

Sepsis: rethinking the approach to clinical research

John C. Marshall¹

Departments of Surgery and Critical Care Medicine and the Li Ka Shing Knowledge Institute,
St. Michael's Hospital, University of Toronto, Toronto, Ontario, Canada

Abstract: The clinical syndrome of sepsis encompasses a highly heterogeneous group of clinical disorders, varying with respect to the site, bacteriology, and even presence of infection and with the clinical syndrome evolving in the host. Clinical trials of strategies to modulate the host response that mediates sepsis were first initiated 25 years ago. A continuing record of disappointment has characterized subsequent work, and only a single new therapy has been licensed for clinical use. Yet, these commercial disappointments obscure a vibrant body of new knowledge that has clarified the biology of the innate immune response whose deranged expression is responsible for sepsis and that has provided important new insights into the failings of the traditional model of clinical research in sepsis. This review highlights advances in basic biology and underlines insights from clinical research that may point to new and more effective ways of translating an understanding of innate immunity into effective treatments for a leading cause of global morbidity and mortality. *J. Leukoc. Biol.* 83: 471–482; 2008.

Key Words: innate immunity · translational research · clinical trials · Toll-like receptors · TNF

The word, “sepsis”, is attributed to Hippocrates (460–370 BCE), who asserted that living tissues could be broken down through one of two contrasting processes. *Pepsis* was the process through which food was digested or grapes fermented to produce wine: It was healthy, life-sustaining, and good. *Sepsis*, on the other hand, described the process through which flesh rotted, swamps generated foul airs at night, and wounds festered: It was rank, disease-producing, and evil [1]. His ideas predated by more than 2 millennia the insights of Pasteur, Koch, and Lister, who established the microbial etiology of the process and fundamentally transformed the management of infection.

Galen of Pergamon (129–216 AD) described the clinical manifestations of localized acute inflammation. To his four cardinal signs of inflammation—*rubor*, *calor*, *dolor*, and *tumor*—Celsus added a fifth: *functio laesa*, or loss of function.

Two concepts have remained intricately interwoven: that infection by exogenous microscopic organisms is responsible for many of the ailments humans suffer and that this suffering arises through acute and life-threatening, physiologic changes in the host. However, it has only been in the past quarter century that it has been appreciated that the morbidity of

infection is not solely a consequence of the cytopathic activity of the microorganism but as importantly, an indirect consequence of the response of the host to the microbial invasion [2]. Thus, the concept of sepsis as a consequence rather than as a manifestation of infection has begun to emerge, and with this new awareness, a new spectrum of therapeutic opportunities has emerged. That we have yet to successfully translate this new paradigm into effective new therapies underscores the complexity of the biologic processes involved but also, the inadequacies of the approaches we have used to accomplish this objective.

Since the first clinical study of an antiserum to LPS in 1982 [3], there have been in excess of six dozen phase II or phase III clinical trials evaluating the use of more than 20 different approaches that target microbial products or specific mediators of the host response in critically ill patients with sepsis [4, 5]. Only one of these has been licensed as a therapy for sepsis—activated protein C, also known as drotrecogin α -activated [6]—and controversy about the risk:benefit ratio of the agent [7] has resulted in European regulatory agencies mandating a further phase III study to secure ongoing approval for its use. Two other agents currently available for clinical use—adrenal corticosteroids [8] and i.v. Ig (IVIG) [9]—have shown apparent efficacy in patients with severe sepsis and septic shock and so, are variably used in clinical management.

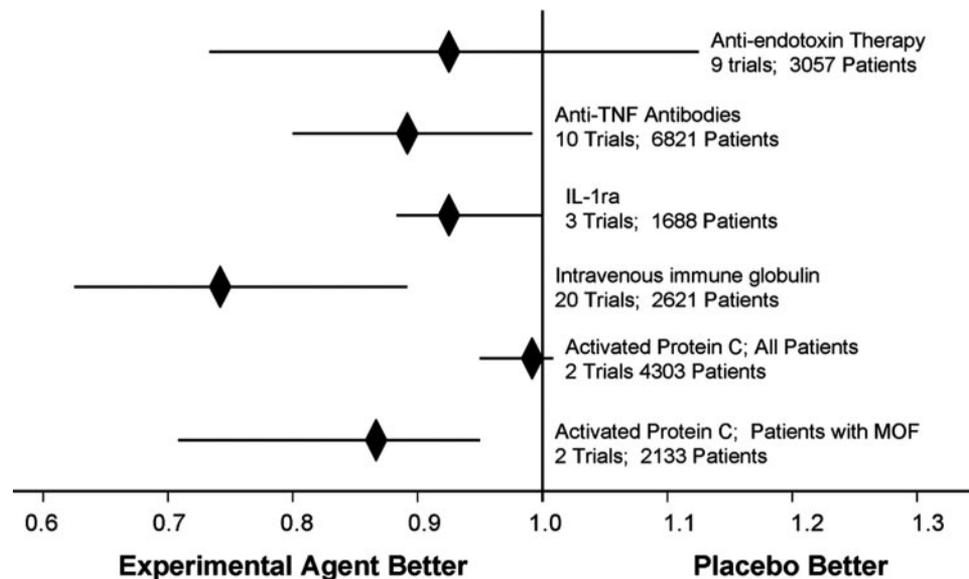
At first glance, the fact that a single licensed but controversial agent is the sole product of a quarter century of research involving tens of thousands of patients and several billions of research dollars seems legitimate grounds for despair. The reality, however, is more complex and in that complexity, much more optimistic. Indeed, for a variety of differing strategies that have been evaluated in sufficiently large cohorts to draw reliable conclusions, there is a small but consistent signal of biologic efficacy that translates into an absolute mortality improvement in the range of 2–4% [4] (**Fig. 1**). Whether the signal achieves statistical significance is typically a question of how the initial analyses are performed, how data from multiple studies are aggregated, and how those aggregated data are interpreted. For example, a large, multicenter-randomized trial of an anti-TNF mAb (afelimomab) was conducted to test the hypothesis that patients with elevated levels of IL-6 would have

¹ Correspondence: St. Michael's Hospital, 4th Floor Bond Wing, Rm. 4-007, 30 Bond Street, Toronto, Ontario M5B 1W3, Canada. E-mail: marshallj@smh.toronto.on.ca

Received June 9, 2007; revised October 18, 2007; accepted October 23, 2007.

doi: 10.1189/jlb.0607380

Fig. 1. Pooling data from clinical trials of five different therapeutic strategies targeting microbial products or endogenous mediators of the host response reveal a consistent signal, suggesting clinical benefit. IL-1ra, IL-1R antagonist; MOF, multiple organ failure.



a survival benefit if treated with afelimomab [10]. The prospectively designed statistical analysis called for an adjusted analysis of survival rates to correct for baseline imbalances in patient demographic characteristics. For patients with elevated IL-6 levels, the adjusted mortality benefit was statistically significant; the unadjusted analysis was not. However, when all enrolled patients were evaluated, there was a statistically significant, overall mortality benefit that became insignificant if the originally specified adjustment was applied. Similarly, a large trial of activated protein C (drotrecogin α -activated) found a statistically significant, overall mortality benefit of 6.1% ($P < 0.006$), which was most striking in patients at high risk of death [6]. A subsequent study in patients at low risk of death found no evidence of benefit for the agent, and when the results of the two trials are pooled, whether they attain statistical significance depends on the type of the meta-analytic model used [11]. Although great passions have been ignited in debating whether either of the agents “works,” the debate is ultimately sterile. However, like the results of the infamous United States presidential election of 2000, much rides on the ultimate conclusion, even if the data are inescapably, statistically ambiguous.

It is not my goal in this brief review to further engage the debate about the efficacy (or lack thereof) of strategies that have been studied in the past. Rather, I will attempt to synthesize an evolving understanding of the biologic complexities of innate immunity with some particularly compelling lessons derived from the conduct of clinical trials in this challenging patient population and so to highlight the future needs of a more successful research agenda.

CLINICAL RESEARCH IN SEPSIS: A HISTORICAL PERSPECTIVE

Contemporary approaches to clinical trials in sepsis are grounded in several key studies undertaken in the 1980s. Elizabeth Ziegler and her colleagues [3] studied 212 patients

with Gram-negative infection and randomized half of them to treatment with an antiserum raised against the core polysaccharide of endotoxin. Entry criteria emphasized clinical judgment—a strategy that proved very effective in identifying patients who subsequently proved to be infected. Treatment with the antiserum improved the survival of infected patients and particularly those in shock. During the same era, a group of investigators led by the late Roger Bone [12] undertook a study evaluating the efficacy of high-dose methylprednisolone in sepsis. They reasoned that early intervention would be more likely to be efficacious and so proposed a constellation of clinical findings that they termed “sepsis syndrome” [12], as the entry criteria for their trial. Sepsis syndrome was defined as suspected or documented infection, in association with hyper- or hypothermia, tachycardia, tachypnea, and evidence of the dysfunction of one or more organs. These criteria were established through the consensus of the small group of investigators, rather than on the basis of data from epidemiologic studies. Their trial failed to show benefit for the use of high-dose corticosteroids [13]. Nonetheless, these criteria, or a modification of them termed the systemic inflammatory response syndrome (SIRS) [14], have been used for every industry-funded study of novel therapeutics in sepsis that has followed. One of the more compelling reasons for the disappointing results of these studies has been their persistent use of inadequate and nonvalidated clinical criteria to define an at-risk population for study.

Defining an at-risk population has proven enormously challenging, and many of our assumptions regarding the epidemiology of sepsis have been proven wrong. It was assumed, for example, that patients meeting criteria for sepsis syndrome would display a common pattern of circulating inflammatory mediators; cohort studies show that this is not the case [15]. Similarly, it was assumed that levels of circulating endotoxin will be highest in patients with Gram-negative infection, so that anti-endotoxin therapies should be targeted here. Epidemiologic studies suggest that not only are endotoxin levels lower in patients with Gram-negative infection [16], but they are ele-

vated in noninfectious acute disorders such as the rupture of an abdominal aortic aneurysm [17] or acute pancreatitis [18], and the majority of critically ill patients has elevated levels of circulating endotoxin at the time of Intensive Care Unit (ICU) admission [19].

If the clinical epidemiology of sepsis were poorly understood a quarter of a century ago, the biologic basis of the process was even more so. Enthusiasm for the use of corticosteroids predated knowledge about the mechanisms that enabled the host to recognize danger, about the cellular processes that were activated in response to danger, and about cytokines and the extraordinarily complex network of host-derived mediators that mediated its clinical phenotype. The role of TNF as an early mediator of systemic inflammation was only established in 1985 [20], and our evolving understanding of the role of TLRs in enabling the host to recognize danger is a product of the last decade [21].

A rapidly evolving understanding of the biology of sepsis and an increasing awareness of the shortcomings of our previous attempts to translate the science of innate immunity into effective therapies can drive a refined and improved clinical research agenda. The challenge, however, is significant.

BIOLOGIC INSIGHTS

The inflammatory response is conserved, complex, and biologically redundant

The capacity to respond effectively to an external threat is a fundamental requirement of all living organisms. Thus, it is perhaps not surprising that genes encoding proteins that mediate this response are highly conserved. Strikingly, genes mediating innate immune responses in higher eukaryotes commonly subserve an important developmental role in lower species. *Toll* in the fruit fly *Drosophila* was identified on the basis of its capacity to direct dorsal-ventral patterning [22]; in mammals, orthologs of Toll encode the TLRs that function as key pattern recognition receptors (PRRs) in the innate host immune response to danger [23]. In the nematode, *Caenorhabditis elegans*, an ortholog of the mammalian gene macrophage migration inhibition factor, is up-regulated in dauer larvae, a larval stage adapted for long-term survival during conditions of exogenous stress [24]. *Ced-3* in *C. elegans* encodes a proapoptotic protein essential for the controlled deletion of 131 cells during the maturation process [25]. Its human ortholog, caspase-1, exerts proapoptotic activity in certain cells [26] but also plays a critical role in the expression of innate immunity through its ability to cleave and activate pro-IL-1 β and pro-IL-18 [27]. Similarly, pre-B cell colony-enhancing factor (visfatin), a protein that has been implicated in inflammation in species as diverse as sponges [28] and humans [29], subserves an important enzymatic role in the synthesis of nicotinamide adenine dinucleotide (NAD) in bacteria [30].

The innate immune response is also enormously complex. The administration of i.v. endotoxin to healthy human volunteers results in the differential expression of 3714 unique genes—fully one-eighth of the human genome [31]; the majority of these is down-regulated. Many of these play a key role in

the evolution of an inflammatory response, as evidenced by the fact that their inhibition (in the case of genes that are overexpressed) or restoration (in the case of genes whose expression is inhibited) can reduce mortality in murine endotoxemia (**Table 1**). The extraordinary interdependence of these distinct genes is underlined by the fact that an alteration in the expression of any single one can significantly change mortality risk. The corollary, however, once the cascade has been activated, is that their inherent complexity makes it less likely that manipulating any single protein will sufficiently perturb the process to provide mortality benefit.

The inflammatory response is not specific for infection

Although infection with microorganisms or viruses is a potent stimulus for activation of an inflammatory response, the endogenous sensing mechanisms that initiate a response do not specifically recognize infection but rather, respond to biochemical signals that suggest an imminent threat to the host. The best characterized of these so-called PRRs is the family of TLRs, widely expressed on cells of the innate immune system [23].

TLRs are mammalian orthologs of the *Drosophila* protein Toll. They comprise a family of 13 distinct receptor proteins characterized by leucine-rich repeats in their extracellular domains and are widely distributed on hematopoietic cells, as well as on epithelial and endothelial cells. Their key role in innate immunity became apparent with the observation that a developmental gene that regulates dorsoventral patterning during embryologic development also regulated the expression of an antifungal protein, drosomycin, in the adult [32] and with the demonstration that a mutant gene that confers endotoxin resistance in the C3H HeJ mouse encodes a TLR protein (TLR4) with an amino acid substitution in its extracellular domain [21].

TLRs recognize distinctive molecular patterns found in microorganisms, and although each receptor recognizes a number of distinct ligands, it does so with quite remarkable specificity (**Table 2**). TLR2 and TLR4, for example, recognize microbial molecules commonly found in Gram-positive and Gram-negative bacteria, respectively. TLR5 responds to bacterial flagellin, TLR9 to conserved CpG motifs in bacterial DNA, and TLR3, -7, and -8 to patterns characteristic of viruses. Engagement of a TLR results in the recruitment of adaptor proteins and activates intracellular signaling pathways that lead to the expression of NF- κ B-dependent genes that are the characteristic mediators of an inflammatory response [33]. The basis of specificity in the recognition of ligands of TLRs is unknown, although it has been suggested that the basis for receptor-ligand interactions depends on the hydrophobicity of the ligand [34]. Other endogenous PRRs have been recognized, including the intracellular nucleotide-binding oligomerization domain receptors, the triggering receptors expressed on myeloid cells (TREM) family, the Siglec molecules, and the C-type lectin receptors (for a recent review, see ref. [35]).

Yet, it is overly simplistic to conceptualize TLRs as a family of specific microbial sensors. It has become apparent that TLRs, particularly TLR2 and TLR4, can be activated by a number endogenous ligands (Table 2), eliciting a response that

TABLE 1. Biochemical Mediators Implicated in the Mortality of Murine Endotoxemia

	Activity increases lethality	Activity decreases lethality
Cytokines and extracellular proteins	IL-1	IL-1ra
	IL-12	IL-4
	IL-18	IL-10
	TNF	IL-13
	IFN	IVIG
	TGF	IFN
	Macrophage migration inhibitory factor (MIF)	Hepatocyte growth factor (HGF)
	MIP-1	Leukemia inhibitory factor (LIF)
	LIF	Vascular endothelial growth factor (VEGF)
	High-mobility group box-1 (HMGB1)	Multifunctional protein-14 (MFP-14)
	LPS-binding protein (LBP)	C-reactive protein
	Parathyroid hormone-related protein	Kallikrein-binding protein
	Low density lipoprotein (LDL)	MCP-1
		Bactericidal permeability increasing protein (BPI)
		Cathelicidin peptide-18 (CAP-18)
		TNF-stimulated gene-14 (TSG-14)
		Apolipoprotein E
		Very LDL
		High-density lipoprotein
		Complement components C3, C4
	C1 inhibitor	
	Fibronectin	
	Melatonin	
	Vasoactive intestinal peptide (VIP)	
	Pituitary adenylate cyclase activation polypeptide	
	Gelsolin	
	Proinsulin C peptide	
	Urocortin	
	Adrenomedullin	
	Cortistatin	
	Calcitonin gene-related peptide	
Coagulation factors	Factor VII	
	Tissue factor	
	Factor V Leiden heterozygosity	
Cell surface receptors	IL-1R	Macrophage FcR
	TNFR	VIP receptor
	Platelet activating factor (PAF) receptor	Adenosine A3 receptor
	LDL receptor	Adenosine receptor A2A
	CD11a	Endothelial protein C receptor
	CD18	A7 nicotinic acetyl choline receptor
	Leukocyte-endothelial cell adhesion molecule (LECAM-1)	
	Triggering receptor expressed on myeloid cell-1 (TREM-1)	
	Kinin receptor	
Signal transduction molecules and other intracellular proteins	MyD88	Kir 6.1 ATP channel
	DAP12	PI-3K
	Hek	Stat-4, Stat-6
	p38	I6B
	NF-κB	Hemoxygenase
	IFN regulatory factor-2 (IRF-2)	Glucose-6-phosphate dehydrogenase
	Cyclo-oxygenase-II	Heat-shock protein 70 (HSP70)
	Inducible NOS	
	5-Lipoxygenase	
	Endothelial NOS	
	Caspase-3	
	HSP gp96	
Miscellaneous	PAF	Vitamin B2
		Vitamin B12
		Vitamin D3
		Oxidized phospholipids

TABLE 2. Exogenous and Endogenous Ligands of TLRs

	Exogenous ligands	Endogenous ligands
TLR1	Triacylated lipopeptide <i>Mycobacterium</i>	
TLR2	Peptidoglycan, lipoteichoic acid Bacterial lipoprotein Zymosan <i>Mycoplasma</i> lipopeptide	HSP60, HSP70, gp96 Hyaluronin Biglycan HMGB1
TLR3	Double-stranded RNA Picornavirus	
TLR4	LPS β -Glucan Coxsackie virus Respiratory syncytial virus	HSP60, HSP70, gp96 HMGB1 Fibronectin extradomain A Heparan sulfate Surfactant protein A β -Defensin LDL Palmitate Heme
TLR5	Flagellin	
TLR6	<i>Mycoplasma</i> lipopeptide	
TLR7	Single-stranded RNA7/8	
TLR8	Influenza virus	
TLR9	Nonmethylated CpG DNA Herpes simplex Cytomegalovirus	HMGB1

is indistinguishable from the engagement of a microbial ligand [36]. TLR2 and TLR4 might well be considered as classic danger receptors, activated by endogenous ligands, when the ligand is encountered in an abnormal environment. Both recognize conserved cell-wall structures in endogenous bacteria that normally populate the epithelial surface of the gastrointestinal tract and so, might well be considered endogenous rather than exogenous ligands. Moreover, TLR4 engagement in gut epithelial cells mediates protection against injury and maintains normal intestinal homeostasis [37], and TLR9 engagement is reported to play a similarly protective role [38]. Further evidence for a role of TLRs in responding to endogenous signals derives from the fact that the IL-1R is a member of the TLR family, and its engagement activates identical cellular responses.

From a biologic perspective then, the family of Toll-like PRRs functions not to differentiate the mammalian from the microbial worlds but rather, to serve as an alarm signal, evoking a cellular response to a potentially injurious ligand that is abnormally present in the immediate external environment of the cell. It follows from this that our concept of sepsis is artificially restrictive—the host responds to whole bacteria, bacterial products such as endotoxin, and intracellular products released from injured tissues with the same potentially harmful response. In fact, only in the context of infection is it likely that the response will prove beneficial to the host, despite the inevitable tissue injury that accompanies it.

Inflammation represents a compromise between containing a threat and damaging the host

Confronted with the alarming clinical reality of a patient in imminent danger of dying of refractory septic shock, it is

difficult to appreciate that the innate immune response driving this lethal disease does not represent some perverse form of divine vengeance but rather, the dysregulated expression of a process that has throughout eukaryotic evolution been fundamental to the survival of multicellular organisms. Expressed in the constrained confines of a small wound, the innate immune response serves the multiple contingent imperatives of stopping hemorrhage from a disrupted blood vessel, delivering antimicrobial mediators and phagocytic cells to the site of injury, containing the challenge to limit its dissemination, eliminating the invading pathogen, and ultimately, once the threat has been overcome, terminating the inflammatory process and initiating tissue repair. Modulating elements of this process has the potential to disrupt normal host defense mechanisms.

The response to the experimental manipulation of endogenous inflammatory mediators is strikingly model-dependent. In a variety of different animal species, neutralization of TNF prior to challenge with LPS or live Gram-negative bacteria attenuates illness severity and significantly improves survival; at the same time, neutralization of TNF does not alter the course of a complex infectious process such as cecal ligation and puncture (CLP) and actually increases mortality following experimental challenge with *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, or *Candida* [39] (**Fig. 2**). These observations are replicated in studies of the use of anti-TNF therapies for inflammatory bowel disease or rheumatoid arthritis; serious infections as a complication of treatment occur twice as often in patients receiving anti-TNF agents [40]. In fact, it has been a common observation in animal models that interventions that improve outcome following challenge with endotoxin often worsen outcome when the experimental challenge is a viable microorganism [41, 42].

From a biological perspective, sepsis may well represent the most challenging disorder to treat with interventions that target the innate immune response, as attenuation of the inflammatory response can blunt the adaptive antimicrobial responses that promote the resolution of infection. It has recently been reported that the inflammatory response to dead cells and tissue injury is mediated through the IL-1R and that blunting this response does not jeopardize antibacterial immunity [43], suggesting that it may be possible to selectively adverse sequelae of systemic infection.

Regulation of inflammation converges on a limited repertoire of intracellular pathways

Although the soluble mediators of an acute inflammatory response are many, their expression is regulated through a relatively small number of intracellular signaling pathways; targeting these, rather than individual products of their activation, is an intuitively, more attractive concept. Signals transduced through TLR4, for example, classically activate pathways that are dependent on or independent of the recruitment of MyD88 (**Fig. 3**), and recruitment of MyD88 adaptor-like [Mal; also known as Toll/IL-1R (TIR) domain-containing adapter protein (TIRAP)] to the cytoplasmic region of TLR4 results in MyD88 recruitment, and the formation of a signaling complex including IL-1R-associated kinase-1 (IRAK1), IRAK4, and TNFR-associated factor 6 (TRAF6), which in turn activate TGF- β -activated kinase-1 (TAK1) [45], initiating a cascade leading to

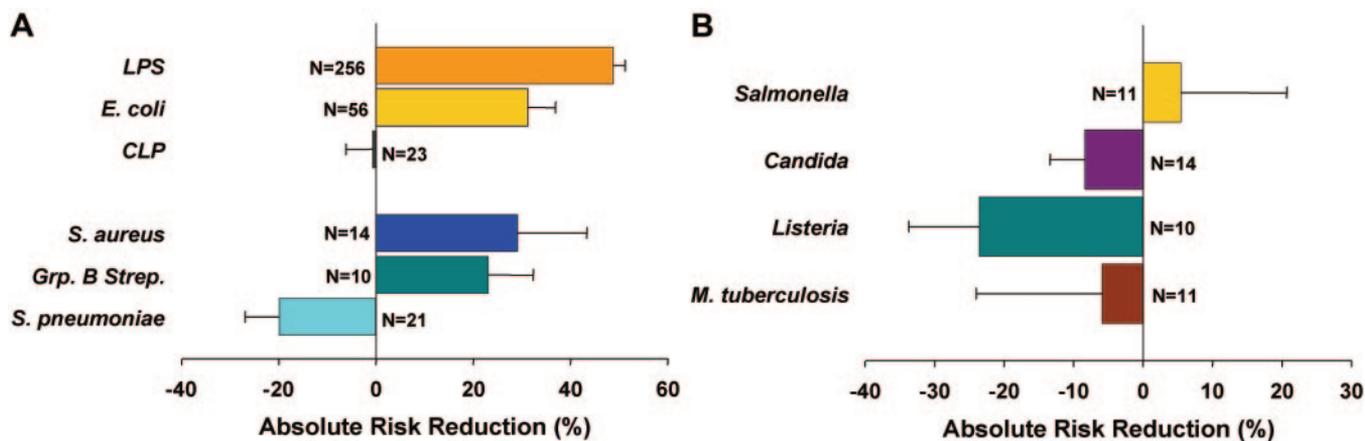


Fig. 2. In a variety of preclinical models of sepsis in eight different species, there is a consistent signal, suggesting that neutralizing TNF improves outcome following systemic challenge with LPS or Gram-negative bacteria and certain Gram-positive organisms (with the exception of *S. pneumoniae*; A) but not following challenge with *Candida*, *M. tuberculosis*, or *Listeria* (B); from ref. [39]. *E. coli*, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; Grp. B Strep., Group B Streptococcus.

the phosphorylation of I κ B and its dissociation from inactive cytoplasmic NF- κ B. Free NF- κ B can translocate to the nucleus, where by binding to its specific consensus sequence, it can up-regulate the transcription of a large number of proinflammatory genes.

The fundamental, evolutionary tradeoff between effective antibacterial activity and harm from the adverse consequences of acute inflammation is further evident in the intracellular signaling pathways downstream of the TLRs. A single nucleotide polymorphism (SNP) involving the substitution of leucine for serine at amino acid 180 in Mal has been shown to be protective for heterozygous individuals in a variety of infectious diseases including tuberculosis, malaria, and pneumococcal disease [46].

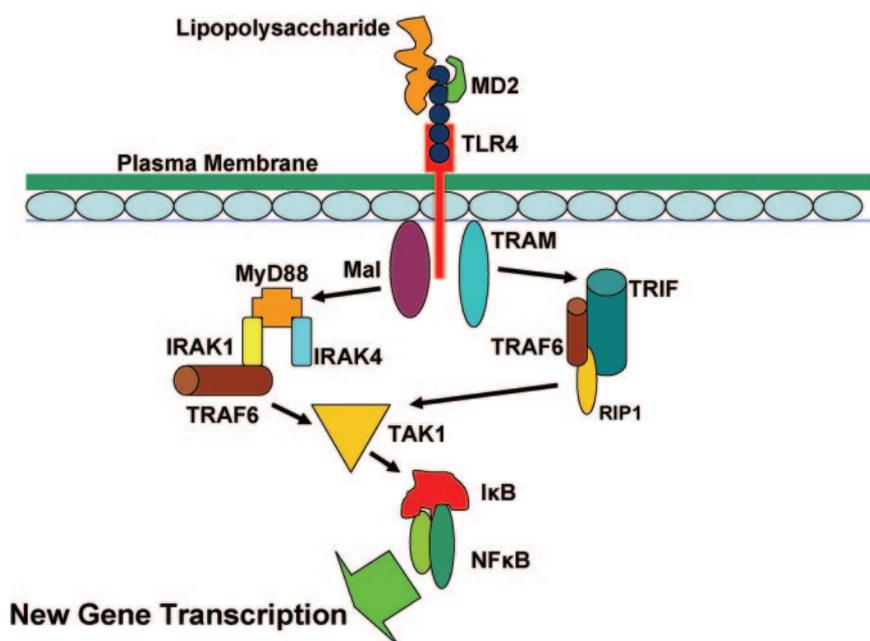
Small molecule inhibitors of these intracellular signaling pathways have not been evaluated in septic patients, but an

inhibitor of the p38 MAPK pathway can attenuate endothelial activation and inhibit fibrinolysis in human volunteers challenged with i.v. endotoxin, although it does not affect other inflammatory pathways [47]. A synthetic lipid A analog that functions as an inhibitor of ligand binding to TLR4 can block or attenuate the clinical and biochemical alterations associated with i.v. endotoxin infusion [48]. This compound is currently being evaluated in a phase-III trial of severe sepsis.

CLINICAL CHALLENGES

As more is learned about the biology of the innate immune response that produces the syndrome of sepsis, the limitations of our knowledge regarding the clinical syndrome and the shortcomings of contemporary approaches to clinical research

Fig. 3. A simplified schematic representation of events following the engagement of TLR4 by endotoxin. Engagement of Mal (TIRAP) by the intracellular tail of TLR4 recruits MyD88 and a complex of proteins including IRAK1, IRAK4, and TRAF6. This complex, in turn, activates TAK1, leading to the phosphorylation of I κ B and the translocation of NF- κ B to the nucleus, where it can promote the transcription of a number of proinflammatory cytokines. TAK1 can also be activated by a MyD88-independent pathway, initiated by the interaction of TLR4 and TIR domain-containing adaptor-inducing IFN- β (TRIF)-related adaptor molecule (TRAM) and leading to the recruitment of TRIF, receptor-interacting protein 1 (RIP1), and TRAF6. Adapted from ref. [44]. MD2, Myeloid differentiation protein 2.



are increasingly evident. These impact research in all domains, from the evaluation of resuscitation strategies to long-term rehabilitation; the focus of this manuscript, however, is novel therapies that target putative mediators of sepsis.

Clinical sepsis is a dynamic process whose epidemiology and natural history are poorly defined

Current estimates suggest the incidence of severe sepsis (sepsis associated with acute organ dysfunction) and septic shock (sepsis associated with cardiovascular compromise) to be between 300,000 and 750,000 cases each year [49, 50]. Responsible for more than 200,000 deaths each year, sepsis claims as many victims as acute myocardial infarction and is one of the 10 leading causes of death in the developed world. In the developing world, acute infectious diseases such as tuberculosis, malaria, HIV, and infectious diarrhea exact a substantial toll in preventable mortality, making sepsis a global health problem of major importance. Yet, although it is true that infection is an important cause of preventable morbidity and mortality and that much of that morbidity is affected through the activation of innate immunity, it will be readily apparent that sepsis is not a single homogeneous disease process but a generic term for a large group of diseases. Moreover, it will be further evident that the morbidity of a significant number of other acute inflammatory disorders, including pancreatitis, autoimmune disease, burns, and trauma, is driven by the same alterations in innate immunity that mediate the acute response to infection.

The nonspecific clinical criteria of SIRS are met by the majority of patients admitted to an ICU [51] and by a significant number of patients who are successfully managed outside the ICU [52]. Many of these have infection as the cause or the consequence of their state of acute illness. Two key questions arise: Does an infectious cause discriminate between patients who might benefit from supportive therapies (other than specific anti-infectious interventions) and those who will not? For patients who have infection, would we expect patients with polymicrobial peritonitis from an anastomotic leak, nosocomial pneumonia from a highly resistant *Pseudomonas*, systemic candidiasis, a urinary tract infection acquired in a nursing home, and *Pneumocystis carinii* pneumonia in the setting of advanced HIV to respond equally well to an intervention that modulates innate immunity? Intuitively, we would not, but we do not have the basic knowledge of the epidemiology of differing causes of sepsis to stratify patients more effectively, and all of these patients would meet the entry criteria for contemporary sepsis trials.

A number of years ago, for example, we sought to delineate the contribution of microbial and host response factors to the mortality of critical illness [53]. We studied a cohort of 211 critically ill patients admitted to a surgical ICU. The diagnosis of infection required the objective documentation of a microbial pathogen, by culture of normally sterile tissue or by the demonstration of pus in a sterile body cavity, and was established on microbiologic criteria alone, without reference to clinical manifestations. The presence and severity of a systemic inflammatory response were quantified using a sepsis score that graded abnormalities in five domains characteristic

of a systemic inflammatory response—temperature, leukocyte count, cardiac output, insulin requirements, and changes in level of consciousness—on a scale of 0 (normal)–3 (markedly abnormal) and that generated a daily score from 0 to 15, which provided a measure of how clinically septic a patient was. Meeting criteria for infection was associated with a significantly increased risk of ICU mortality; only four of 123 patients without infection died (3.3%), compared with 23 of 107 (21.5%) patients with microbiologic evidence of infection (odds ratio, 8.1; 95% confidence interval 2.7–24.4; $P < 0.0001$). Increasing maximal values of sepsis score similarly showed a strong association with mortality risk (Fig. 4). When we assessed patients with infection, nonsurvivors had significantly higher sepsis scores than survivors (Fig. 4). Yet, when the corollary analysis was performed, evaluating only patients with elevated sepsis scores (seven or higher), we were unable to identify any infection-related variable that discriminated survivors and nonsurvivors, suggesting that it is the magnitude of the response rather than the trigger that elicited that response that is the major determinant of outcome.

Advances in the management of cardiovascular diseases have been crucially dependent on the availability of large, longitudinal, epidemiologic studies such as the Framingham study to define risk factors for adverse outcome and so, to

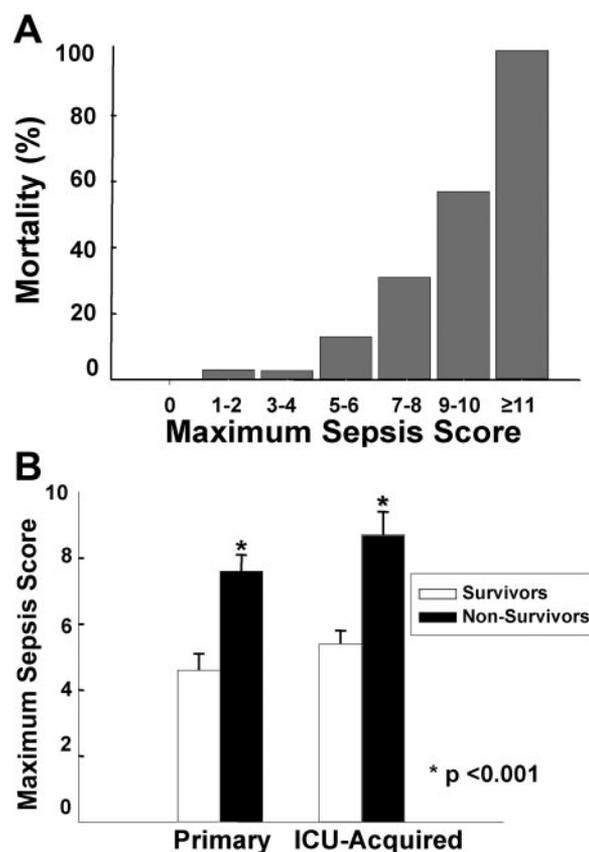


Fig. 4. Mortality increased with increasing maximal values of a sepsis score, from 0% at a score of 0–100% when the score was greater than 10, indicating that the intensity of the systemic inflammatory response correlates with risk of death (A). For patients with infection at the time of ICU admission (Primary) or infection acquired within the ICU (ICU-Acquired), nonsurvivors had higher sepsis scores than survivors. Adapted from ref. [53].

identify promising approaches to intervention [54]. In the field of oncology, successful adjuvant therapy only became possible following large international initiatives to define the natural history of specific kinds of cancer and the risk factors for recurrence or death [55]. Progress in the adjuvant therapy of sepsis will require that we move from our current approach of attempting to define disease through a process of expert consensus to one of characterizing the clinical and biochemical course of acute inflammation through large, epidemiologic studies and using the insights deriving from these to inform future attempts at disease stratification. The questions for such studies are many; some of the more basic are outlined in **Table 3**.

The phenotype of sepsis is shaped by the initial insult and subsequent clinical interventions

The clinical syndromes that evolve in critically ill patients in an ICU differ from diseases that have affected humans over the course of our existence in one fundamental regard: They arise in patients who, in the absence of ICU care, would have died a rapid death. They are, therefore, quintessentially iatrogenic disorders. They arise in the survivors of otherwise lethal disease, and the very act of subverting that lethality dictates that their subsequent course will be shaped by the medical decisions that are being made.

The natural history of sepsis, therefore, is not only poorly characterized but driven by the initial insult and the decisions made by the treating physicians. ARDS, a disorder characterized by the development of diffuse pulmonary infiltrates and impairment of oxygen uptake in the lung, is recognized to be a complication of sepsis, the pulmonary manifestation of the generalized capillary permeability increase that accompanies systemic inflammation [56]. However, the lung injury of ARDS can be exacerbated by over distention of the lung during mechanical ventilation [57], an insult that triggers the release of proinflammatory cytokines [58] and in the experimental animal, induces remote epithelial cell apoptosis and organ dysfunction [59]. The transfusion of RBCs [60], the liberal use of sedation [61], and the overuse of antibiotics [62], common sources of variability in ICU clinical care, are associated with exacerbation of organ dysfunction or worsening of the prognosis for ultimate survival.

TABLE 3. Key Questions in the Epidemiology of Sepsis and Systemic Inflammation

-
- Which individual biomarkers or groups of biomarkers at the time of initial presentation predict failure to respond to early intervention, and an increased risk of new organ dysfunction and ultimate mortality?
 - What clinical features present early in the course of illness predict a failure to respond to early intervention, and an increased risk of new organ dysfunction and death?
 - What microbiologic factors predict failure to respond, new organ dysfunction, and death?
 - What clinical variables present early in the course of disease predict each of the specific characteristic late complications of sepsis including nosocomial infection, prolonged cardiovascular dysfunction, acute respiratory distress syndrome (ARDS), and acute renal failure?
 - To what extent do the above predictors vary from one country to the next?
-

Clinical studies of agents that target innate immunity, therefore, must take into consideration not only the initial insult that elicited the response but also the modification of the course of that response by subsequent clinical intervention. It is recognized that the mortality of sepsis differs significantly throughout the countries of Europe [63]; variability in clinical practice is likely responsible for at least some of that outcome variability. Understanding the epidemiology of variable clinical care is yet another prerequisite to the design of informative clinical trials in sepsis. Similarly, during the conduct of those trials, care must be taken to ensure that clinical management is consistent with current knowledge about best practice [64].

The objectives of therapeutic intervention are poorly defined

It may seem self-evident that the therapeutic objective in treating a patient with a lethal disorder such as sepsis is to increase the odds of survival, and regulatory agencies have been resolute in their insistence that 28-day all-cause mortality is the appropriate primary outcome measure for pivotal trials of new therapies [65], but elevating the short-term mortality benefit to the position of primary endpoint for clinical research poses three major challenges.

First, mortality is an insensitive measure of biologic activity. In the experimental animal, where resuscitation is minimal, physiologic support is rarely provided, and the experimental circumstances can be manipulated to maximize a signal, short-term mortality is a simple and feasible measure of biologic activity. To use mortality as a measure of biologic activity in humans, for example, to determine optimal dose or duration of therapy or to identify potential toxicity, is at best inefficient and at worst, unethical. Yet, preliminary human studies to document activity and infer optimal dosing are rarely performed; rather, activity and dosing are inferred from animal data or from preliminary work in healthy humans. It has been all too common that large phase III trials generate indeterminate results, which on closer scrutiny, may reflect shortcomings of study design or conduct, rather than a failure of the therapeutic concept. For example, a phase-III trial of an antibody to TNF that enrolled 1879 patients was unable to demonstrate improved survival in treated patients [66]. Assay of bioactive TNF in the serum of a subset of these patients showed no evidence of reduced bioactivity in treated patients: It is entirely possible that the trial was conducted with an inactive antibody or with a suboptimal dose of an active one. A phase-III study of inhibition of NO generation by N(G)-methyl-L-arginine (L-NMMA) was terminated early because of increased mortality in the treated group [67]. A review of the data from that study shows that the mean arterial blood pressure target in the treated patients was ~85 mm Hg and that although mortality was increased in patients receiving the highest doses of L-NMMA, it was significantly reduced in those who had received the lowest doses. Finally, a phase-III study of the recombinant tissue factor pathway inhibitor, an endogenous anticoagulant, reported an absolute mortality benefit of almost 10% in patients in the first half of the study and an inexplicable increase in mortality of the same magnitude in the second half, so that the overall study results showed no effect

[68]. The discrepancy, highly improbable on the basis of random variability, remains unexplained.

Second, the morbidity and mortality of sepsis evolve over a prolonged period of time, and although deaths occur in the first few days, the increased risk of death persists for a period of years [69, 70]. Thus, an improvement in short-term survival may not translate into a longer-term survival benefit, and an improvement in health-related quality of life in a disease whose morbidity is chronic may not be reflected in mortality status. As many as 40% of surviving patients enrolled in sepsis trials remain in the ICU for at least 1 month [6]. When mortality is evaluated, a longer-time window, perhaps 90 days, appears to be more appropriate [71].

Finally, sepsis commonly occurs as a complication of other diseases that are associated with a significant short-term mortality risk and with a marked diminution in quality of life. For the patient who develops sepsis as a consequence of a pneumonia acquired in a nursing home or a soft-tissue infection associated with malignancy or peripheral vascular disease, death may not be the most feared outcome, and measures of independence and quality of life may assume primary importance.

The development and evaluation of new therapies for sepsis entail the integration of multiple lines of study to show proof of concept, to define optimal patient populations for intervention, to establish optimal dose and duration of treatment, to document short-term efficacy, and to establish long-term benefit in mortality and morbidity [72]. Too often these aspects have been ignored in studies whose primary goal is expedited drug registration.

Patients with sepsis are highly heterogeneous with respect to risk and the potential to benefit from specific therapies

An underlying theme to the preceding discussion is that sepsis is not a simple disease with uniform expression in all patients but a complex process that displays a tremendous degree of heterogeneity and that this heterogeneity, by increasing the inherent noise of clinical research, has obscured signals that would indicate therapeutic efficacy. A key challenge, therefore, is to reduce that heterogeneity by appropriately stratifying patients for the purposes of research and clinical management.

The problem of clinical heterogeneity is not unique to sepsis but rather, is an inherent property of complex diseases or diseases with highly variable expression and multiple therapeutic options. Recognition that patients with cancer do not share a single disease but rather have many different diseases whose optimal management depends on the extent of the disease at the time therapy is instituted led oncologists to appreciate the importance of developing validated staging systems to be used to stratify patients enrolled in therapeutic studies [73]. Cancer staging proceeds from the assumption that patient populations must first be segregated by the anatomic and histologic nature of the cancer and then staged based on the extent to which the tumor has spread beyond the primary. The most widely used such system, the tumor, nodes, and metastases (TNM) system, stratifies patients based on the size and invasiveness of the primary tumor, the presence and extent of regional nodal spread, and the presence of remote metastases.

In doing so, it not only groups patients based on their risk of dying from cancer but also stratifies them by their potential to benefit from specific classes of therapy. A localized tumor will respond to surgical management alone. If there is regional spread, adjuvant therapies can improve prognosis, whereas if there are distant metastases, surgery is unlikely to be beneficial, and more aggressive forms of therapy can provide palliation.

Using these concepts from oncology as a model, it has been proposed that sepsis might be staged in a similar manner on the basis predisposition, insult, response, and organ dysfunction (PIRO; **Table 4**) [74, 75]. The so-called PIRO model exists as an untested hypothesis rather than as a clinical tool, although initiatives to evaluate its feasibility and use are in progress. Multiple lines of evidence, however, indicate that each of these domains can impact not only prognosis but also the potential for differential benefit or harm following intervention.

There is evidence that each of these variables can impact the outcome following the neutralization of TNF. A number of epidemiologic studies have shown that prognosis in sepsis is significantly impacted by genetic factors [76] and specifically by the presence of SNPs in innate immune response genes [77]. A SNP in the promoter region of the TNF- α gene that replaces a guanine nucleotide with an adenine is associated with an increased risk of mortality from sepsis in some but not all studies [78]; perhaps more importantly, as anti-TNF therapies become more widely used in rheumatology, it has become apparent that patients most likely to benefit from treatment are those who have this polymorphism [79]. In animal models, the impact of neutralizing TNF is highly dependent on the challenge organism (Fig. 2) [39]. Clinical studies have suggested that the impact of TNF neutralization may be greatest in patients with elevated levels of IL-6 [10, 80] and also that the greatest benefit accrues to patients who do not have significant organ dysfunction at baseline [81].

It will require an enormous amount of work to transform the PIRO concept into a useful stratification tool for clinical research and therapeutic decision-making. Nonetheless, the de-

TABLE 4. The PIRO Model

Domain	Measures
Predisposition	Genetic polymorphisms Comorbidities Sex Age Cultural/religious beliefs
Insult	Infection—microbiology, site Intoxication—e.g., endotoxemia Injury Ischemia
Response	Physiologic (e.g., temperature, heart rate) Nonspecific markers of inflammation (e.g., C-reactive protein, procalcitonin, IL-6) Specific measures of therapeutic target (e.g., TNF, endotoxin)
Organ Dysfunction	Physiologic measures of dysfunction Use of specific therapies Biochemical markers of deranged processes (e.g., apoptosis)

velopment of staging systems for sepsis is intuitively appealing and pragmatically necessary if we are to progress.

CONCLUSIONS: TOWARD A MORE EFFECTIVE APPROACH TO CLINICAL RESEARCH

The concept of sepsis is disarmingly simple and the clinical syndrome, only too familiar to all who have cared for the acutely ill. Yet, beyond the treatment of infection by source control and antibiotics and the support of the acute physiologic derangements by cardiovascular resuscitation and ICU-based organ support, advances in management have proven elusive. Our failure to make progress reflects in no small part a persistent reluctance to change our approach to clinical research in response to a new, biologic understanding and to insights gained from clinical trials that have been undertaken over the past quarter century. At the risk of oversimplifying an admittedly complex challenge, I would suggest that the possibility of success in the future hinges on our ability to address 10 key issues:

1. Our understanding of the biological and clinical natural history of acute illness is inadequate. We need large, intensive, and unbiased epidemiologic studies of acutely ill patients to define clinical and biochemical natural history. These studies should not start from arbitrarily defined, syndromic categories such as sepsis or ARDS but rather, seek to determine the biochemical profiles associated with adverse outcome and the clinical phenotypes that associate with these biochemical profiles.

2. We do not understand the impact of clinical practice on the phenotype of critical illness. We need large, international studies to characterize practice variability in patient management and to correlate this variability with subsequent clinical course.

3. We need to develop plausible, validated systems to stratify acutely ill patients in those domains that predict increased risk of adverse outcome and differential potential to respond to therapy. The PIRO model can serve as an initial template for such an initiative, but the final architecture must be dictated by data.

4. Our therapeutic targets in innate immunity may be sub-optimal. We need to evaluate interventions that target processes arising later in the course of illness or processes such as signal transduction that can modify the expression of multiple endogenous mediators.

5. A substantial body of information has been accumulated that can inform the design of future trials targeting classical mediators such as TNF, LPS, or IL-1. We are now in a position to treat acute TNF- or IL-1-mediated disease rather than sepsis. We need to refine entry criteria for clinical trials to reflect this knowledge about patient populations who will optimally benefit from intervention.

6. In early-phase clinical research, we need to focus first on establishing proof of concept in acutely ill patients by performing small studies whose objective is to show that intervention can alter levels of relevant biomarkers or affect acute changes in physiology [72].

7. We need to focus our efforts in early-phase research on rescuing patients with disease refractory to available therapy as further proof of concept.

8. Having characterized promising populations of patients who might benefit from a particular therapy, we need to show in adequately powered phase II trials that intervention can alter levels of biomarkers related to the intervention and produce more rapid or complete reversal of study entry criteria.

9. Having accomplished the above, we should focus on demonstrating clinical effectiveness, reflected not only in short-term mortality but also in longer-term mortality and improvement in quality of life.

10. Finally, we need to study the long-term morbidity and mortality associated with intervention, to evaluate the cost-effectiveness of therapy, and to re-evaluate the continuing use of the approach in a changing therapeutic environment.

Albert Einstein defined insanity as “doing the same thing over and over, and expecting a different result.” It is time to try something different.

ACKNOWLEDGMENT

The work was presented at the 7th World Congress on Trauma, Shock, Inflammation and Sepsis, March 2007.

REFERENCES

1. Majno, G. (1991) The ancient riddle of (sepsis). *J. Infect. Dis.* **163**, 937–945.
2. Michalek, S. M., Moore, R. N., McGhee, J. R., Rosenstreich, D. L., Mergenhagen, S. E. (1980) The primary role of lymphoreticular cells in the mediation of host responses to bacterial endotoxin. *J. Infect. Dis.* **141**, 55–63.
3. Ziegler, E. J., McCutchan, J. A., Fierer, J., Glauser, M. P., Sadoff, J. C., Douglas, H., Braude, A. I. (1982) Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. *N. Engl. J. Med.* **307**, 1225–1230.
4. Marshall, J. C. (2003) Such stuff as dreams are made on: mediator-targeted therapy in sepsis. *Nat. Rev. Drug Discov.* **2**, 391–405.
5. Vincent, J-L., Sun, Q., Dubois, M-J. (2002) Clinical trials of immunomodulatory therapies in severe sepsis and septic shock. *Clin. Infect. Dis.* **34**, 1084–1093.
6. Bernard, G. R., Vincent, J-L., Laterre, P. F., LaRosa, S. P., Dhainaut, J-F., Lopez-Rodriguez, A., Steingrub, J. S., Garber, G. E., Helterbrand, J. D., Ely, E. W., Fisher Jr., C. J., Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) Study Group (2001) Efficacy and safety of recombinant human activated protein C for severe sepsis. *N. Engl. J. Med.* **344**, 699–709.
7. Eichacker, P. Q., Natanson, C. (2003) Recombinant human activated protein C in sepsis: inconsistent trial results, an unclear mechanism of action, and safety concerns resulted in labeling restrictions and the need for phase IV trials. *Crit. Care Med.* **31** (Suppl. 1), S94–S96.
8. Annane, D., Sebille, V., Charpentier, C., Bollaert, P-E., Francois, B., Korach, J-M., Capellier, G., Cohen, Y., Azoulay, E., Troche, G., et al. (2002) Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. *JAMA* **288**, 862–871.
9. Turgeon, A. F., Hutton, B., Fergusson, D. A., McIntyre, L., Tinmouth, A. A., Cameron, D. W., Hébert, P. C. (2007) Meta-analysis: intravenous immunoglobulin in critically ill adult patients with sepsis. *Ann. Intern. Med.* **146**, 193–203.
10. Panacek, E. A., Marshall, J. C., Albertson, T. E., Johnson, D. H., Johnson, S. B., MacArthur, R. D., Miller, M. A., Barchuk, W. T., Fischkoff, S., Kaul, M., et al. (2004) Efficacy and safety of the monoclonal anti-TNF antibody F(ab')₂ fragment in patients with severe sepsis stratified by IL-6 level. *Crit. Care Med.* **32**, 2173–2182.

11. Friedrich, J. O. (2006) Drotrecogin α (activated) in severe sepsis. *N. Engl. J. Med.* **354**, 94–96.
12. Bone, R. C., Fisher, C. J., Clemmer, T. P., Slotman, G. J., Metz, C. A., Balk, R. A. (1989) Sepsis syndrome: a valid clinical entity. Methylprednisolone Severe Sepsis Study Group. *Crit. Care Med.* **17**, 389–393.
13. Bone, R. C., Fisher, C. J., Clemmer, T. P., Slotman, G. J., Metz, C. A., Balk, R. A. (1987) A controlled clinical trial of high-dose methylprednisolone in the treatment of severe sepsis and septic shock. *N. Engl. J. Med.* **317**, 653–658.
14. Bone, R. C., Balk, R. A., Cerra, F. B., Dellinger, R. P., Fein, A. M., Knaus, W. A., Schein, R. M. H., Sibbald, W. J. (1992) Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* **101**, 1644–1655.
15. Casey, L. C., Balk, R. A., Bone, R. C. (1993) Plasma cytokines and endotoxin levels correlate with survival in patients with the sepsis syndrome. *Ann. Intern. Med.* **119**, 771–778.
16. Bates, D. W., Parsonnet, J., Ketchum, P. A., Miller, E. B., Novitsky, T. J., Sands, K., Hibberd, P. L., Graman, P. S., Lanken, P. N., Schwartz, J. S., et al. (1998) Limulus amoebocyte lysate assay for detection of endotoxin in patients with sepsis syndrome. AMCC Sepsis Project Working Group. *Clin. Infect. Dis.* **27**, 582–591.
17. Roumen, R. M. H., Frieling, J. T. M., van Tits, H. W., van der Vliet, J. A., Goris, R. J. (1993) Endotoxemia after major vascular operations. *J. Vasc. Surg.* **18**, 853–857.
18. Buttenschoen, K., Berger, D., Hiki, N., Buttenschoen, D. C., Vasilescu, C., Chikh-Torab, F., Seidelmann, M., Beger, H. G. (2000) Endotoxin and antiendotoxin antibodies in patients with acute pancreatitis. *Eur. J. Surg.* **166**, 459–466.
19. Marshall, J. C., Foster, D., Vincent, J.-L., Cook, D. J., Dellinger, R. P., Opal, S., Abraham, E., Brett, S., Smith, T., Mehta, S., Derzko, A., Romaschin, A. (2004) Diagnostic and prognostic implications of endotoxemia in critical illness: results of the MEDIC study. *J. Infect. Dis.* **190**, 527–534.
20. Beutler, B., Milsark, I. W., Cerami, A. C. (1985) Passive immunization against cachectin tumor necrosis factor protects mice from lethal effect of endotoxin. *Science* **229**, 869–871.
21. Poltorak, A., He, X., Smirnova, I., Liu, M.-Y., van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., et al. (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in the *Tlr4* gene. *Science* **282**, 2085–2088.
22. Anderson, K. V., Jürgens, G., Nüsslein-Vollhard, C. (1985) Establishment of dorsal-ventral polarity in the *Drosophila* embryo: genetic studies on the role of the Toll gene product. *Cell* **42**, 779–789.
23. Akira, S., Uematsu, S., Takeuchi, O. (2006) Pathogen recognition and innate immunity. *Cell* **124**, 783–801.
24. Marson, A. L., Tarr, D. E., Scott, A. L. (2001) Macrophage migration inhibitory factor (MIF) transcription is significantly elevated in *Caenorhabditis elegans* dauer larvae. *Gene* **278**, 53–62.
25. Yuan, J. Y., Horvitz, H. R. (1990) The *Caenorhabditis elegans* genes *ced-3* and *ced-4* act cell autonomously to cause programmed cell death. *Dev. Biol.* **138**, 33–41.
26. Miura, M., Zhu, H., Rotello, R., Hartweg, E. A., Yuan, J. (1993) Induction of apoptosis in fibroblasts by IL-1 β -converting enzyme, a mammalian homolog of the *C. elegans* cell death gene *ced-3*. *Cell* **75**, 653–660.
27. Nhan, T. Q., Liles, W. C., Schwartz, S. M. (2006) Physiological functions of caspases beyond cell death. *Am. J. Pathol.* **169**, 729–737.
28. Muller, W. E., Perovic, S., Wilkesman, J., Kruse, M., Muller, I. M., Batel, R. (1999) Increased gene expression of a cytokine-related molecule and profilin after activation of *Suberites domuncula* cells with xenogeneic sponge molecule(s). *DNA Cell Biol.* **18**, 885–893.
29. Jia, S. H., Li, Y., Parodo, J., Kapus, A., Fan, L., Rotstein, O. D., Marshall, J. C. (2004) Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. *J. Clin. Invest.* **113**, 1318–1327.
30. Martin, P. R., Shea, R. J., Mulks, M. H. (2001) Identification of a plasmid-encoded gene from *Haemophilus ducreyi* which confers NAD independence. *J. Bacteriol.* **183**, 1168–1174.
31. Calvano, S. E., Xiao, W., Richards, D. R., Feliciano, R. M., Baker, H. V., Cho, R. J., Chen, R. O., Brownstein, B. H., Cobb, J. P., Tschoeke, S. K., et al. (2005) A network-based analysis of systemic inflammation in humans. *Nature* **437**, 1032–1037.
32. Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J. M., Hoffmann, J. A. (1996) The dorsoventral regulatory gene cassette *spätzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults. *Cell* **86**, 973–983.
33. O'Neill, L. A., Bowie, A. G. (2007) The family of five: TIR-domain-containing adaptors in Toll-like receptor signaling. *Nat. Rev. Immunol.* **7**, 353–364.
34. Seong, S. Y., Matzinger, P. (2004) Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. *Nat. Rev. Immunol.* **4**, 469–478.
35. Sansonetti, P. J. (2006) The innate signaling of dangers and the dangers of innate signaling. *Nat. Immunol.* **7**, 1237–1242.
36. Miyake, K. (2007) Innate immune sensing of pathogens and danger signals by cell surface Toll-like receptors. *Semin. Immunol.* **19**, 3–10.
37. Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S., Medzhitov, R. (2004) Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* **118**, 229–241.
38. Lee, J., Mo, J. H., Shen, C., Rucker, A. N., Raz, E. (2007) Toll-like receptor signaling in intestinal epithelial cells contributes to colonic homeostasis. *Curr. Opin. Gastroenterol.* **23**, 27–31.
39. Lorente, J. A., Marshall, J. C. (2005) Neutralization of tumor necrosis factor (TNF) in pre-clinical models of sepsis. *Shock* **24** (Suppl. 1), 107–119.
40. Bongartz, T., Sutton, A. J., Sweeting, M. J., Buchan, I., Matteson, E. L., Montori, V. (2006) Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA* **295**, 2275–2285.
41. Schaedler, R. W., Dubos, R. J. (1962) The fecal flora of various strains of mice. Its bearing on their susceptibility to endotoxin. *J. Exp. Med.* **115**, 1149–1159.
42. Marshall, J. C., Deitch, E. A., Moldawer, L. L., Opal, S., Redl, H., van der Poll, T. (2005) Pre-clinical models of sepsis: what can they tell us? *Shock* **24** (Suppl. 1), 107–119.
43. Aarts, M. A., Granton, J., Cook, D., Bohnen, J., Marshall, J. C. (2007) Empiric antimicrobial therapy in critical illness: results of a surgical infection society survey. *Surg. Infect. (Larchmt)* **8**, 329–336.
44. Takeuchi, O., Akira, S. (2007) Signaling pathways activated by microorganisms. *Curr. Opin. Cell Biol.* **19**, 185–191.
45. O'Neill, L. A., Dunne, A., Edjeback, M., Gray, P., Jefferies, C., Wietek, C. (2003) Mal and MyD88: adapter proteins involved in signal transduction by Toll-like receptors. *J. Endotoxin Res.* **9**, 55–59.
46. Khor, C. C., Chapman, S. J., Vannberg, F. O., Dunne, A., Murphy, C., Ling, E. Y., Frodsham, A. J., Walley, A. J., Kyrieleis, O., Khan, A., et al. (2007) A Mal functional variant is associated with protection against invasive pneumococcal disease, bacteremia, malaria and tuberculosis. *Nat. Genet.* **39**, 523–528.
47. Branger, J., van den Blink, B., Weijer, S., Gupta, A., van Deventer, S. J., Hack, C. E., Peppelenbosch, M. P., van der Poll, T. (2003) Inhibition of coagulation, fibrinolysis, and endothelial cell activation by a p38 mitogen-activated protein kinase inhibitor during human endotoxemia. *Blood* **101**, 4446–4448.
48. Lynn, M., Rossignol, D. P., Wheeler, J. L., Kao, R. J., Perdomo, C. A., Noveck, R., Vargas, R., D'Angelo, T., Gotzkowsky, S., McMahon, F. G. (2003) Blocking of responses to endotoxin by E5564 in healthy volunteers with experimental endotoxemia. *J. Infect. Dis.* **187**, 631–639.
49. Martin, G. S., Mannino, D. M., Eaton, S., Moss, M. (2003) The epidemiology of sepsis in the United States from 1979 through 2000. *N. Engl. J. Med.* **348**, 1546–1554.
50. Angus, D. C., Linde-Zwirble, W. T., Lidicker, J., Clermont, G., Carcillo, J., Pinsky, M. R. (2001) Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit. Care Med.* **29**, 1303–1310.
51. Muckart, D. J., Bhagwanjee, S. (1997) American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference definitions of the systemic inflammatory response syndrome and allied disorders in relation to critically injured patients. *Crit. Care Med.* **25**, 1789–1795.
52. Rangel-Frausto, M. S., Pittet, D., Costigan, M., Hwang, T., Davis, C. S., Wenzel, R. P. (1995) The natural history of the systemic inflammatory response syndrome (SIRS): a prospective study. *JAMA* **273**, 117–123.
53. Marshall, J. C., Sweeney, D. (1990) Microbial infection and the septic response in critical surgical illness. Sepsis, not infection, determines outcome. *Arch. Surg.* **125**, 17–22.
54. Kannel, W. B. (1999) Historic perspectives on the relative contributions of diastolic and systolic blood pressure elevation to cardiovascular risk profile. *Am. Heart J.* **138**, 205–210.
55. Gospodarowicz, M., Benedet, L., Hutter, R. V., Fleming, I., Henson, D. E., Sobin, L. H. (1998) History and international developments in cancer staging. *Cancer Prev. Control* **2**, 262–268.
56. Wheeler, A. P., Bernard, G. R. (2007) Acute lung injury and the acute respiratory distress syndrome: a clinical review. *Lancet* **369**, 1553–1564.

57. (2000) Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network. *N. Engl. J. Med.* **342**, 1301–1308.
58. Ranieri, V. M., Suter, P. M., Tortorella, C., de Tullio, R., Dayer, J. M., Brienza, A., Bruno, F., Slutsky, A. S. (1999) Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome. A randomized controlled trial. *JAMA* **282**, 54–61.
59. Imai, Y., Parodo, J., Kajikawa, O., de Perrot, M., Fischer, S., Edwards, V., Cutz, E., Liu, M., Keshavjee, S., Martin, T. R., et al. (2003) Injurious mechanical ventilation and end-organ epithelial cell apoptosis and organ dysfunction in an experimental model of acute respiratory distress syndrome. *JAMA* **289**, 2104–2112.
60. Hebert, P. C., Wells, G., Blajchman, M. A., Marshall, J., Martin, C., Pagliarello, G., Tweeddale, M., Schweitzer, I., Yetisir, E. (1999) A multicentre randomized controlled clinical trial of transfusion requirements in critical care. Transfusion Requirements in Critical Care Investigators for the Canadian Critical Care Trials Group. *N. Engl. J. Med.* **340**, 409–417.
61. Kress, J. P., Pohlman, A. S., O'Connor, M. F., Hall, J. B. (2000) Daily interruption of sedative infusions in critically ill patients undergoing mechanical ventilation. *N. Engl. J. Med.* **342**, 1471–1477.
62. Chastre, J., Fagon, J. Y., Soler, P., Bornet, M., Domart, Y., Trouillet, J. L., Gibert, C., Hance, A. J. (1988) Diagnosis of nosocomial bacterial pneumonia in intubated patients undergoing ventilation: comparison of the usefulness of bronchoalveolar lavage and protected specimen brush. *Am. J. Med.* **85**, 499–506.
63. Vincent, J-L., Sakr, Y., Sprung, C. L., Ranieri, V. M., Reinhart, K., Gerlach, H., Moreno, R., Carlet, J., Le Gall, R., Payen, D. (2006) Sepsis in European intensive care units: results of the SOAP study. *Crit. Care Med.* **34**, 344–353.
64. Dellinger, R. P., Carlet, J. M., Masur, H., Gerlach, H., Calandra, T., Cohen, J., Moreno, R., Carlet, J., Le Gall, R., Payen, D. (2004) Surviving sepsis campaign guidelines for management of severe sepsis and septic shock. *Crit. Care Med.* **32**, 858–873.
65. Schwieterman, W., Roberts, R. (1997) FDA perspective on study design for therapies for severe sepsis. *Sepsis* **1**, 69.
66. Abraham, E., Anzueto, A., Gutierrez, G., Tessler, S., San Pedro, G., Wunderink, R., Dal Nogare, A., Nasraway, S., Berman, S., Cooney, R., et al. (1998) Double-blind randomized controlled trial of monoclonal antibody to human tumor necrosis factor in treatment of septic shock. NORASEPT II Study Group. *Lancet* **351**, 929–933.
67. Lopez, A., Lorente, J. A., Steingrub, J., Bakker, J., McLuckie, A., Willatts, S., Brockway, M., Anzueto, A., Holzapfel, L., Breen, D., et al. (2004) Multiple-center, randomized, placebo-controlled, double-blind study of the nitric oxide synthase inhibitor 546C88: effect on survival in patients with septic shock. *Crit. Care Med.* **32**, 21–30.
68. Abraham, E., Reinhart, K., Opal, S., Demeyer, I., Doig, C., Rodriguez, A. L., Beale, R., Svoboda, P., Laterre, P. F., Simon, S., et al. (2003) Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis: a randomized controlled trial. *JAMA* **290**, 233–247.
69. Perl, T. M., Dvorak, L., Hwang, T., Wenzel, R. P. (1995) Long-term survival and function after suspected gram-negative sepsis. *JAMA* **274**, 338–345.
70. Yende, S., Angus, D. C., Ali, I. S., Somes, G., Newman, A. B., Bauer, D., Garcia, M., Harris, T. B., Kritchevsky, S. B. (2007) Influence of comorbid conditions on long-term mortality after pneumonia in older people. *J. Am. Geriatr. Soc.* **55**, 518–525.
71. Cohen, J., Guyatt, G., Bernard, G. R., Calandra, T., Cook, D., Elebourne, D., Marshall, J., Nunn, A., Opal, S. (2001) New strategies for clinical trials in patients with sepsis and septic shock. *Crit. Care Med.* **29**, 880–886.
72. Marshall, J. C., Vincent, J. L., Guyatt, G., Angus, D. C., Abraham, E., Bernard, G., Bombardier, C., Calandra, T., Jorgensen, H. S., Sylvester, R., Boers, M. (2005) Outcome measures for clinical research in sepsis: a report of the 2nd Cambridge Colloquium of the International Sepsis Forum. *Crit. Care Med.* **33**, 1708–1716.
73. Mackillop, W. J., O'Sullivan, B., Gospodarowicz, M. (1998) The role of cancer staging in evidence-based medicine. *Cancer Prev. Control* **2**, 269–277.
74. Marshall, J. C., Vincent, J-L., Fink, M. P., Cook, D. J., Rubenfeld, G., Foster, D., Faist, E., Reinhart, K. (2003) Measures, markers, and mediators: towards a staging system for clinical sepsis. *Crit. Care Med.* **31**, 1560–1567.
75. Levy, M. M., Fink, M., Marshall, J. C., Abraham, E., Angus, D., Cook, D., Cohen, J., Opal, S. M., Vincent, J-L., Ramsay, G. (2003) 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit. Care Med.* **31**, 1250–1256.
76. Sørensen, T. I., Nielsen, G. G., Andersen, P. K., Teasdale, P. W. (1988) Genetic and environmental influences on premature death in adult adoptees. *N. Engl. J. Med.* **318**, 727–732.
77. Holmes, C. L., Russell, J. A., Walley, K. R. (2003) Genetic polymorphisms in sepsis and septic shock: role in prognosis and potential for therapy. *Chest* **124**, 1103–1115.
78. Mira, J-P., Cariou, A., Grall, F., Delclaux, C., Losser, M-R., Heshmati, F., Cheval, C., Monchi, M., Teboul, J-L., Riche, F., et al. (1999) Association of *TNF2*, a *TNF-α* promoter polymorphism, with septic shock susceptibility and mortality. *JAMA* **282**, 561–568.
79. Lee, Y. H., Rho, Y. H., Choi, S. J., Ji, J. D., Song, G. G. (2006) Association of *TNF-α* -308 G/A polymorphism with responsiveness to *TNF-α*-blockers in rheumatoid arthritis: a meta-analysis. *Rheumatol. Int.* **27**, 157–161.
80. Reinhart, K., Wiegand-Löhnert, C., Grimminger, F., Kaul, M., Withington, S., Treacher, D., Eckart, J., Willatts, S., Bouza, C., Krausch, D., et al. (1996) Assessment of the safety and efficacy of the monoclonal anti-tumor necrosis factor antibody-fragment, MAK 195F, in patients with sepsis and septic shock: a multicenter, randomized, placebo-controlled, dose-ranging study. *Crit. Care Med.* **24**, 733–742.
81. Marshall, J. C., Albertson, T. E., Barchuk, W., van Meter, L. (2001) Effect of anti-TNF antibody on organ-specific components of MOD score. Afelimomab Study Group. *Am. J. Respir. Crit. Care Med.* **163**, A820.