Review

Bench-to-bedside review: Sepsis, severe sepsis and septic shock – does the nature of the infecting organism matter?

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Abstract

International guidelines concerning the management of patients with sepsis, septic shock and multiple organ failure make no reference to the nature of the infecting organism. Indeed, most clinical signs of sepsis are nonspecific. In contrast, *in vitro* data suggest that there are mechanistic differences between bacterial, viral and fungal sepsis, and imply that pathogenetic differences may exist between subclasses such as Gram-negative and Gram-positive bacteria. These differences are reflected in different cytokine profiles and mortality rates associated with Gram-positive and Gram-negative sepsis in humans. They also suggest that putative anti-mediator therapies may act differently according to the nature of an infecting organism. Data from some clinical trials conducted in severe sepsis support this hypothesis. It is likely that potential new therapies targeting, for example, Toll-like receptor pathways will require knowledge of the infecting organism. The advent of new technologies that accelerate the identification of infectious agents and their antimicrobial sensitivities may allow better tailored anti-mediator therapies and administration of antibiotics with narrow spectra and known efficacy.

Introduction

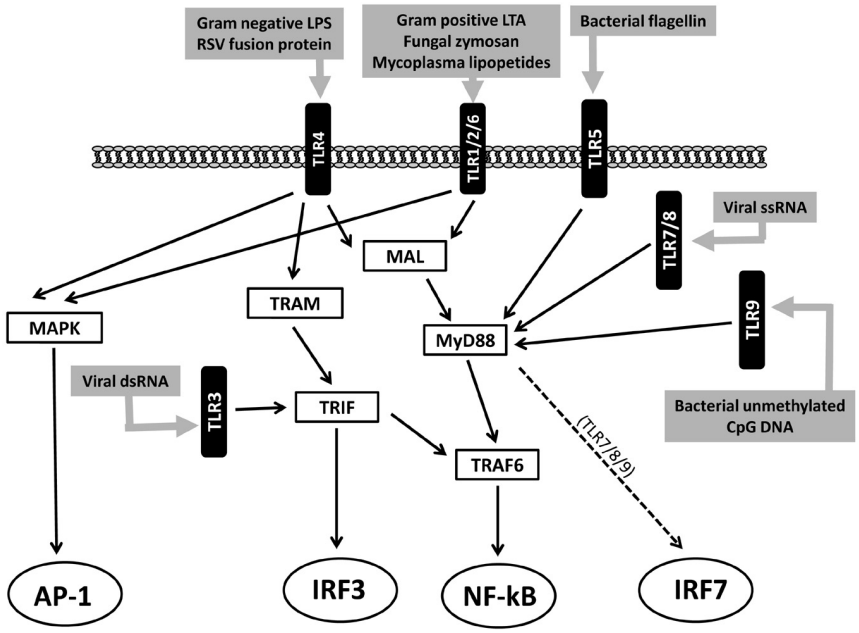
Sepsis and its sequelae, namely severe sepsis, septic shock and multiple organ failure, dominate the case load of non-coronary intensive care units (ICUs). Despite a fall in mortality, deaths attributable to sepsis have risen in developed countries as the incidence increases in an ageing population [1,2]. Moreover, patients who survive suffer considerable morbidity and score poorly in many domains of health-related quality of life assessments [3,4]. Hence, sepsis is the focus of many quality improvement initiatives. The US Institute for Healthcare Improvement's '5 million lives' campaign aims to reduce the incidence of nosocomial sepsis [5]. Furthermore, the Surviving Sepsis Campaign (instigated by the European Society of Intensive Care Medicine, International Sepsis Forum and Society of Critical Care Medicine) aims to harmonize the clinical management of patients with established sepsis using the best evidence available currently [6].

Louis Pasteur was the first to link micro-organisms with human disease when he identified the streptococcal aetiology of puerperal sepsis [7]. It is now known that sepsis also arises after infections with a range of micro-organisms that include viruses, fungi and protozoa. However, neither the Surviving Sepsis Campaign nor the guidelines of the American College of Chest Physicians and Society of Critical Care Medicine [8] make any reference to whether specific infectious agents influence the natural history or therapy of an episode of sepsis. Similarly, standard definitions do not focus on the site of infection. Thus, sepsis is often considered as a single entity, with little or no reference to the causative agent or the anatomical focus of infection. Does this mean that the nature of the organism has no influence?

Clinically, the nature of the organism is critical in that many possess specific virulence factors that have considerable prognostic significance. For example, Panton-Valentine leukocidin secreted by staphylococci contributes to the development of a rapidly progressive haemorrhagic necrotizing pneumonia in immunocompetent patients [9] and a particularly high mortality rate [10]. It is likely that other microbial and host factors influence the effects of Panton-Valentine leukocidin [11,12]. Similarly, other bacterial subgroups secrete toxins such as superantigenic toxic shock syndrome toxin 1, exfoliative toxin, botulinum toxin and tetanus toxin. All are associated with additional mortality above that attributable to bacterial infection *per se*. However, aside from virulence factors specific to certain organisms, differences are also detectable in association with broader microbial classifications. Most data exist for differences between Gram-positive and Gram-negative infections [13].

ICU = intensive care unit; IFN = interferon; IL = interleukin; LPS = lipopolysaccharide; PCR = polymerase chain reaction; TLR = Toll-like receptor; TNF = tumour necrosis factor.

Figure 1



Simplified schematic of intracellular signalling for TLRs. AP, activator protein; CpG DNA, cytosine-guanine dinucleotides; dsRNA, double-stranded ribonucleic acid; IRF, interferon response factor; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MAL, MyD88-adaptor-like; MAPK, mitogen-activated protein kinase; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor-κB; ssRNA, single-stranded ribonucleic acid; TLR, Toll-like receptor; TRAM, Toll-receptor-associated molecule; TRIF, Toll-receptor-associated activator of interferon.

Differences in the host response

Infectious pathogens are detected by the innate immune system via Toll-like receptors (TLRs). Ten TLRs have been identified, through which most pathogens can be detected. Recognition does not require previous exposure to a pathogen or an enormous range of genome-encoded receptors, such as is associated with the T-cell receptor. TLRs respond to molecular patterns such as unmethylated CpG dinucleotides that are common in bacteria but uncommon in the host. Mammalian DNA methyltransferases result in methylation of 70% to 80% of CpG cytosines [14]. Similarly, TLR4 and TLR2 recognize lipopolysaccharide (LPS) and lipoteichoic acid, structural molecules that are unique to the cell walls of Gram-negative and Gram-positive bacteria, respectively. Whereas bacterial components signal via a single TLR, it is unlikely that whole bacteria signal so exclusively. Indeed, cell wall extracts from Gram-positive and Gram-negative organisms contain components that can activate both receptors [15,16]. This lack of absolute dependence on a single receptor has obvious benefits for the host. However, mice deficient in TLR2 and TLR4 are more prone to infections with staphylococci [17] and *Salmonella* spp. [18], respectively, which suggests that Gram-positive infection may have a TLR2-dominant signal, whereas Gram-negative infections have a TLR4-dominant signal.

The intracellular signalling cascades of the TLRs are illustrated in Figure 1. These converge through common adaptor molecules onto three transcription factors: nuclear factor-κB, activator protein-1, and interferon response factor-1. All three factors result in the upregulation of genes for pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α, IL-1, and the IFNs. However, this convergence of signalling cascades is not reflected *in vitro*. Specific ligands for receptors result in different but overlapping responses. For example, TLR4 but not TLR2 agonists prolong neutrophil survival [19]. Additionally, cytokine release differs in human trophoblasts [20] and peripheral blood mononuclear cells [21-23] according to bacterial component. Although whole bacteria may signal via several TLRs, there remains divergence in cytokine responses to whole bacteria *in vitro* [24]. Heat-killed streptococci induce greater IFN-γ but less IL-10 release than heat-killed *Escherichia coli* in a whole blood model [25]. Other investigators have demonstrated that heat-killed staphylococci induce less IL-6, IL-8, IL-1β and TNF-α from neonatal blood than *E. coli* [26].

These *in vitro* observations can be extended to the results of clinical studies. Microarray data from 52 patients suggest that different but overlapping sets of genes are upregulated and these sets include genes that are implicated in the inflammatory response [21]. The patient numbers were too small to exclude host interactions. Nevertheless, it is possible that patterns of gene expression in the host could be exploited therapeutically or as a diagnostic tool. Gram-negative disease has been shown to result in greater plasma levels of TNF-α

than Gram-positive infection [25,27]. Gram-negative meningococcal septicaemia is associated with greater plasma IL-10 and lower IFN-γ than Gram-positive sepsis [25]. Others have identified differences in IL-6, IL-18 and procalcitonin levels [21]. However, such differences in cytokine profiles do not manifest overtly in either physiological or clinical differences. Signs such as fever, hypotension and tachycardia, and widely used biochemical markers (for example, raised C-reactive protein) and leucocytosis are nonspecific. By contrast, there may be differences in mortality afforded by the nature of the infecting organism. These differences have not remained constant over time, because it has been observed that the incidence of Gram-negative sepsis is falling whereas that of Gram-positive sepsis has remained steady [1]. Moreover, univariate analyses have suggested that Gram-positive or staphylococcal infections appear to be associated with greater mortality [28-30]. In another multivariate analysis [30] only pseudomonal infections appeared to carry a sigificantly different (higher) mortality rate.

These findings are important because the aetiology of sepsis has changed over time. In the 1980s the most frequently identified organisms were Gram-negative bacteria, often of gastrointestinal origin. More recently Gram-positive bacteria have accounted for the greatest proportion of hospital admissions with sepsis in which an organism is identified [1,30]. It is not clear whether this is a consequence of greater use of prostheses and invasive vascular devices [31] or of increasing prevalence of multiresistant organisms (for example, methicillin-resistant *Staphylococcus aureus*) [32]. Methicillin-resistant *S. aureus* is associated with increased ICU length of stay, postoperative complications, treatment costs and mortality [32]. The incidence of fungal sepsis has also increased. In a study of 49 US hospitals, fungi accounted for 11.7% of bloodstream infections in ICUs [1,33], with an associated mortality of 45% [33,34]. There are few data describing the cytokine profiles of severe fungaemia or viraemia relative to that of bacterial sepsis. Finally, in around 40% of cases no organism is identified as the cause of sepsis [30], possibly because of lack of samples, previous antibiotic therapy, or deficiencies in microbiological techniques. It is not known how the different microbial groups are represented within this important subgroup [35].

In summary, the nature of an infectious pathogen influences the mechanism of the host response. This appears teleologically intuitive, because a common strategy would not allow the host to exclude all viruses, intracellular infections, extracellular infections and microbial structures. The corollary is that the effects of any specific anti-mediator therapies may vary according to the nature of the infection.

Differences in the response to therapeutic intervention

The nature of the infecting organism is critical, primarily for the selection of appropriate antimicrobial agents. Observational studies have demonstrated that the appropriateness of such therapy has the greatest impact on outcome in sepsis [35].

Patients with Gram-positive or Gram-negative infections have responded differently in some clinical trials targeting mediators of the inflammatory response [36]. Unfortunately, not all have reported efficacy according to the nature of the infecting organism. However, in a randomized, double-blind, placebo-controlled trial of a soluble fusion protein of TNF-α receptor, no adverse events were observed in patients with Gram-negative infection, whereas patients with Gram-positive infection tended to have increased mortality [37]. In contrast, a murine monoclonal antibody directed against human TNF-α tended to reduce mortality in Gram-positive infection, whereas that in Gram-negative infection mortality tended to increase [38]. The platelet-activating factor receptor antagonist BN52021 and the bradykinin antagonist CP-0127 both resulted in reduced mortality in Gram-negative disease, with no effect in patients with Gram-positive infection [39,40]. Finally, patients with Gram-positive disease have potentially been harmed in trials of IL-1 receptor antagonists [41] and anti-LPS (HA-1A) [42]. To date, drotrecogin alfa (activated) is the only therapy that has been demonstrated to be efficacious in severe sepsis by a large, randomized, double-blind, placebo-controlled trial. Drotrecogin alfa appears to be equally effective in patient with the broader classifications of Gram-positive, Gram-negative, or fungal sepsis [43,44]. When examined at the level of individual organisms, the data suggest that some differences in therapeutic response may exist. Indeed, patients with *Streptococcus pneumoniae* infection may have the greatest reduction in mortality with drotrecogin alfa therapy [44], although this observation was not formally evaluated.

There is considerable interest in the therapeutic opportunities afforded by the discovery of TLRs. Inhibition of signalling pathways may limit an over-exuberant and possibly damaging host inflammatory response. Several therapies targeting the TLR4 pathway are under development. Being directed at TLR4, these therapies may be efficacious only in bacterial Gram-negative sepsis, and their effectiveness will thus be critically dependent on the nature of the infecting organism. For example, TAK-242 is a small molecule antagonist that reduces LPS-induced production of nitric oxide, IL-1β, IL-6 and TNF-α by human blood mononuclear cells [45,46]. It is selective for TLR4 and not TLR2, TLR3 or TLR9 signalling. *In vivo*, it improves survival when it is administered to mice even after a normally fatal LPS challenge [47]. TAK-242 is currently undergoing phase III evaluation in a multicentre, randomized, placebo-controlled study of patients treated within 36 hours of the onset of severe sepsis and concomitant respiratory and cardiovascular failure [48]. The primary end-point of the study is 28-day all-cause mortality. An earlier study of TAK-242 [49] was stopped after enrolling 277 patients; data are yet to be reported. Alternatively, E5564, or

eritoran, is a synthetic lipodisaccharide that antagonizes LPS [50]. *In vivo*, E5564 blocks the induction of cytokines by LPS and reduces lethality after injection of LPS or bacteria into mice [50]. 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 count, TNF-α, and IL-6 after LPS injection [51]. E5564 is being evaluated in a phase III, double blind, placebo-controlled study conducted in patients within 12 hours of onset of severe sepsis [52]. The primary outcome measure is 28-day survival. Finally, two other agents yet to be investigated are CRX-526 (a synthetic lipid A mimetic and thus TLR4 agonist) [53] and soluble decoy TLRs [54-56].

Determination of the infecting organism

Current standard microbiological techniques identify infecting organisms after culture of a clinical isolate in conditions suitable for replication of the infectious agent. This may be difficult with fastidious organisms or if patients have received antibiotics. Preliminary classification is usually possible within 24 hours, with full species identification and antimicrobial sensitivity data becoming available 48 to 72 hours after blood sampling. The slowness of the investigation usually mandates the use of 'best guess', and often broad spectrum, antibiotics while awaiting results.

Several techniques are being developed that accelerate the identification of infecting organisms. Many detect nucleotide sequences specific to pathogens in blood after standard culture. Techniques include fluorescent *in situ* hybridization and PCR assays [57]. The wide range of possible pathogens requires the use of many PCR conditions; this can be circumvented by using custom printed DNA microarrays. Typically, these detect panels of 20 to 40 gene sequences to discern the most common isolates [58]. Furthermore, sequences that correlate with antimicrobial resistance can be detected to guide appropriate therapy. It is theoretically possible to undertake PCR-based amplification of sufficient magnitude to detect low copy numbers of DNA sequences, thereby eliminating the requirement for an initial period of standard culture. The utility of these techniques is limited currently by difficulties in differentiating contaminants and nonliving or degraded bacteria from clinically relevant isolates. Finally, infrared vibrational spectroscopy allows the identification of bacterial specific proteins in whole blood [59]. This emerging technique does not require amplification or extraction of the proteins.

No system has been evaluated extensively in clinical practice, but they offer considerable potential advantages. First, they may facilitate the use of antibiotics with narrower spectra but known efficacy against a particular organism; this may minimize the development of multidrug resistant bacteria and infections such as *Clostridium difficile* diarrhoea. Second, they promote better understanding of the heterogeneity of infection in sepsis. Finally, they may allow the use of some of the specific anti-mediator therapies that are being investigated.

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Conclusion

The nature of an infecting organism is critically important. Clinically, specific virulence factors such as exotoxins influence the manifestations, morbidity and mortality of sepsis. Furthermore, the nature of the pathogens influences the mechanism of the host response and therefore the response to any therapy. From the perspective of the physician, early identification of an infectious agent will allow confirmation that infection underlies an inflammatory process, allow the use of efficacious and narrow spectrum antibiotics, and may open the door to new therapies targeted at pathogen-specific inflammatory pathways.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HG, TE, and SF planned, drafted, read, and approved the final manuscript.

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