistent with the concept of sepsis as poisoning, Magendie reported in 1823 that the sepsis syndrome could be induced by injection of extracts of purulent material [12]. In 1856, Panum extracted and distilled purulent material and found that a water-soluble but not an alcohol-soluble fraction induced the sepsis syndrome. Panum called this toxic fraction the 'putrid gift' [13]. In 1863, Pasteur discovered that putrefaction was caused by microbial infection [14]. In 1892, Pfeiffer showed that the poison is integral to bacteria, whether they are alive or dead, and he accordingly named it 'endotoxin' [15].

During the following 50 years, those interested in sepsis and the sepsis syndrome tried to identify the chemical nature of endotoxin (for a review of studies from this period, see [16]). Boivin and Mesrobeanu devised better methods for extracting, isolating and purifying endotoxin from bacterial cell walls and discovered that a lipid–polysaccharide–protein–phospholipid complex caused toxicity. Goebel *et al.* showed that this complex could be dissociated by acid into a non-toxic polysaccharide fraction and a toxic lipoprotein fraction, and by alkali into a non-toxic protein fraction and a toxic polysaccharide–lipid fraction. Westphal and Luderitz showed that toxicity is due to a protein-free macromolecule consisting of lipid and saccharide (lipopolysaccharide, LPS). The polysaccharide portion of the LPS molecule (O-antigen) varies greatly in composition, a property that Kauffmann and White exploited to serotype bacteria. The lipid portion of LPS is highly conserved, and extraction of lipid from bacteria that cannot synthesize polysaccharide proved the lipid (lipid A) to be the source of toxicity. With these advances, Pfeiffer's bacterial endotoxin became one of the most studied compounds in the world.

Elucidating the structure of endotoxin was difficult, and elucidating the basis of its toxicity was not less challenging. A crucial question was how LPS acts on cells and tissues to cause toxicity. LPS might stimulate a receptor on cell membranes, or perturb the hydrophobic

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Available online 18 November 2005

 1471-4914/$ - see front matter doi:10.1016/j.molmed.2005.11.002

environment of cell membranes [17], or both. A decisive step towards determining how LPS acts was taken by Sultzer in 1968 [18] with the discovery that an inbred strain of mice resists poisoning by endotoxin. Watson and Riblet [19] mapped resistance or lack thereof to a genetic locus, which they called *Lps*. Although these findings suggested the existence of a receptor for LPS, other studies cast doubt on this notion. For example, LPS bound equally well to lymphoid cells from *Lps*-mutant and wild-type mice [17] and it activated platelets from both mouse strains. These findings suggested that LPS does not function through a receptor, but might non-specifically disrupt cell membranes by insertion of lipid A [17], or that *Lps* controls systemic and not cellular responses.

However, two subsequent discoveries built a compelling case for the existence of an LPS receptor. In 1989, Wright *et al.* [20] identified a serum protein that could bind LPS and enhance macrophage responses to LPS; they called the protein LPS-binding protein (LBP). One year later, the same investigators discovered that the leukocyte antigen CD14 binds LBP–LPS complexes [21]. This finding led some to think that CD14 is 'the' LPS receptor; however, such a conclusion was premature. CD14 is anchored to cells only by lipid and lacks transmembrane and intracellular components needed to transmit a signal. Subsequently, CD14 was shown to be a co-receptor and not 'the' receptor for LPS [22].

Finally, in the late 1990s, a series of discoveries clarified the nature of the LPS receptor and brought the canonical model of sepsis into focus (Table 1). In 1997, Medzhitov *et al.* [23] cloned the human homolog of the *Drosophila* Toll gene, the product of which controls development of the body plan [24]. Medzhitov *et al.* reported that transfection of a mutated form of the Toll receptor homolog constitutively activated NF-κB function and thus expression of NF-κB-controlled inflammatory cytokines [23]. In 1998, Rock *et al.* [25] showed that the human homolog of *Drosophila* Toll consists of a family of Toll-like receptors (TLR). Also in 1998, Poltorak *et al.* [26] discovered that the *Lps* locus encodes the mouse homolog of TLR4, directly linking endotoxin poisoning and sepsis to LPS acting on TLR4. Various investigators showed that TLR are stimulated by microbial products [27], which were referred to as pathogen-associated molecular patterns (PAMPs) [28]. That LPS binds to cell surface structures other than TLR4 [21,29] and that it activates complement [30] offer alternative explanations for the non-specific membrane interactions noted earlier.

Table 1. Key references uncovering the molecular basis of sepsis

Authors	Findings	Refs
Panum	The 'sepsis-inducing substance' is a water-soluble 'toxin' in purulent material	[13]
Pfieffer	The toxin is integral to bacterial cell walls; hence the name 'endotoxin'	[15]
Sultzer	Some inbred mice are resistant to endotoxin, hence susceptibility is a genetic trait	[18]
Watson and Riblet	LPS susceptibility maps to the '*Lps*' locus	[19]
Medzhitov *et al.*	The human homolog of the *Drosophila* Toll gene is cloned and activity of the gene product is linked to inflammation	[23]
Rock *et al.*	Toll-like receptors are encoded by a 'TLR family' of genes	[25]
Poltorak *et al.*	The mouse *Lps* locus encodes TLR4, linking sepsis to endotoxin acting on TLR4	[26]

The canonical model of sepsis emerged from these discoveries in microbiology, biochemistry and genetics over a period of 100 years. The 'putrid gift' was shown to be LPS or a related PAMP, which act on TLR encoded by the *Lps* locus delivering archetypal signals that cause the fever, shock and death of sepsis (Figure 1). This canonical model has been acclaimed [11] and yet, as we shall consider, it fails to explain the sepsis syndrome.

Limitations to the canonical model of sepsis

Etiology of SIRS

The etiology of SIRS poses an important challenge to the canonical model of sepsis. SIRS is commonly observed in conditions such as cancer, pancreatitis and multi-organ trauma; it exhibits pathophysiological features that are indistinguishable from those of sepsis [9] and it requires TLR4 [31]. But clinicians and scientists have been puzzled by how SIRS arises without infection, or a source of LPS or other PAMPs [7]. Some have suggested that SIRS results from occult infection, perhaps from defects in the intestinal barrier [32]. Finding LPS in the blood of some patients with severe trauma or burns supported this idea [33]. However, LPS is not detected in most subjects with SIRS and the level of LPS predicts neither the severity nor the outcome of SIRS in humans or in mice [33]. Thus, something other than LPS or PAMPs probably causes SIRS.

Bacterial infection and the epidemiology of sepsis

Another challenge to the canonical model of sepsis stems from the epidemiology of the condition: the sepsis syndrome more often complicates infection caused by Gram-positive bacteria or fungi, which do not produce LPS, than those caused by Gram-negative bacteria, which do [1]. This apparent contradiction seems to be easily explained because Gram-positive bacteria produce peptidoglycan and lipoteichoic acid and fungi produce zymosan, which can stimulate TLR2 and TLR6, generating the same intracellular signals as TLR4 [27].

However, there are reasons to question whether peptidoglycan or lipoteichoic acid induce the sepsis syndrome. Peptidoglycan can activate inflammatory responses in human cells *in vitro* [34] and can induce the sepsis syndrome in rats [35], but the quantity of peptidoglycan needed to induce the syndrome is large (10 mg per kilogram body weight). Furthermore, peptidoglycan, similar to zymosan, might act via complement activation [36] rather than directly via TLR.

Lipoteichoic acid seems even less likely than peptidoglycan to cause the sepsis syndrome. Although highly purified lipoteichoic acid triggers release of tumor necrosis factor α (TNFα) from isolated human monocytes, this release is potently inhibited by proteins in the blood [37]. Indeed, Yipp *et al.* [38] showed that administration of up to 50 mg of lipoteichoic acid per kilogram body weight fails to induce any sign of SIRS in mice. Lipoteichoic acid might act in synergy with peptidoglycan [39]; however, the

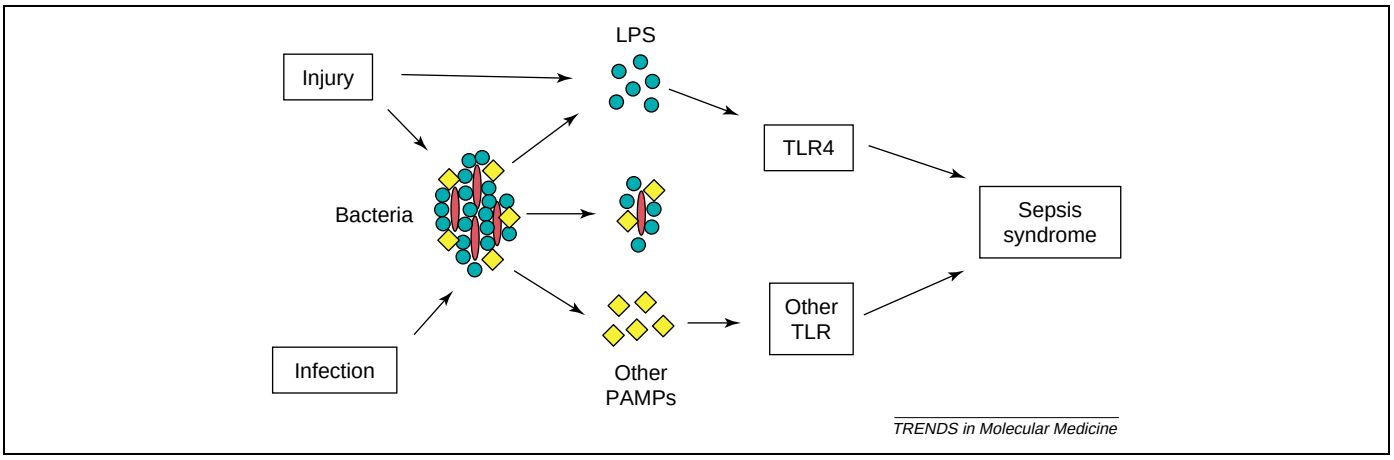

Figure 1. The canonical model of sepsis. This model emerged following research spanning >150 years. Injury or infection of tissues leads to entry of microorganisms such as bacteria. Microorganisms subsequently introduce poisons consisting of LPS or other PAMPs, which act on TLR. In some cases, injury introduces bacterial poisons without infection, causing sepsis syndrome or SIRS. TLR deliver cellular signals that ultimately lead to fever, tachycardia, shock and death, which characterize the sepsis syndrome.

lipoteichoic acid used in synergy studies was contaminated by LPS [40]. Thus, it is not clear whether peptidoglycan or lipoteichoic acid actually induce the sepsis syndrome, or whether they do so by stimulating TLR.

Role of LPS in sepsis

Although LPS can induce the sepsis syndrome when injected into animals [41] and humans [16,42], it has been difficult to demonstrate that LPS causes the syndrome in those infected with Gram-negative bacteria. As with SIRS, the level of LPS in the blood of septic patients does not predict the manifestations of sepsis or its outcome [43], and agents that block LPS do not lessen the manifestations or improve the outcome of sepsis [44,45]. For example, McCloskey *et al.* [46] showed that 32% of patients with Gram-negative sepsis treated using anti-LPS antibodies and 33% of control subjects died. Furthermore, the outcome of sepsis is not improved by LPS-neutralizing agents such as taurolidine or polymyxin B [44]. Of course, Gram-negative bacteria might have agonists other than LPS for TLR4, or for other TLR. However, this would cast doubt on the role of LPS and TLR4 in the canonical model of sepsis.

Role of TLR4 in sepsis

The classical model of Gram-negative sepsis places TLR4 in a pivotal position: when stimulated by LPS or other PAMPs from microorganisms, TLR4 causes fever, shock and death in sepsis. This concept is supported by the fact that Sultzer's mutant mice (C3H/HeJ) [41], which have non-functional TLR4, and mice lacking TLR4 [47] do not develop shock and do not die when given LPS. However, when C3H/HeJ mice are infected with Gram-negative bacteria, the manifestations of sepsis worsen and the rate of death increases. Thus, the LD50 (defined as the amount of a material, given all at once, that causes death of 50% of the animals) for virulent *Escherichia coli* in C3H/HeJ mice is less than ten organisms, whereas the LD50 for wild-type mice is 10 000 organisms [48]. Thus, TLR4 appears to protect against rather than cause shock in sepsis.

TLR4 also paradoxically protects humans from Gram-negative infection. Smirnova *et al.* [49] found that TLR4 mutations, which are rare and probably disadvantageous in humans [50], are over-represented in patients with meningococcal meningitis or septicemia. Similarly, Lorenz *et al.* [51] genotyped 91 patients with septic shock and found that some of those with the most severe manifestations had TLR4 mutations (Asp299Gly and/or Thr399Ile) that impaired responses to LPS [52]. Thus, contrary to the predictions of the canonical model of sepsis, TLR4 function might prevent the sepsis syndrome.

Increased prevalence or severity of the sepsis syndrome in animals and humans with defective TLR4 function might be explained if TLR4 both protects the host (e.g. by sequestering infectious organisms) and causes manifestations of sepsis when protection is overwhelmed (e.g. those organisms escape localization) [53]. However, if TLR4 protects against sepsis and the sepsis syndrome, then treatment with anti-LPS therapies should make sepsis and the sepsis syndrome worse, by impairing the protection conferred by TLR4. In fact, anti-LPS therapies used in clinical trials do not fulfill this prediction: the manifestations and mortality of sepsis are neither better nor worse in those treated with anti-LPS agents [46]. Moreover, the notion that LPS acts on TLR4 to contain infection does not explain why animals with mutated TLR4 have an increased rate of death from Gram-positive infection [54]. If LPS does indeed act on TLR4 to contain infection, then one might justly criticize those companies, investigators and regulatory agencies that conducted and sanctioned the trials of anti-LPS agents.

TLR and adaptive immunity

Another challenge to the canonical model of sepsis concerns the idea that innate immunity via TLR helps initiate T-cell responses [6,55]. Activation of naïve T cells requires their interaction with activated dendritic cells, and TLR provide the most reliable way to activate dendritic cells. Indeed, some have considered TLR to be a receptor for 'danger signals' [56] that link innate to adaptive immunity. However, T cells attack mainly

viruses, tumors and transplants, none of which releases PAMPs. Some viruses have double-stranded RNA that can stimulate TLR3 [57] or single-stranded RNA that can stimulate TLR7 or TLR8 [58,59]. However, the dendritic cells that initiate antiviral responses do not express TLR3 [60] and respond to single-stranded RNA only following active viral infection [59] or if the RNA is complexed to a lipid carrier [58]. Thus, the action of known PAMPs on TLR cannot explain how cell-mediated immunity arises against viruses, tumors and transplants.

Addressing the challenges to the canonical model of sepsis

Endogenous agonists

Some of the most difficult challenges to the canonical model of sepsis might be addressed if endogenous substances instead of LPS or other PAMPs could activate TLR. Activation of TLR by endogenous agonists could explain how the sepsis syndrome arises in Gram-positive and fungal infections, and in Gram-negative infections when little LPS is present. It could also explain the etiology of SIRS and how TLR initiate immune responses to viruses, tumors and transplants [3,61].

The concept of endogenous agonists emerged in part from efforts to explain how dendritic cells are activated in the absence of infection. We found that heparan sulfate, a biologically active saccharide released from cell surfaces and extracellular matrices by almost every type of inflammation [62,63], activates dendritic cells [64] and promotes alloimmune responses [65] as fully as LPS does. Moreover, heparan sulfate and fragments of hyaluronic acid, a related saccharide, act via TLR4 [3,66] (Table 2). Some have questioned whether these agonists might have been contaminated with minute amounts of LPS [27]. However, this possibility was excluded because selective digestion of heparan sulfate abrogates agonist activity [67], whereas LPS antagonists do not [3,31]. Indeed, because LPS is almost always contaminated with other substances [68], similar objections might be raised to the canonical model.

Table 2. Some endogenous activators of Toll-like receptors [82]

Endogenous agonist of TLR	Receptor	Location
Heparan sulfate	TLR4	Extracellular matrix, cell surfaces
Hyaluronic acid	TLR4	Extracellular matrix, synovium
Fibronectin extra-domain A	TLR4	Extracellular matrix, serum
Heat-shock protein 60	TLR4	Mitochondria
Heat-shock protein 70	TLR2 and TLR4	Cytoplasm
Gp96	TLR2 and TLR4	Endoplasmic reticulum
β-Defensin 2	TLR4	Epithelial surfaces
Fibrinogen	TLR4	Serum
Surfactant protein-A	TLR4	Lung epithelium
Chromatin–IgG complexes	TLR9	Nucleus and serum
Lauric acid [83]	TLR4	Serum
Biglycan [84]	TLR2 and TLR4	Extracellular matrix, macrophages

Because heparan sulfate can activate TLR4, it was logical to ask whether it can induce SIRS. We have shown that administration of heparan sulfate induces SIRS in mice with functional TLR4 but not in mice with non-functional TLR4 [31]. SIRS was not caused by contamination of heparan sulfate, because anti-LPS factor of the horseshoe crab *Limulus* blocked SIRS induced by injection of LPS but had no impact on SIRS induced by heparan sulfate. Thus, endogenous agonists link innate to adaptive immunity and explain how the sepsis syndrome occurs in the absence of infection.

Endogenous agonists and control of TLR4

Endogenous agonists for TLR4 might solve some conceptual problems, but they raise two difficult questions. How do endogenous agonists become available to stimulate TLR4? And what prevents SIRS from occurring spontaneously in healthy individuals if endogenous agonists and TLR4 exist naturally at the same place and time?

To determine how TLR4 and endogenous agonists might interact, we developed a model system in which cells expressing TLR4 and co-receptors are grown in a microenvironment containing heparan sulfate [67]. Cells bearing TLR4 are strikingly resistant to LPS or heparan sulfate if these cells are in a microenvironment containing heparan sulfate. However, when the cells are first treated with elastase, a protease released from activated neutrophils in inflammation that cleaves the core proteins of heparan sulfate proteoglycan [63], TLR4 is fully activated by heparan sulfate or LPS. Thus, contrary to the simple model in Figure 1, TLR4 is not necessarily ready to respond to LPS or other agonists but is naturally constrained from signaling; signaling might first require release from inhibition, perhaps through activation of complement and/or recruitment of neutrophils. Furthermore, the inflammation that releases TLR4 from inhibition can also liberate enough heparan sulfate to serve as an agonist [67]. This suggests that, in at least some circumstances, proteases and not PAMPs could incite the sepsis syndrome (Figure 2).

Because administration of LPS causes sepsis syndrome, one might reasonably ask whether suppression of TLR4 is biologically important. In fact, suppression of TLR4 explains several curious aspects of responses to LPS. First, although large doses of LPS can cause sepsis syndrome, LPS is usually injected with D-galactosamine, which amplifies responses 1000-fold [69]. How D-galactosamine 'conditions' the response to LPS is not precisely known, but the conditioning is specific for TLR4 because mice that lack TLR4 are not harmed by D-galactosamine [31]. Bolmer *et al.* [70] showed that D-galactosamine reduces the concentration of two protease inhibitors in serum. We suspect that this decrease in protease inhibition might relieve suppression of TLR4. Second, in the absence of D-galactosamine, larger doses of LPS might activate both platelets [71] and complement [30], thus stimulating protease activity. Humans are thought to be more sensitive to LPS than rodents and some non-human primates [72]. Yet, cells from species with different sensitivities to LPS respond similarly to it *in vitro* [3,73]. This discrepancy between *in vivo* and *in vitro* sensitivity

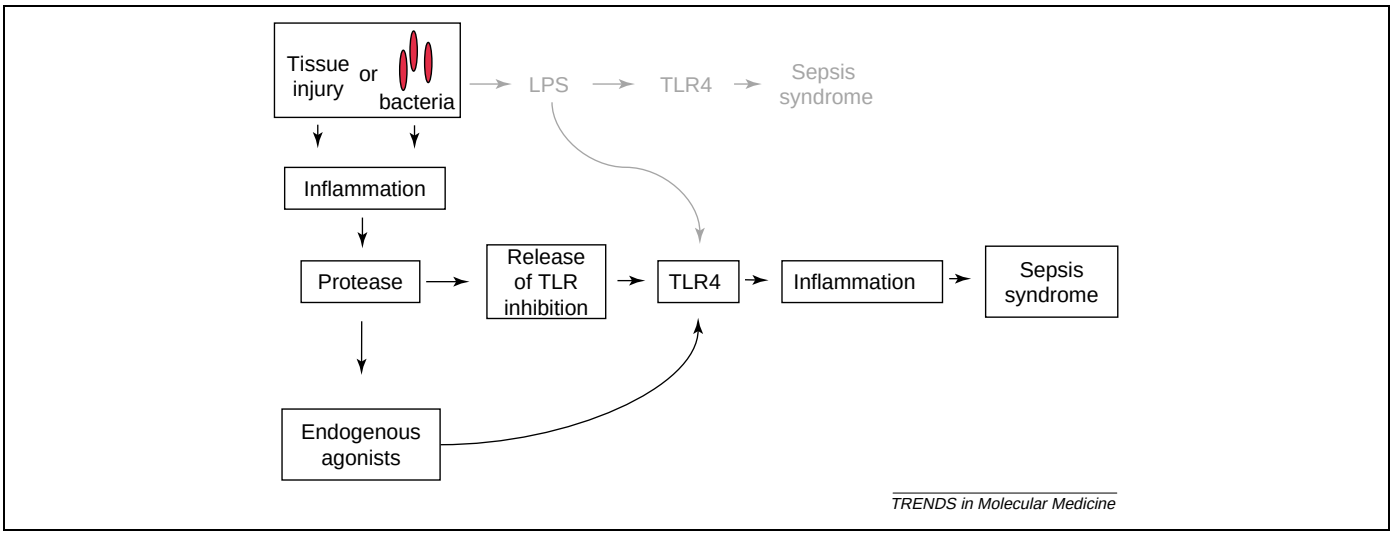

Figure 2. A new model of sepsis. This model of sepsis suggests that TLR4, and perhaps other TLR, are not ready to respond to PAMPs such as LPS, but must first be released from constitutive suppression. Injury or infection incite inflammation, which activates one or more proteases. Protease activation releases TLR4 from constitutive inhibition, and also liberates endogenous agonists of the receptor. Once released, TLR responds to endogenous and/or exogenous activators to amplify inflammation and initiate the sepsis syndrome. The canonical pathways of sepsis are shown in grey; pathways of the new model are shown in black. Box 2 gives examples of inflammatory mediators that might initiate and affect sepsis. This new model explains: (i) how sepsis is initiated by microorganisms lacking PAMPs; (ii) how TLR link innate and adaptive immunity against viruses, tumors and transplants; and (iii) how SIRS occurs in the absence of microbial infection.

has not been explained. Our suggestion is that differences in sensitivity might be better explained by differences in the activity of proteases, protease inhibitors and/or complement.

Proteases have been implicated previously in the pathophysiology of the sepsis syndrome and of SIRS. Dubois *et al.* [74] showed that the LD50 for LPS is increased fivefold in mice deficient in matrix metalloproteinase 9 (MMP-9). Steinberg *et al.* [75] found that rats made septic by cecal ligation and puncture are protected from death when treated with a modified tetracycline that inhibits MMP-9. Some have concluded that proteases cause harm by damaging organs in sepsis [76]. Our results suggest that proteases also remove inhibition on TLR4, enabling initiation of inflammatory reactions.

How does the microenvironment constrain activation of TLR4? One mechanism could involve direct interaction between heparan sulfate and TLR4, limiting mobility of the receptor in the plasma membrane. Consistent with this concept, Medzhitov [23] showed that mutant TLR4 that has the extracellular domain of CD4 signals constitutively. An alternative mechanism comes from our recent discovery that chemokine (C–X–C motif) receptor 4 (CXCR4) suppresses activation of TLR4 by LPS [77]. Moreover, SDF-1, the agonist for CXCR4, amplifies TLR4 suppression. Because SDF-1 associates with heparan sulfate [78] and is inactivated by elastase *in vivo* [79], SDF-1 could be the target through which proteases relieve suppression.

Concluding remarks

Ironically, the first scientific investigation of sepsis might have offered the best insight. The 'putrid gift' of Panum was soluble in water and insoluble in alcohol. This property characterizes heparan sulfate and hyaluronate better than it does LPS, which forms micelles both in water and alcohol [80]. Although illuminating, subsequent investigation focusing on LPS and the canonical model of sepsis might have obscured the most useful questions. For example, given the extraordinary potency of LPS *in vitro*, one might ask why the sepsis syndrome does not complicate most infections and why it does not complicate the bacteremia that follows dental work or manipulation of the intestines. And if sepsis syndrome is a common cause of death, why are infections more lethal in the absence of TLR4? If the sepsis syndrome simply reflects the 'spilling over' of exogenous TLR agonists into the systemic circulation, what prevents the manifestations of sepsis in those infections that generate immune responses (because immune responses presumably reflect the escape of TLR agonists to systemic lymphoid tissues where B-cell responses arise)? Based on recent observations, we would argue that sepsis syndrome does not complicate most infections because TLR4 signaling is suppressed. Thus, sepsis syndrome and SIRS are exceptions rather than the rule and these conditions result from failure to contain TLR4 activation (sepsis syndrome) or from systemic release of endogenous TLR4 activators (SIRS).

Several recent observations seemingly turn the etiology of sepsis inside out. First, the sepsis syndrome can be, and perhaps often is, caused by endogenous substances. If this is correct then LPS might be more a marker than a trigger of the sepsis syndrome. Second, sensitivity to sepsis and related conditions might soon be predicted at a molecular level by variations in activity or control of proteases. If this prediction proves correct, then inflammatory reactions thought to be the effectors of the sepsis syndrome might turn out to initiate the sepsis syndrome.

Our new model of sepsis raises new and perhaps urgent questions (Box 1). What is the biological importance of the control of TLR4 activation? If release from inhibition is needed for TLR4 activation, is that release mediated by complement, coagulation, degranulation of leukocytes and/or tissue endoproteases? If activation of TLR4 in tissues is inhibited, as our work suggests [67], can the same be said for TLR4 on leukocytes? And if TLR4 on

Box 1. Questions sparked by the new model for sepsis

(i) What releases TLR from inhibition in sepsis and SIRS? Is it complement or coagulation, proteases released by phagocytes, or matrix endoproteases?
(ii) Is TLR4 on circulating cells regulated in the same way as TLR4 in tissues? What are the regulators?
(iii) Which endogenous agonists of TLR4 trigger important responses such as sepsis syndrome, SIRS and immune responses? Is LPS more a marker than a mediator of the sepsis syndrome?

Box 2. Inflammation as an initiator and an effector of sepsis

Cascades and products postulated to cause systemic manifestations of sepsis [45] that might also initiate sepsis:

- Coagulation cascade
- Complement cascade (involving proteases and anaphylatoxin)
- Protease(s) released by phagocytes (e.g. elastase)
- Protease(s) released by tissues and/or endothelium (e.g. metalloproteinases)
- Cytokines (e.g. TNF, interleukins)

leukocytes is inhibited, what mediates that inhibition? If endogenous substances can stimulate TLR4, to what extent do they do so? And do endogenous agonists make the study of LPS obsolete?

Answering these questions will not only expand our understanding of sepsis, but also might generate more specific and effective therapies. Focusing on the control of TLR4 function rather than on the availability of a putative agonist (LPS) makes it possible to focus therapeutics on systemic reactions and inciting events (Box 2). Furthermore, focusing on endogenous agonists (or inhibitors) of TLR4, rather than on LPS, might enable more widely useful therapies to be devised; thus, therapies for conditions such as ischemia-reperfusion [2], atherosclerosis [4] and osteoporosis [81] might be found.

Acknowledgements

This work was supported by grants from the National Institutes of Health (HL46810 and AI53733).

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